Crude black pepper phytochemical 3D printed cell based miniaturized hydrazine electrochemical sensing platform

Khairunnisa Amreen [1], Mary Salve [1], Sanket Goel *

MEMS, Microfluidics and Nanoelectronics Laboratory, Department of Electrical Engineering, Birla Institute of Technology and Science-Pilani, Hyderabad, India

A R T I C L E   I N F O

A B S T R A C T

Herein, a glassy carbon electrode (GCE) modified with a hybrid of crude black pepper-graphitized mesoporous carbon (GCE/GMC@pep) for an electrochemical detection of hydrazine is presented. The GCE/GMC@pep shows a surface-confined redox peak at an electrode potential $E_{1/2} = + 235$ mV vs.Ag/AgCl in pH 7 PBS with a surface excess value $\Gamma_{e-pep} = 13.19 \times 10^{-9}$ mol^{-1} cm^2. The physico-chemical characterization unveils the interaction of GMC with piperine. Further, GCE/GMC@pep manifests a highly selective electro-catalytic activity towards Hydrazine in neutral pH at $+257$ mV in a linear detection range of 70 μM-7 mM with lower limit of detection of 40 μM(with cyclic voltammetry) and 30 μM-5 mM with lower limit of detection of 0.5 μM (with chronoamperometry). More so, the fabricated electrode system provides an insignificant interference from co-existing biochemical / chemicals. Real samples, tobacco, cigarette smoke, lake water and human blood serum, are further analyzed via standard addition approach. Overall, this study describes a prototype module for tuning the redox active behavior of selective phytochemical for electrochemical sensing applications.

1. Introduction

xploring the electroactive nature and electron transfer mechanism of natural products is a herculean task. Natural products are a concoction of many phytochemical [1] owing to which, studying electro-chemical behavior of the selective species is challenging. However, with a fine-tuning and electrode surface modification with suitable matrices [2,3], one can entrap the desired phytochemical and its surface confined redox behavior can be studied.One of the significant phytochemicals whichis utilized in everyday life is piperine, which is a major component of black pepper [4]. Crude black pepper isa significant spice used globally [5].Apart from its culinary uses, the literature reveals its potential by harnessing its analgesic, antipyretic, antioxidant, antimicrobial and anti-inflammatory properties [6]. It was also reported that piperine acts as anticonvulsive and a vasodilator, due to which it effects the reproductive and nervous system [5]. Although there are many studies based on the isolations and uses of piperine and crude pepper, but meager studies have been reported regarding its electro chemical activity.The chemical composition of pepper is an alkaloid called piperine (Scheme I) which gives the pungency [7]. The aroma and flavor is constituted by a volatile essential oil present in pepper [8].In this study, an attempt was made to trap the piperine from crude extract of black pepper onto a graphitized mesoporous carbon matrix over a GCE electrode surface to analyze its redox activity in a miniaturized plat form.

A plug-and-play miniaturized system provides an attractive analytical platform due to its features of low sample volume, high-throughput analysis and ability to integrate with different detection system. The use of robust micro-devices [9] for electrochemical detection has become popular for several analytes as it allows direct and label-free detection without any derivatization step. 3D Printing [10] is an attractive approach for the rapid prototype development for fabricating 3D configuration in a layer-by-layer manner. Off lately, it is emerging as a potential tool to revolutionize the fabrication of miniaturized/microfluidic devices for variable applications.Due to the salient features like complete automation, varied commercial applications in industry and academia, ease to build required designs, opens a pathway for manufacturing novel materials, electrodes and electrochemical sensing platforms.However, development ofthese materials, electrodes as sensor has been recently developed due to the availability of the conductive material to be appliedin the process [11,12]. The processinvolves, designing of a 3D solid object built using a Computer Aided Design software (CAD). This then needs to be changed to (STL) Standard Triangle Language file format i.e.; compatible with the 3D printer software. This changes a 3D image into a corresponding 2D layer of the object giving a G-code file. Ultimately, the 3D printer is able to print the object in a layer-by-layer manner by depositing the conductive material [12]. Various sensorshave been developed using 3D printed device combined with optical, electrochemical and electrochemiluminescencetechniques [13–15].

* Corresponding author.
  E-mail address: sgoel@hyderabad.bit-pilani.ac.in. (S. Goel).
[1] These authors contributed equally to this work.

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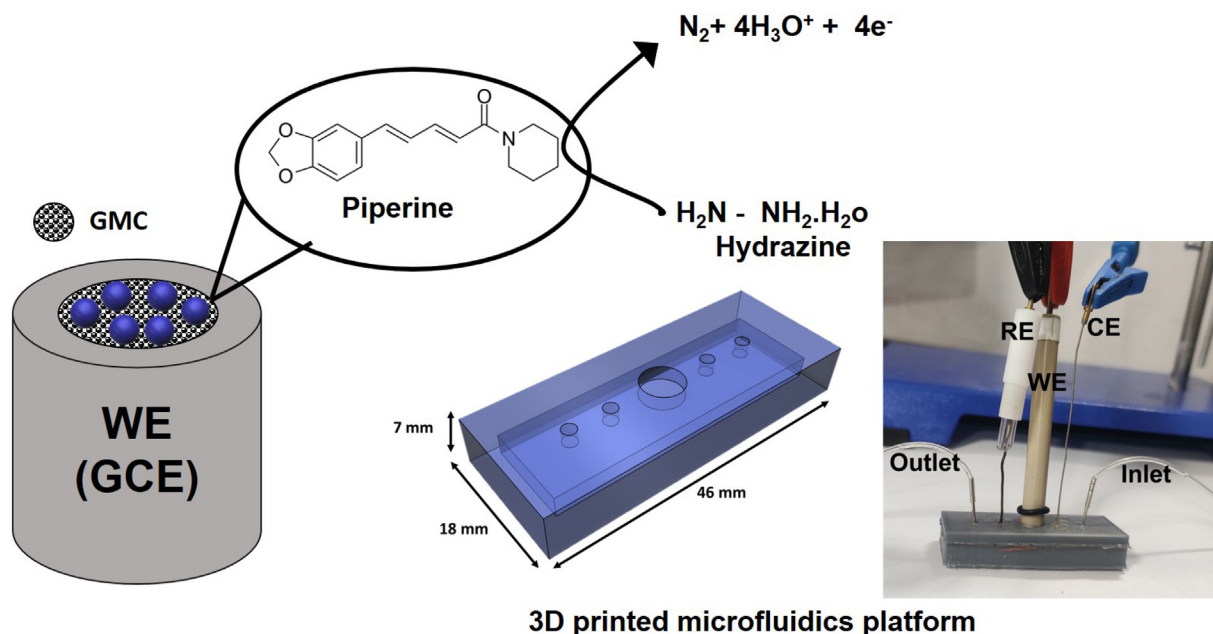
Scheme I. Schematic representationand the real image of the electrode modification, 3D printed miniaturized three-electrode platform and the reaction mechanism of electro-catalytic oxidation of hydrazine via GCE/GMC@pep.

The integration of electrochemical instrumentation using 3D printed cell has gained interest as they are relatively cost-effective,robust, ease to handle, provide biosensing compatible platform and several other features that are needed for developing point for care testing devices [16]. The pioneer application of 3D-printing for electrochemical sensing was given by Snowden et al., in 2010. They developed a flow-cell which can be integrated with the electrode of choice for electrochemical sensing using a redox probeferrocenylmethyltrimethylammoniumhexaflorophosphate (FcTMA$^+$) [17]. Since then, substantial amount of 3D printed electrodes for biomedical applications [18], optical applications [19], conductive materials [20], electrochemical sensors [21], microfluidic devices [22] have been fabricated. Recently, our group has developed 3D printed microfluidic device for electrochemical detection of hydrogen peroxide using pencil graphite electrode modified with silver nanoparticle [14].

Meanwhile, the electrochemical activity of the natural product; piperine is not much explored.Wang et al.,studied thequantification of piperine in black pepper [4] using a voltammetry technique in a bulk system with GCE as a working electrode. Herein, they were successful to get an irreversible peak which was directly proportional to the concentration of piperine. However, to the best of our knowledge, the studies on immobilization of piperine as a redox mediator for further electrochemical sensing application is not reported so far.

As a model system, application of GCE/GMC@pep for electro-catalytic, selective sensing of hydrazine [23],was carried out. Hydrazine and its derivatives are latent reducing agents and are deployed as a basic material for varied products including emulsifiers, pesticides, explosive, rocket propellant for ignition, corrosion inhibitor in industrial plant and photographic chemicals [24].Regardless of its broad spectrum applications, it is one of the hazardous pollutants and Group B2 carcinogen [25]. With the increase in industrialization, there is a significant upsurge in the usage of it. Therefore, its pollution in water bodies, soil and ultimately to food crops has significantly augmented. In further, certain human consumption products like tobacco and cigarette contains high amount of this hazardous compound. Ultimately, it tends to enter the blood system. Therefore, a sensitive and selective detection of hydrazine is in demand for protection of human health and environmental remediation. Though there are conventional spectroscopic and color-based analytical methods for detection, yet electro analytical methods are preferred due to

their simplicity and ease-of-use and remarkable limit-of-detection [26,27]. There are different carbon nanomaterials, organic, inorganic chemical, metal complex like prussian blue, metal-hexacyanoferrate, aromatic hydrocarbons, polymers etc. based electrochemical sensors for hydrazinereported in the literature specifically in bulk systems. For instance, Jayasri et al., in 2007 reported an amperometric based detection of hydrazine with manganese hexacyanoferrate- graphite powder and wax composite electrode. The analysis was done in bulk solution with ferricyanide as redox probe [28]. Likewise, Luo et al., reported cobalt hexacyanoferrate and graphene nanocomposite for simultaneous detection of hydrazine and nitrite. The analysis was carried out in a conventional bulk volume three-electrode system. Further, the real sample analysis was done in tap water and river water in a slightly acidic pH supporting electrolyte of PBS [29]. Kang et al. developed a unique electrode based on silicate sol-gel matrix-modified indium tin oxide which are pre adsorbed with $[Fe(CN)_6]^{3-}$ ions for hydrazine sensing in bulk system [30].In a similar work, Ramaraj et al., in 2017, fabricated a screen printed disposable electrode with Polydimethyldiallylamine stabilized Copper(*II*)hexacyanoferrate nanocubes modification.However, a tedious method of electrode preparation was adopted with multiple steps [31]. Vishnu et al., reported nickel hexacyanoferrate complex' in situ modification on carbon nanotube as redox mediator for bulk volume conventional three-electrode based hydrazine detection [32]. In a same way, numerous reports are available with chemical mediators and bulk sample analysis, however, negligible reports are available using a non-hazardous, natural product; phytochemical as a redox probe for hydrazine detection in microfluidic cell.

In the present work, GCE/GMC@pep has been fabricated showing an excellent electro-catalytic oxidation of hydrazine. In addition to this, effect of varying concentration was examined which gave a substantial limit-of-detection. Effect of interference from other biochemicals was also studied and real sample analysis (detection in cigarette smoke, tobacco and blood serum of smoker) was also performed to examine the practicality of the fabricated system. The results obtained portrayed that the designed electrochemical miniaturizedplatform, using crude pepper modified electrode, for an effective electrochemical sensing was highly stable and selective. Further, it is extendable for point-of-care analysis of hydrazine in real samples.

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2. Materials and methods

2.1. Chemicals and reagents

Crude pepper was purchased from DMart supermarket, Hyderabad, India. Commercially available piperine with 97% purity was obtained from Sigma Aldrich. Carbon nanomaterials used: Graphitized mesoporous carbon (GMC) (50 nm size and 99.95% purity), Multiwalled Carbon Nanotube (MWCNT) (99.9%), Singlewalled Carbon Nanotube (SWCNT), Graphite powder (GP), Carbon Nanofibre (CNF) were acquired from Sigma Aldrich.Hydrazinesulphate was also procured from Sigma Aldrich.All the other chemicals used for the supporting electrolyte, sensing etc.were of analytical grade and used without any further purification.

2.2. Design and fabrication of 3-D printed miniaturized device

A commercial desktop 3D printer based on Fused Deposition Modelling (FDM) from Flashforge Maker was used. The computer-aided design (CAD) of the miniaturized device was created in 123D design software (Autodesk) and converted to required 3Dprinter format (.stlfile). The printing material used in this work is acrylonitrile butadiene styrene (ABS) filament of 1.75 mm diameter which was compatible with printer. The optimized parameter for printing of ABS filament was 240 °C extruder temperature, 120°Cbed temperature with a layer height of 100 mm and 100% infill. Scheme I show device schematic diagram and real image of the developed 3D printed miniaturized device with overall dimensions of 40 mm × 20 mm × 5 mm (length × breadth × height) with a close rectangular reservoir (30 mm × 8 mm × 3 mm) for the fluid. The volume of the consumed reagent was 750 μl for the reservoir to getfilled completely. The inlet and outlet ports for the fluid were realized on the top layer of diameter 1 mm and for the 3 electrode ports for GCE (5 mm diameter), Pt wire (0.8 mm diameter) and Ag/AgCl wire (0.8 mm diameter). The electrode holes were made of appropriate dimension in the 3D printed cell, so that while inserting the electrode in the 3D printed cell, it gets properly fit so that the leakage can be avoided. Further, the top layer of the cell, where the insertion holes are made for the fluid insertion, was gluedusing a glue gun to make the device leakage-proof.The continuous flow of electrolyte was made by introducing the solution into the fabricated cell using an external syringe pump at a flow rate of 500 μl/min. Scheme I depicts the modified surface of GCE. The developed platform was fully reusable, and after each experiment the platform was cleaned using water gun and dried in oven at 40 °C for 30 s.The benefits of using this type of 3D printed cell include the amenability to perform the electrochemical sensing with low volume of electrolyteconsumption (μL), high-throughput analysis and ability to integrate with different detection systems.

2.3. Apparatus

Cyclic Voltammetry (CV) measurements were performed using OrigaLsysElectrochem (OrigaFlex 500). A miniaturized 3D printed platform,with a three electrode based system, was used for fabrication of electrochemical sensing system. A three electrode system comprising of chemically modified glassy carbon electrode as a working electrode, Platinum wire as a counter and Ag/AgCl(3 M KCl) as areference electrode with 700 μL working volume was used.cApreoLoVac scanning electron microscope (SEM) was used for microscopic analysis of the developed electrode.Likewise, FTIR-4200 from Jasco, RigakuUltima IV, UV-VIS Spectrophotometer V-650 JASCO, uniRAM −3300 Micro Raman Mapping spectrophotometer were used for FTIR, UV–Vis, and Raman spectroscopy. A syringe pump was acquired from the Holmarc Pvt. Ltd. for the continuous flow of the fluids.

2.4. Preparation of GCE/nanomaterial@pep

Around 10 g crude pepper was crushed, using motar and pestle, into a fine powder. 0.1 g of the grounded powder was weighed and mixed with 10 mL of pH 7 phosphate buffer solution (PBS). The mixture was sonicated for 30 min and this solution was used as an electrolyte.Electrochemical pretreatment of GCE was carried out by manual cleaning with alumina powder/scrub pad followed by electro-chemical pre-treatment in pH 7 PBS in a potential window of − 200 to 1000 mV vs. Ag/AgCl for $n = 20$ at 50 mV/s. 2 mg of the carbon nanomaterial was dispersed in 500 μL of ethanol and kept in ultra-sonication bath for 15–20 min. 2 μL of the dispersed solution was drop-casted on clean GCE and left for air drying for about 2–3 min. The carbon nanomaterial modified electrode was E-cycled in the pepper solution (700 μL) in the potential window − 400 to 800 mV vs Ag/AgCl for 40 cycles at 50 mV/s. Post 40 cycles, the electrode was cleaned gently with distilled water and shifted to blank solution (PBS pH 7) and the CV was performed in the same parameters for confirming the stability.

2.5. Electro-catalysis of hydrazine with GCE/GMC@pep

The fabricated GCE/GMC@pep was subjected to the electrocatalytic sensing of hydrazine by dipping the modified electrode in 1mMsolution with platinum and Ag/AgCl electrodes. CV measurements were carried out at the mentioned parameters for 2 cycles at 10 mV/s.

2.6. Real sample preparation

Hydrazine detection in cigarette smoke, cigarette tobacco and blood serum sample was demonstrated. The cigarette sample and tobacco samples were prepared using a standard protocol from the literature [33,34]. A cigarette pack was procured from local shop and used as received. The cigarette smoke was collected in pH 7 PBS using a dreschel's bottle. Herein, a burning cigarette was placed on one end of the glass tube of the dreschel's bottle which was then immersed in 100 mL pH 7 PBS. The other glass tube of the bottle was connected with a suction pump. The smoke got dissolved in PBS which was further serially diluted and used. Same cigarettes were cut open and tobacco was collected. 5 mg of the tobacco as sonicated in 10 mL pH 7 PBS for 20 mins, which was used for analysis.In addition to this, blood serum sample of a chain smoker volunteers was also analyzed to detect the traces.The already treated blood serum samples were taken from the medical center and used without any further treatment. The dilution was 1:5 in PBS.

3. Results and discussion

3.1. Piperine immobilization over GCE/GMC

Cyclic voltammetry technique was used for electrochemical deposition of piperine from the pepper solution at 50 mVs^{-1}. In the present study, the piperine modified electrode (GCE/GMC@pep) showed a well-defined and a stable redox peak at $E_{1/2} = + 235$ mV vs. Ag/AgCl. The obtained redox peak was also stable in a blank pH 7 PBS with a surface excess value Țe-pep $= 13.19 \times 10^{-9}$ mol^{-1} cm^{2}. The feasible mechanism of piperine immobilization onto GMC could be due to $\pi - \pi$ interaction of graphitic composition of GMC and the aromatic ring of piperine [1]. As can be seen in Fig. 1A (curve a), GCE/GMC upon e-cycling in the pepper solution, gave a well-defined, continually growing (up to $n = 40$), redox peak at $E_{1/2} + 235$ mV vs Ag/AgCl in a potential window of − 400 to 800 mV. Whereas, the blank solution (GCE/GMC), in PBS pH 7 alone, failed to give any such peak (curve b). Consequently, it was validated that GCE/GMC interacts with the piperine present in the solution. To further check the stability post immobilization, GCE/GMC@pep was transferred to a blank (without pepper) PBS. CV was performed ($n = 20$) at 50 mV/s. The peak retained with (~3%) decrease in the current. Henceforth, the GCE/GMC@pep was fabricated (Fig. 1B).

In order to verify that the redox peak obtained was due to piperine alone form the crude pepper but not due to any other component, a control experiment using purified commercially available pepper was performed. 0.1 g of piperine (commercial) was taken in 10 mL of PBS as a stock to perform CV at optimized parameters. Interestingly, the peak obtained is at $E_{1/2} =$
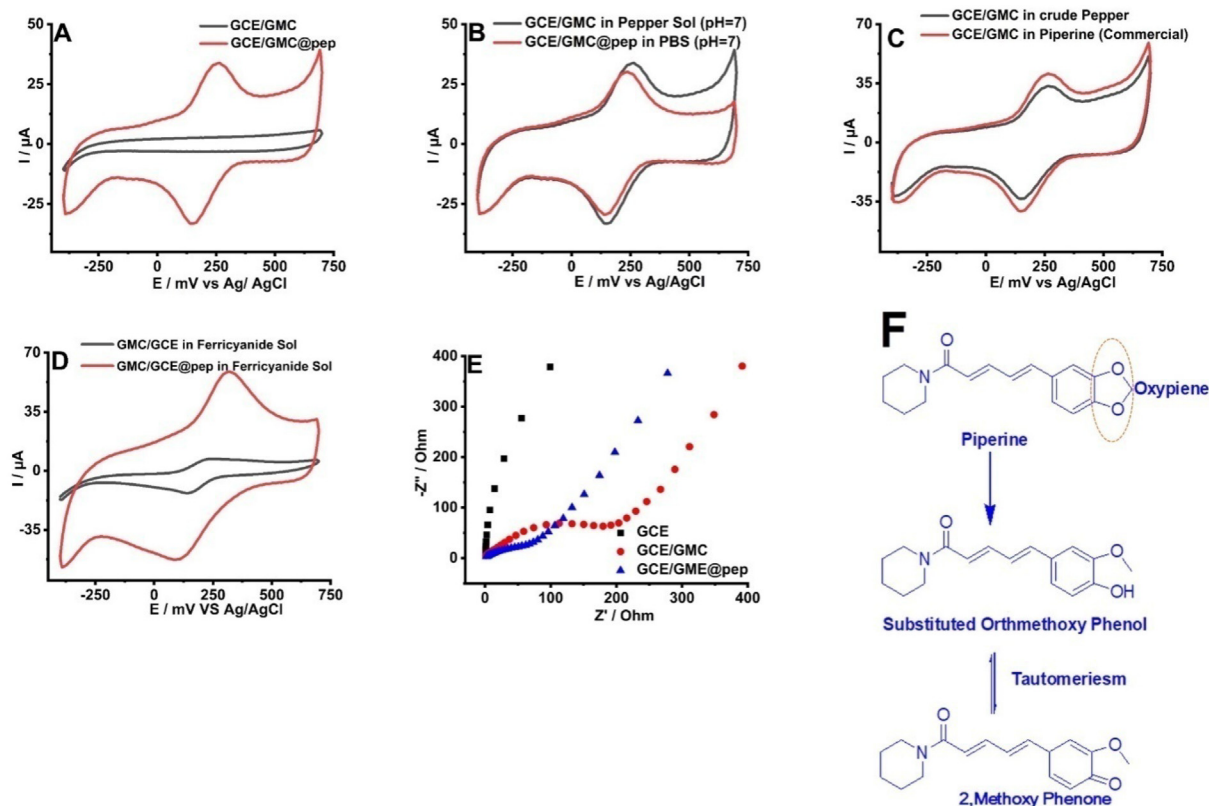
Fig. 1. (A) CV response of GCE/GMC in pH 7 PBS and pepper solution stock at 50 mV/s vs. Ag/AgCl. (B) GCE/GMC@pep in stock pepper solution and blank pH 7 phosphate buffer solution at 50 mV/s. (C) Comparative CV response of GCE/GMC modified with crude pepper and commercial piperine. (D) CV response of GCE/GMC alone and GCE/GMC@pep in 5 mM Potassium Ferricyanide at 50 mV/s vs. Ag/AgCl. (E) Electrochemical Impedance spectra of bare and modified electrodes in 5 mM ferricyanide solution. (F) Plausible mechanism of the piperine redox mechanism.

+235 mV vs. Ag/AgCl and co-insides with the peak attained by crude pepper (Fig.1C). However, the commercial piperine showed slightly higher current value than crude pepper plausibly due to isolation and purity. Henceforth, it was established that there was a selective immobilization of piperine from the concoction of pepper.

3.2. Electrochemical studies of GCE/GMC@pep

Using a standard ferricyanide solution (5 mM), the electrochemical activity of the prepared electrode was observed by performing the CV. The results obtained from the CV, clearly depicted that the GCE/GMC@pep gave higher current with the redox mediator as compared to the GCE/GMC alone. Therefore, it can be concluded that immobilization of piperine improves the electron-transfer behavior (Fig.1D).

Fig. 1E gives the electrochemical impedance spectra of the GCE/GMC@pep in 5 mM Ferricyanide solution. It is clearly depicted from the spectra that post modification with GMC followed by Piperine, the conductivity of the bare (unmodified GCE) increased. This is plausible due to the fact that with each modified layer, the shuttling of electrons is enhanced.The mechanism of piperine oxidation and reduction is given in Fig. 1F. Based on the reported literature, it is presumed that the benzodioxypienering moiety of the piperine molecule undergoes redox mechanism upon applying potential cycle. Ring opening takes place causing ring cleavage to give a substituted orthomethoxy phenol as an intermediate which further undergoes, the keto-enol tautomerism, to give 2, methoxy phenone. Gandhi et al., reported Sesamol-carbon nanoblack based electrochemical oxidation wherein, oxypiene moiety undergoes redox action. In reference of that, the proposed mechanism was considered as the redox peak obtained is similar [35].

3.3. Physico-chemical characterization

For validating the immobilization of piperine, the fabricated electrode GCE/GMC@pep, control electrodes: GCE/GMC and piperine (commercially available) were subjected to Raman, UV–Vis, FT-IR and SEM characterization. Fig.2 (A-C) are the SEM images and EDX analysis. (A) is the SEM of bare GMC wherein porous nature is observed, whereas, in (B) i.e.; GMC@pep, porous surface is masked by spherical particles of probable of piperine. (C) EDX shows absence of any metal except carbon and oxygen.The other carbons SEM images with piperine were also recorded. (Fig. S1, Supplementary). The spherical structures as observed in GMC were not observed with other carbons. Further, Fig.2D shows the UV–Vis characterization. Bare electrode (GCE/GMC) gave the response at 224 nm and piperine alone gave at 342 nm. Whereas, the composite of GCE@pep clearly showed two distinctive peaks at 224 and 342 nm, authenticating the immobilization of piperine over GMC. Fig.2E, represents the FTIR spectrum of the optimized and blank electrodes. The peaks obtained by Piperine alone, 3378 (-OH str), 2936 (-CH str), 1638 (-NH bnd), 1437 (-C=C), 1257 (-C-N) str), 1028 (-C-O str) and GMC alone, 3439 (-OH), 1582 (C=C), 1425 (-OH bnd) were exactly retained in the optimal electrode GMC@pep, 3432 (-OH str), 2920 (-CH str), 1579 (-NH), 1432 (-C=C), 1241 (-C-N), 1096 (-C-O str), ratifying the immobilization of piperine over GMC. Fig.2F shows the Raman characterization, post and pre modifications. The bare GMC gave D and G bands at 1301 and 1512 cm^{-1}characteristic to the graphitic and distorted nature of the underlying carbon, whereas, after immobilization of piperine, the band intensities were clearly changed. The calculated ID/IG ratio (based on the area under the peak) [36] shows a substantial change from 0.148 to 0.22, this change signifies the modification of underlying carbon with piperine, plausible due to π-π interaction [36].
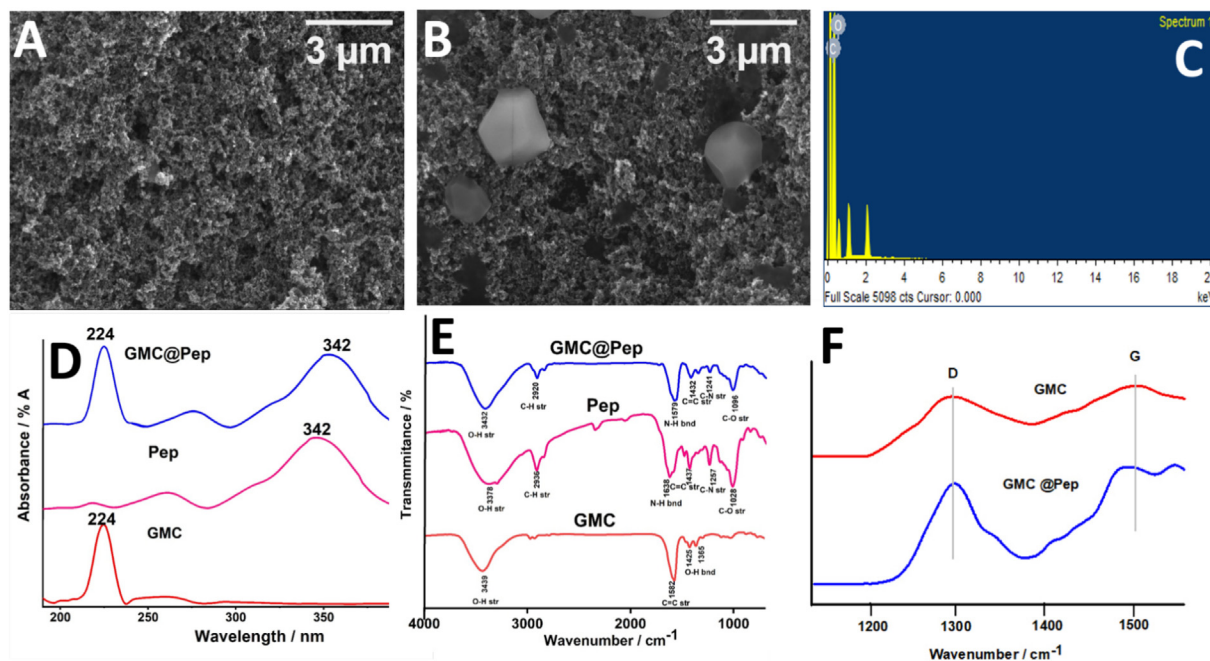
Fig. 2. (A) SEM image for bare GMC. (B) SEM image for GMC@pep. (C) *E*-DAX analysis for GMC@pep. (D) UV–Vis spectroscopy. (E) FT-IR spectrum. (F) Raman spectroscopy.

3.4. Effect of underlying carbon

To study the key role of underlying carbon in tuning the redox property of piperine, the CV experiments were conducted by modifying the working electrode with various carbon nanomaterials. The parameters like scan rate, potential window, pepper solution and pH were kept constant while carbon nanomaterial were screened. Different carbon nanomaterials known for improving the electron-transfer mechanism, like multi walled carbon nanotube, single walled nanotube, graphite nano-powder, carbon nanofiber, graphene oxide, were tested. It was discovered that these materials showed no electrochemical redox peak corresponding to piperine (Fig.3). Thus, it can be concluded that the porous nature of graphitized mesoporous carbon not only assisted the entrapment of piperine but also enhanced it' redox electrochemical behavior [1].

3.5. Effect of scan rate

Effect of scan rate on the CV GCE/GMC@pep was also studied. Fig.4A shows a comparative CV response from 10 to 340 mV. An ordered increment in both anodic and cathodic peak current was observed. A base-line corrected peak current vs. scan rate plot exhibited a linearity starting from the origin representing a surface-confined electron-transfer mechanism of the modified electrode (Fig.4(B)). This also indicates an excellent immobilization and an active electron-transfer mechanism of piperine
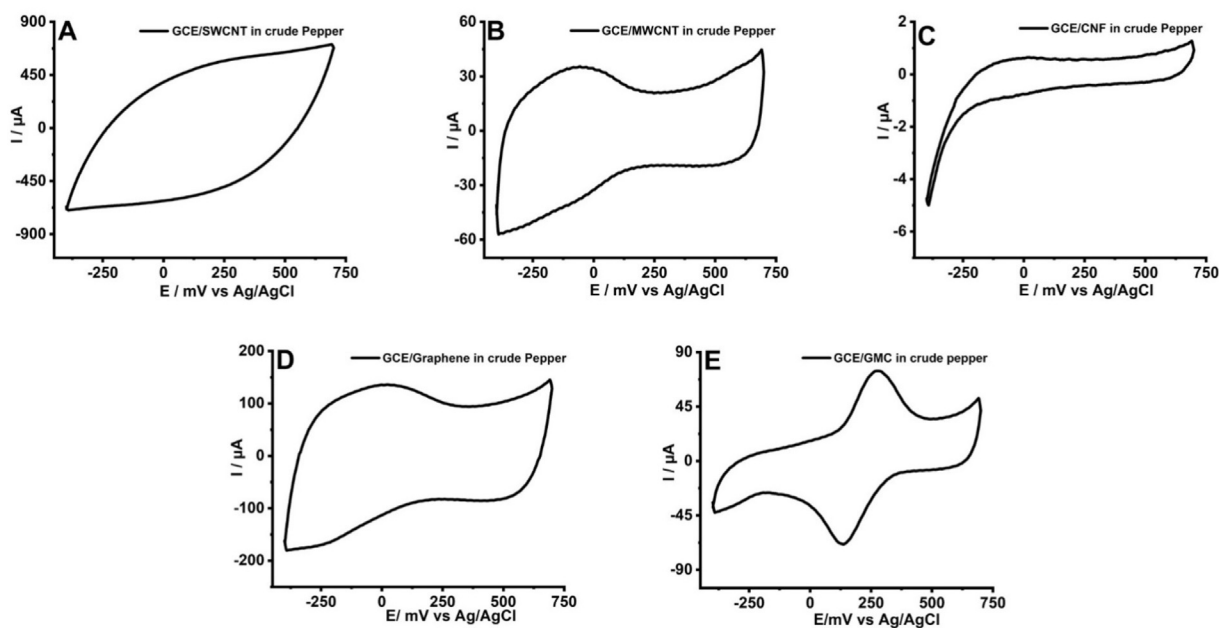

Fig. 3. Comparative CV response of various carbons (A) Single walled carbon nano tube (SWCNT), (B) multi walled carbon nano tube (MWCNT) (C) Carbon nano fiber (CNF), (D) Graphene (E) Carbon mesoporous hydrophilic in pepper solution at 50 mV/s (pH 7) vs Ag/AgCl.
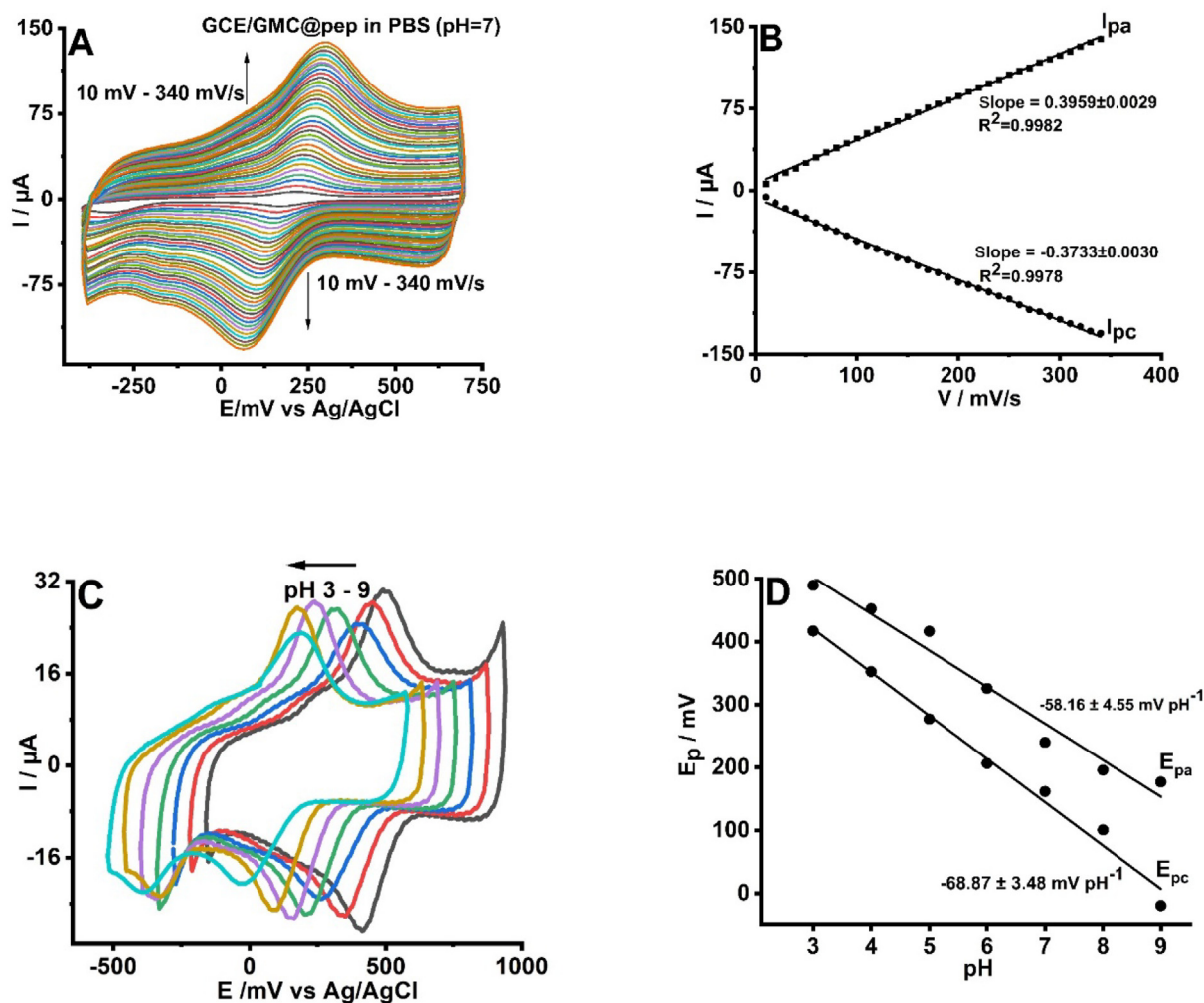
Fig. 4. (A) Effect of varying scan rate on GCE/GMC@pep in pH 7 PBS. (B)Corresponding plot of I_{pa}/I_{pc}vs υ. (C) Comparative CV response of GCE/GMC@pep in various pH phosphate buffer solutions (3–9 pH). (D) Corresponding plot of E_{pa} and E_{pc}vs pH.

with GMC. The results obtained are in co-ordinance with the following equation for a reversible reaction [37].

$$I_{pa} \text{ or } i_{pc} = n^2 F^2 A_{\,e-pep} \upsilon / 4RT \qquad (1)$$

wherein, n is the number of electrons involved, F = faraday constant (96500), A = geometrical surface area of the electrode, υ = scan rate.

3.6. Effect of solution pH

The effect of solution pH on the redox activity of GCE/GMC@pep was explored by performing CV in various pH PBS (2–9 pH).Fig.4C, is a typical CV response from 2 to 9 pH solutions which shows a negativeNernstian shift in the redox peak potential in contradiction of pH [29].A plot of E_{pa} and E_{pc} vs. pH (Fig. 4D) appears linear and has a slop value of -58 ± 1 mV pH^{-1}. This evidently illustrates a characteristic Nernstian behavior with equal number of protons and electrons participating in the reaction [29].

3.7. Electro-catalytic activity towards hydrazine

GCE/GMC@pep was subjected to study the electro-catalytic oxidation of hydrazine in pH 7 PBS. Fig.5A, is a distinctive CV response of GCE/GMC@pep without and with 1 mM of analytein neutral pH PBS. The optimized electrode showed a typical oxidation peak at E' + 257 mV vs. Ag/AgCl corresponding to hydrazine electro-catalytic oxidation [38–41]. Whereas, the control experiment (without analyte) failed to give any such

response (Fig.5A). However, a small bump due to dissolved oxygen in PBS was observed as the experiment was carried out at low scan rate (10 mV/s). Likewise, the control experiment wherein, GCE/GMC bare control electrode in 1 mM Hydrazine also failed to give any response.

Subsequently, the effect of varying scan rate on electrocatalytic oxidation via GCE/GMC@pep was also explored. It was found that oxidation current increases linearly upto 100 mV/spost which slight decrement in the current started indicating the electrode saturation (Fig. 5B). Corresponding plot of i_{pa} vs υ(mV/s) was linear (Fig. 5C).Further, for the analytical application purpose, the effect of varying hydrazine concentration was examined to find out the upper and lower limit range.Both CV and chronoamperometry where used for studies.Fig. 5 D,Eare the CV experiments of the lower and higher concentrations. The electrode showed a remarkable electro-catalytic activity in a linear range of 70 μM – 7 mM with an appreciable limit of detection (LOD) as 40 μMwas in accordance with the reported literature (Table 1). Since no reports are available using phytochemical as a redox mediator for Hydrazine sensing, a couple of recent publications with other chemical redox mediators were explored in Table 1. Although these literatures showed appreciable LOD, but involvedelectrode modifications with non-ecofriendly chemicals.Whereas, the present work is first of its kind that has a phytochemical based electrode modification, hence, involves green chemistry. In further, Fig. 5F,gives the chronoamperometry response of the modified system, GCE/GMC@pep in the concentration range of 30 μM to 5 mM. Corresponding calibration plot (Fig. 5F) showed linearity with LOD as 0.5 μM.
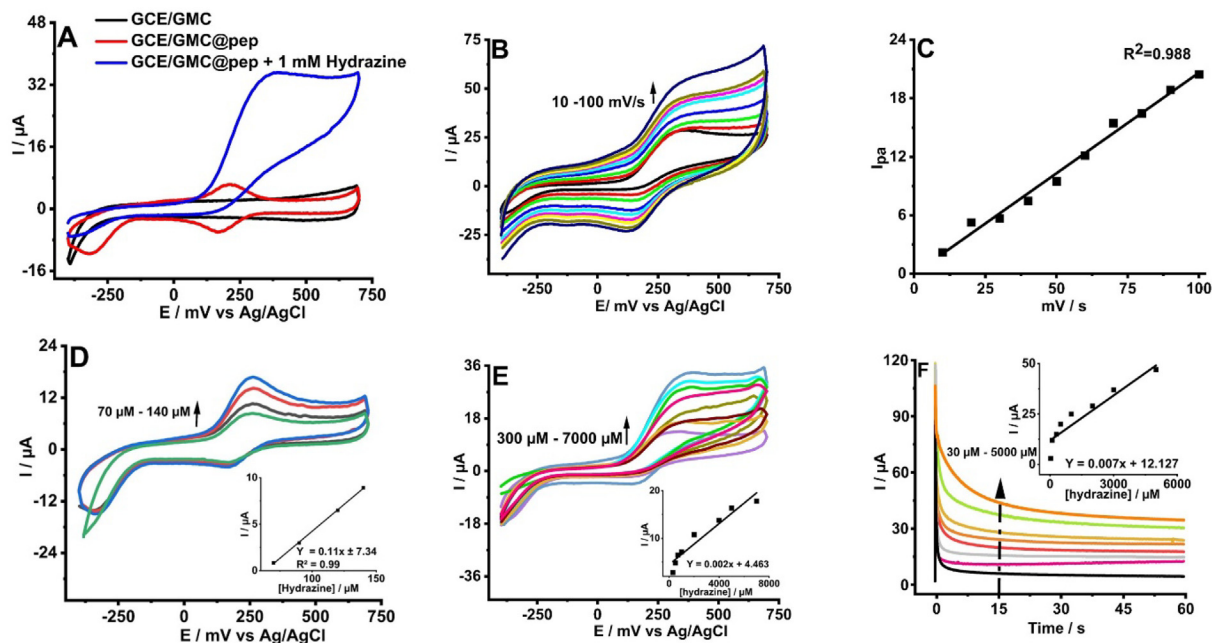
Fig. 5. (A) Comparative CV response of GCE/GMC@ pep in pH 7 PBS and 1 mM Hydrazine at 10 mV/s. (B) Effect of varying scan rate on GCE/GMC@pep in 1 mM Hydrazine and (C) corresponding plot of I_{pa} vs. υ mV/s with base line corrected values. (D)CV of lower Hydrazine concentration ranges (70–140 μM), inset corresponding calibration plot. (E) CV of higher hydrazine concentration ranges (300–7000 μM), inset corresponding calibration plot.(F) Chronoamperometic response of various hydrazine concentrations in linear range (30-5000 μM), inset is the calibration plot.

Table 1
Literature reports regarding hydrazine sensing using phytochemicals.

Modified electrode	Linearity/μM	LOD (μM)	Technique
GCE/MWCNT@EA [19]	500–9000	0.86	Amperometric i-t curve
GCE/MWCNT@Qn [16]	100–3000	0.136	Flow injection analysis (FIA)
MnHCF@graphite–wax composite electrode [28]	3.33×10^{-5} M 8.18×10^{-3} M	6.65	Amperometric i-t curve
GCE/graphene–cobalt hexacyanoferrate [29]	0.25–100	0.069	Amperometric i-t curve
ITO/SSG-$[Fe(CN)_6]^{3-}$/Cohcf [30]	100–700	–	Linear sweep Voltammetry (LSV)
SPCE@PDDA/copper(II) hexacyanoferrate [31]	0.03 to 533.6	0.01	Amperometric i-t curve
GCE/CNT*NiHCF [32]	20–200	0.8	Amperometric i-t curve
GCE/MWCNT@HQ [42]	100–1000	78	Cyclic Voltammetry (CV)
SC/Poly-Tannic acid [43]	0–100	0.1	Cyclic Voltammetry (CV)
GCE/MWCNT@carbazole [44]	25–250	16.3	Amperometric i-t curve
GCE/PEDOT/LS [45]	15–290	9.8	Chronoamperommetry
PGE/Pyrocatechol [46]	0.2–500	0.08	Flow injection amperometry
GCE/GMC@pep(this work)	70–7000	40	Cyclic Voltammetry (CV), chronoamperometry (CA)
	30 to 5000	0.5	

Qn = Quercetin, HQ = Hydroquinone, Ellagic acid, SC = Screen printed carbon electrode, LS = Lignosulfonate, PGE = pencil graphite electrode, MnHCF = Manganese hexacyanoferrate, SPCE = screen printed carbon electrode, PDDA = poly(diallyldimethylammonium chloride), ITO = Indium Titanium Oxide, SSG = silicate sol-gel matrix Cohcf = cobalt hexacyanoferrate, NiHCF = Nickel Hexacyanoferrate.

3.8. Reproducibility and stability

Fig. 6A represents the reproducibility of the fabricated GCE/GMC@pep for the sensing. The fabricated electrode was tested for successive 7 times with 1 mM hydrazine in pH 7 PBS. As can be seen, upto 7 times in a row, the sensing current obtained was same with negligible difference. However, after the 7th attempt, the electrode modifications leached out. Fig. 6B gives the stability of the fabricated electrode. The electrode was prepared and stored in pH 7 PBS, for a period of 60 days. The CV of the fabricated electrode on 1st day vs. CV on the 60th day gave current difference > 7%, however, its sensing ability was intact. Therefore, we found that the electrode could be stored upto 60 days and used upto 7 times effectively.

3.9. Effect of interference

Further to validate the selectivity, effect of various interfering biochemicals, was analyzed. Biochemicals like Uric acid (UA), Ascorbic acid (AA),Cysteine (Cys), Glucose (Glu), Xanthine (Xn), Hypoxanthine (Hxn) were tested in presence of 1 mM of hydrazine. No interference was observed leading to the conclusion that the prepared sensor showed a highly selective sensing suitable for real time application (Fig.6C). Fig. S2 (Supplementary) is the individual CV responses of all the analytes separately, wherein no additional peak (exceptpiperine redox peak) was observed in the optimized potential window further authenticating zero interference.
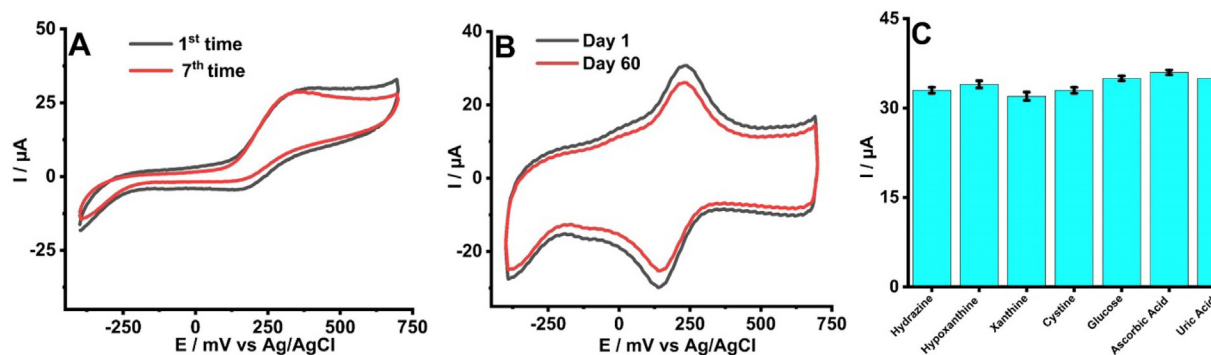
Fig. 6. (A) Comparative CV for reproducibility of Hydrazine sensing. (B) Stability of themodified electrode. (C)Effect of interference from various bio-chemicals, bar- graph for the current response in presence of various bio-chemicals.

Table 2
Real Sample analysis.

Sample	Detected (μM)	Added (μM)	Found in Sample (μM)	Recovery %	Trial 1 (μA)	Trial 2 (μA)	Trial 3 (μA)	Average (μA)	Std Dev.
Serum	1.0 = "<LOD"	70	72	100.27	9.12	9.23	9.06	9.137	0.0862
		140	139	98.02	12.569	12.684	12.587	12.613	0.0619
		210	211	99.62	14.114	14.248	14.354	14.239	0.1203
Tobacco	55.8	70	125.8	100.23	12.16	12.25	12.114	12.175	0.0692
		140	198	101.27	13.134	13.254	13.159	13.182	0.0633
		210	263.5	99.24	14.7816	14.614	14.741	14.712	0.0874
Smoke		70	77.2	101.17	9.801	9.967	9.984	9.917	0.1011
	7.2	140	145	99.11	12.84	12.94	12.892	12.891	0.0500
	"<LOD"	210	218	100.78	14.241	14.25	14.381	14.291	0.0784

3.10. Real sample analysis

The modified electrode (GCE/GMC@pep) was tested in various real samples like smoke of cigarette, tobacco and blood serum by standard addition approach. Since real samples, like blood serum of a chain smoker and live smoke from a burning cigarette, may have lesser concentration of hydrazine (probably <LOD of sensor), therefore, to avoid the error, standard addition approach was adapted. Further, based on the current values from the triplicated experiments, the approximate concentration of hydrazine in real samples was back calculated. The detected concentration values post back calculations is indicated by "<LOD". Table 2 represents the results obtained. It was found that selective detection of hydrazine was done in the real samples with significant recovery values.

4. Conclusion

In this work a selective electrochemical redox property of piperine from crude pepper was examined. A graphitized mesoporous carbon modified glassy carbon electrode (GCE/GMC@pep) was used as a working, and platinum and Ag/AgCl were used as a counter and a reference electrode respectively in neutral pH phosphate buffer solution. The studies were carried out in a 3D printed miniaturized platform (40 mm × 20 mm × 5 mm) with a typical compact three electrode. The prepared GCE/GMC@pep gave a highly stable, reversible redox peak at $E_{1/2}$ + 235 mV vs Ag/AgCl corresponding to electrochemical behavior of piperine. Effect of carbon, scan rate and solution pH were also analyzed. The physico-chemical and microscopic characterization, by UV–Vis, Raman, FT-IR and SEM, revealed the interaction of piperine with GMC. Further, the modified phytochemical based electrode was used for electrochemical detection of hydrazine in neutral pH. Effect of variable concentrations, and interference from bio-chemicals were also explored. To further ensure the validity of the fabricated electrode in real time analysis, various samples, like cigarette smoke, tobacco and blood serum, were analyzed. This work is an archetype of utilizing phytochemicals for electrochemical sensing, which can be leveraged as a green, environment friendly redox mediators, avoiding usage of conventional chemical based ones.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jelechem.2020.114761.

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ORIGINAL ARTICLE

Newborn screening and single nucleotide variation profiling of *TSHR*, *TPO*, *TG* and *DUOX2* candidate genes for congenital hypothyroidism

Yedukondalu Kollati[1] · Radha Rama Devi Akella[2,3] · Shaik Mohammad Naushad[3] · Divya Borkar[3] · Maunika Thalla[3] · Swapna Nagalingam[4] · Lokesh Lingappa[5] · Rajesh K. Patel[6] · G. Bhanuprakash Reddy[4] · Vijaya R. Dirisala[1]

Abstract

High prevalence of congenital hypothyroidism (CH) among Indian newborns prompted us to establish population-specific reference ranges of TSH and to explore the contribution of the common genetic variants in *TSHR*, *TPO*, *TG* and *DUOX2* genes towards CH. A total of 1144 newborns (593 males and 551 females) were screened for CH. SNV profiling (n = 22) spanning three candidate genes, i.e. *TSHR*, *TPO* and *TG* was carried out in confirmed CH cases (n = 45). In screen negative cases (n = 700), ten *TSHR* variants were explored to establish association with CH. No mutation found in *DUOX2*. The 2.5th to 97.5th percentiles of TSH in these newborns were 0.5 to 12.2 mU/L. In newborns with optimal birth weight, the cut-off was 10 mU/L. Lower or higher birth weight resulted in slightly higher TSH. Two *TSHR* variants, i.e. rs7144481 and rs17630128 were associated with agenesis, hypoplasia and goiter. The rs2268477 was associated with agenesis and hypoplasia. The rs1991517, rs2075176 and rs2241119 were associated with agenesis only. The rs7144481, rs17630128, rs1991517 and rs2268477 were associated with 2.17, 4.62, 2.91 and 2.29-fold increased risk for CH, respectively. Among the *TPO* variants, rs867983 and rs2175977 were associated with agenesis and goiter, respectively. Among the *TG* variants, rs2076740 showed association with agenesis and goiter. Two rare variants i.e. *TPO* g.IVS14-19 G>C and *TG* c.1262 C>T were observed in CH cases. No genetic variant identified in the two exons of *DUOX2*. To conclude, the current study established Indian population-specific normative values for TSH and demonstrates specific genotype–phenotype correlations among three candidate genes.

Keywords Congenital hypothyroidism · TSHR · TPO · TG · DUOX2 · Newborn screening

Introduction

Congenital hypothyroidism (CH) is one of the most prevalent metabolic disorders in the newborn with a prevalence of 1:4000 globally [1, 2] and 1:1100 in India [3]. CH is classified into permanent CH and transient CH. The permanent CH can be subdivided into primary CH (PCH) and

Yedukondalu Kollati and Radha Rama Devi Akella contributed equally to this study.

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✉ G. Bhanuprakash Reddy
reddyg.bp@icmr.gov.in

✉ Vijaya R. Dirisala
drdirisala@gmail.com

1 Department of Biotechnology, Vignan's Foundation for Science, Technology & Research (Deemed to be University), Vadlamudi, Guntur, Andhra Pradesh 522213, India

2 Department of Genetics, Rainbow Children's Hospital, Banjara Hills, Hyderabad, Telangana 500009, India

3 Department of Biochemical Genetics and Pharmacogenomics, Sandor Speciality Diagnostics Pvt. Ltd, Banjara Hills, Hyderabad, Telangana 500034, India

4 Biochemistry Division, National Institute of Nutrition, Hyderabad, Telangana 500007, India

5 Department of Pediatric Neurology, Rainbow Children's Hospital, Banjara Hills, Hyderabad, Telangana 500009, India

6 Department of Genetics, Genetic Group of Gujarat Diagnostic Centre, Mehsana, Gujarat 384002, India

secondary CH (SCH) [4, 5]. PCH is further classified into thyroid dysgenesis and thyroid dyshormonogenesis [4, 6]. The majority of these cases (80–85%) have defects in thyroid gland development known as thyroid dysgenesis [7]. Thyroid dysgenesis is ranging from the lack of thyroid gland (agenesis; 35–40%), to located in sublingual position (ectopic; 55–60%) or small thyroid gland or remnants thyroid gland in the normal position (hypoplasia; 5%) [8]. Remaining 15–20% cases are caused by defects in the thyroid hormone biosynthesis known as dyshormonogenesis [8]. Defects in various genes such as a thyroid stimulating hormone receptor (*TSHR*), paired box 8 (*PAX8*), thyroid transcription factor 1 (*TTF-1*) and thyroid transcription factor 2 (*TTF-2*) were reported to have association with thyroid dysgenesis [9] while defects in thyroid peroxidase (*TPO*), thyroglobulin (*TG*), solute carrier family 5 member 5 transporter (*SLC5A5,* encoding *NIS*), solute carrier family 26 member 4 transporter (*SLC26A4* encoding *PDS)*, Dual oxidase (*DUOX1* and *DUOX2*), Dual oxidase maturation factors (*DUOXA1* and *DUOXA2*), and iodotyrosine dehalogenase 1 (*DEHAL1*) were reported to have association with dyshormonogenesis [7].

Thyroid stimulating hormone (TSH) binds to *TSHR* and prompts the release of two signal transduction pathways: G-alpha (s) (G_s) and G_q proteins followed by activation of adenylate cyclase-cyclic adenosine monophosphate (cAMP) and phospholipase C (PLC)-calcium cascade, respectively [10, 11]. These pathways are involved *TG* iodination and cell proliferation. The pathway of Gs is responsible for thyrocytes iodine uptake regulation [11]. The G_q-phospholipase C-calcium cascade activates the *DUOX2* protein function [10]. TSH and its receptors play an important role in the maturation and differentiation of thyroid development in the late stage [12]. The *TPO* is involved in catalyzing iodide oxidation, iodination of tyrosine residues and coupling of mono- and di-iodotyrosyl in *TG* molecule to synthesize triiodothyronine (T_3) and thyroxine (T_4) [13]. This activity needs the presence of hydrogen peroxide (H_2O_2) which is generated by *DUOX2* [7].

Materials and methods

Recruitment of subjects

This project is an offshoot of the newborn screening (NBS) program that is being carried out by the lead investigator from 2001 to till date where in 49,432 newborns were screened [3, 14], out of which 45 CH cases were diagnosed (incidence: 1:1098). From this cohort, 1099 screen negative cases were randomly selected along with the 45 confirmed cases of CH (593 males and 551 females) (Fig. 1).

In the newborn screening, TSH levels were measured using a commercial neonatal TSH kit (Perkin Elmer GSP neonatal kit). The diagnosis of CH was confirmed if the infant has a low T_4 (< 10 μg/dL) and TSH > 40 mU/L. The biochemically diagnosed cases were subjected to other optional tests, including thyroid ultrasonography, thyroid uptake and scan. Presence of an ectopic gland was suggestive of permanent congenital hypothyroidism. Absence of thyroid gland uptake was suggestive of thyroid aplasia or hypoplasia. Normal scan or a goiter was suggestive of a genetic defect in T_4 synthesis. All the diagnosed cases of CH were further evaluated genetically using the candidate genes, i.e. *TSHR*, *TPO*, *TG* and *DUOX2*. A total of 700 screen negative cases was evaluated genetically for *TSHR* variants. The study protocol was approved by the institutional Ethical committee of Rainbow Children's Hospital (RCHBH/066/02-2018), Hyderabad. Informed consent was obtained from the parents or guardians of the enrolled neonates. The exclusion criteria were associated congenital malformations, Down's syndrome and Transient neonatal hyperthyrotropinemia.

DNA isolation and amplification

The DNA was isolated from buffy coat peripheral white blood cells by QIAamp blood kit (QIAGEN, Hilden, Germany). Quality and quantity of isolated DNA were analyzed by using a NanoDrop™ 1000 (Thermo Fisher Scientific, Waltham, USA).

Gene Tool 1.0 [15] computer program was used for primer designing. As shown in the Supplementary Table 1, the specific primers were used in amplifying *TSHR*, *TPO*, *TG* and *DUOX2* gene exons and their exon–intron boundaries. Primers for the amplification of exon 8 of the *TPO* gene were synthesized based on the previous research report [16].

As shown in the Supplementary Table 1, the exonic regions of the *TSHR*, *TPO*, *TG* and *DUOX2* genes, including the splicing regions, were amplified by PCR. The PCR reaction mixture contained 50–100 ng of genomic DNA, 10 pmol of each forward and reverse primers, 1.5 mM $MgCl_2$, 10 mM of each dNTP, 5 μL 10 × PCR buffer, 5U Taq DNA polymerase (Thermo Fisher Scientific, Waltham, USA) at a final volume of 50 μL as in other reports [17]. The conditions for PCR amplification were an initial denaturation of 5 min at 95 °C, followed by 30 cycles of amplification consisting of denaturation at 95 °C for 30 s, annealing at 51–65 °C (based on suitable annealing temperature for each of the primer) for 30 s and extension at 72 °C for 30–60 s and with a final extension at 72 °C for 5 min. The amplified PCR products were analyzed in 2% agarose gel and followed by purified with ExoSAP-IT™ PCR Product purification kit (Applied Biosystems, Foster City, USA) as described in our previous report [18].

Fig. 1 Process flow from newborn screening to molecular testing. This illustrates the process of various stages of testing and the respective sample size at each stage
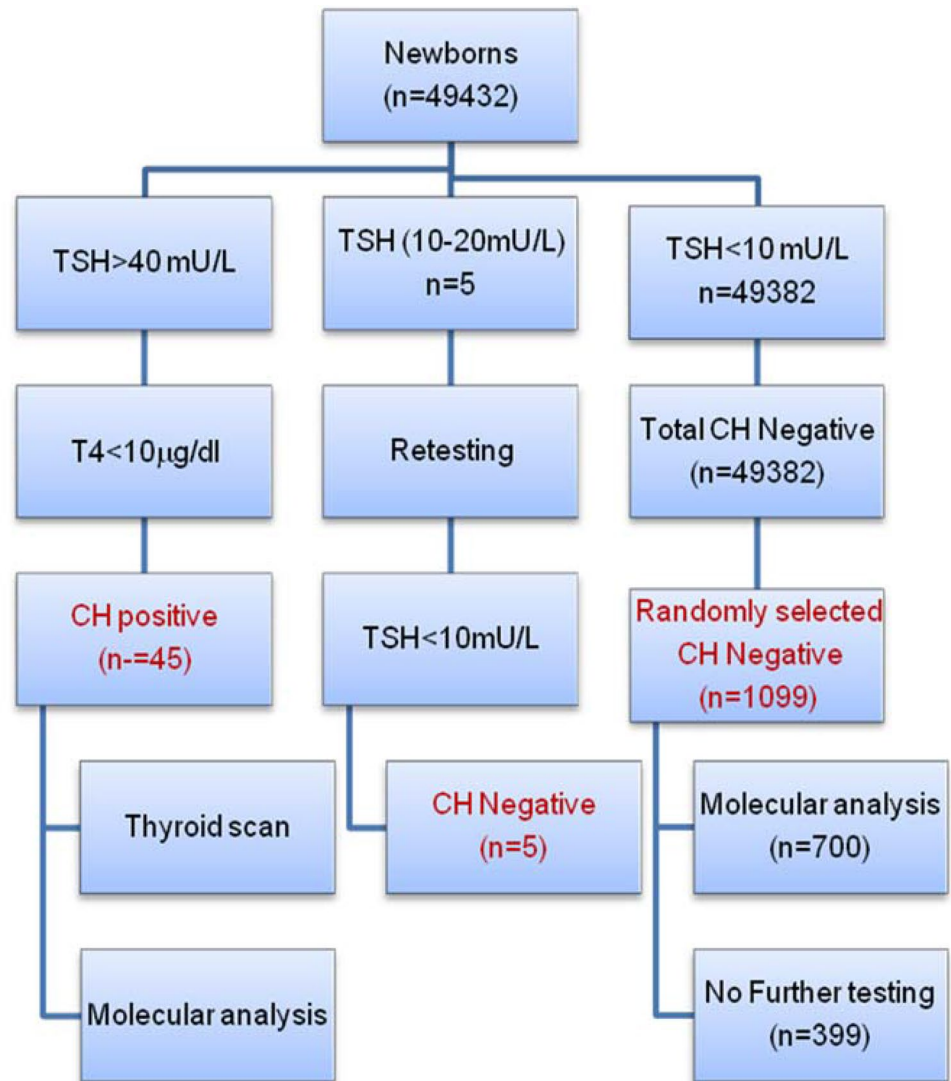
DNA sequencing and data analysis

The DNA sequencing was performed with ABI 3730xLDNA Analyser (Applied Biosystems, Foster City, USA). The sequence variants nomenclature refers to the NCBI human *TSHR*, *TPO*, *TG* and *DUOX2* reference nucleotide sequence (NCBI accession number NM_000369, NM_000547, NM_003235 and NM_014080, respectively). The 'A' of the ATG of the start codon denoted as nucleotide + 1 and the start codon methionine is denoted as codon 1.

Sequence chromatogram data results were analyzed using Chromas 2.6.2 and BLAST (https://www.ncbi.nlm.nih.gov/blast/) software programs. Nucleotide alterations were compared with the 1000 Genomes (https://www.1000genomes.org/) and dbSNP (https://www.ncbi.nlm.nih.gov/snp/) databases as described in our previous report [18].

Sequence variants frequencies and prediction of functional effects

The minor allele frequencies (MAF) of genetic variants were computed from a genome aggregation database (gnomAD; http:// https://gnomad.broadinstitute.org/) and 1000 Genomes Projects (https://www.internationalgenome.org) based on their rs IDs.

The functional effects of the genetic variants were explored using three different in silico tools: SIFT (https://sift-dna.org), PolyPhen-2 (https://genetics.bwh.harvard.edu/pph2/) and PROVEAN (https://provean.jcvi.org.) were used.

Statistical analysis

Fisher exact test was used to establish genetic associations where in 2×2 contingency table was computed based on the presence or absence of genetic variation in the presence

or absence of a particular phenotype. In view of multiple genetic variants, the data representation was restricted to minor allele frequencies and p values. The odds ratios and 95% confidence intervals were not depicted. Student t-test and analysis of variance were used for exploring the differences in mean $\pm$ SD among two or more groups, respectively. Heat-map analysis was carried out to demonstrate the genotype–phenotype associations with respect to thyroid scan results.

Results

Establishment of reference intervals for TSH through newborn screening data

In screen negative newborns, the 2.5th to 97.5th percentiles of TSH in this cohort were 0.5–12.2 mU/L. The data was segregated based on gender (male and female) and birth weight (< 2.0 kg, 2.0–3.5 kg and > 3.5 kg) to explore physiological variations in TSH. It was observed that neonates with lower (male: 0.30–12.0 mU/L; female: 0.92–10.36 mU/L) or higher (male: 0.55–12.34 mU/L; female: 0.5–12.24 mU/L) birth weight exhibit a slight increase in TSH levels compared to those with optimal weight (male: 0.60–10.23 mU/L; female: 0.40–9.90 mU/L) (Fig. 2). However, this increase is not statistically significant.
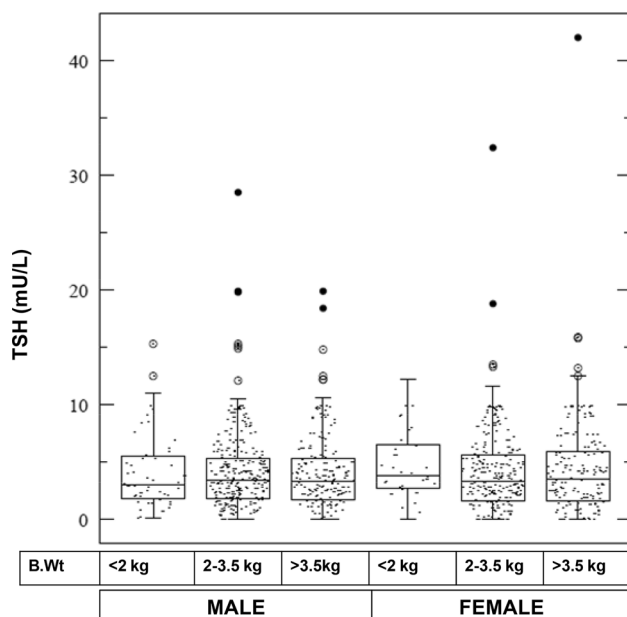
Thyroid scan in confirmed CH cases

Out of 45 confirmed CH cases, 35 opted for thyroid scan and the results are as follows: agenesis ($n = 7$), hypoplasia ($n = 5$), goiter ($n = 7$), no significant abnormalities ($n = 16$). Cases with goiter showed maximum increase in TSH (206.71 ± 119.28 mU/L) and lowest T_4 (1.18 ± 0.73 µg/dL). In cases with hypoplasia, TSH levels were 156.06 ± 94.18 mU/L and T_4 levels were 1.45 ± 0.92 µg/dL. In thyroid agenesis, TSH levels were the lowest (119.4 ± 99.49 mU/L) and T_4 levels were maximum (2.89 ± 2.39 µg/dL) (Fig. 3; Supplementary Table 2).

Genetic analysis

A total of 22 genetic variants was tested in three candidate genes: *TSHR* ($n = 10$), *TPO* ($n = 8$), *TG* ($n = 4$). The MAFs of cases with no significant thyroid gland anomalies were considered as base line to compare the MAFs of cases with agenesis, hypoplasia and goiter (Fig. 4).

As shown in the Supplementary Table 3, novel variants alleles were found to be very rare on the basis of control data of the datasets are shown. MAFs of Asian, global and total populations were computed based on the datasets of gnomAD and 1000 Genome projects. Out of 22 sequence variants, two missense variants, i.e. rs2175977 and rs2069548 were predicted to be deleterious. Remaining variants were benign.
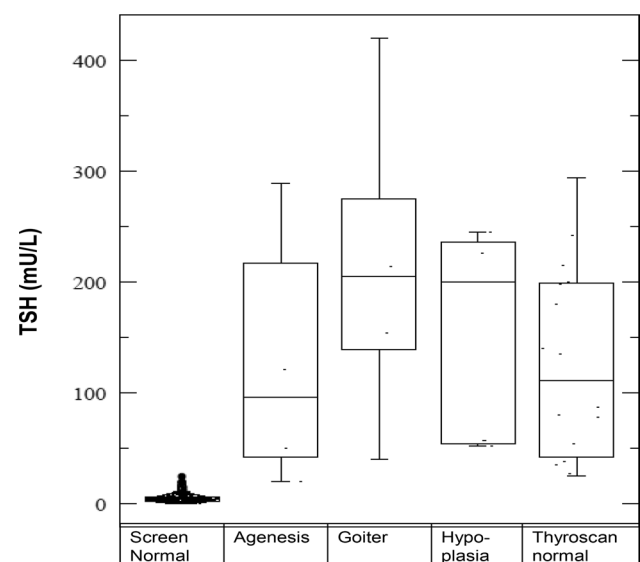

Fig. 2 Distribution of TSH levels based on gender and birth weight. In both genders, newborns with optimal birth weight showed a TSH cut-off of 10 mU/L while newborns with lower and higher birth weight had a cut-off higher by 2 mU/L

Fig. 3 Distribution of TSH levels among screen negative and confirmed CH cases. The screen normal newborns have TSH values < 20 mU/L. Goiter and hypoplasia were associated with higher TSH levels than agenesis and normal thyroscan

Fig. 4 Heat map showing the distribution of 22 genetic variants across three candidate genes in different CH phenotypes. The wild, heterozygous and homozygous mutant genotypes were represented as green, yellow and red colors, respectively. The TSH and T_4 values were graded according to the levels from green (lowest) to red (highest)

TSHR variants

The rs7144481 variant was absent in cases with normal thyroid scan while its MAFs were higher in cases with abnormal thyroid scan, i.e. agenesis (78.57%), goiter (57.14%) and hypoplasia (100.0%) suggesting a strong association of this variant with CH (p < 0.0001). The rs17630128 variant was absent in cases with normal thyroid scan while exhibiting higher MAFs in agenesis (MAF: 35.71%, p = 0.003), hypoplasia (MAF: 30.0%, p = 0.02) and goiter (MAF: 28.57%, p = 0.01). The rs2268477 was absent in cases with normal thyroid scan, but had higher MAFs in hypoplasia (MAF: 50.0%, p = 0.001) and agenesis (MAF: 28.57%, p = 0.01). However, this variant showed no association with goiter (p = 0.18). The rs1991517 variant was associated with agenesis (MAF: 42.86% vs. 9.38%, p = 0.03) while showing null association with goiter and hypoplasia. The rs2075176 and rs2241119 variants showed borderline association with agenesis (p = 0.04) (Table 1).

We have performed *TSHR* sequencing in 700 screening negative newborns to establish baseline frequencies of the investigated variants and to explore the association with CH

in Toto. The rs7144481 variant (Cases vs. controls MAFs: 34.44% vs. 19.50%) showed 2.17-fold (95% CI 1.38–3.42) increased risk for CH. The rs17630128 (MAFs: 12.22% vs. 2.93%) was associated with 4.62-fold increased risk for CH (95% CI 2.29–9.32). The rs1991517 (MAFs: 21.11% vs. 8.43%) variant was associated with 2.91-fold increased risk for CH (95% CI 1.69–4.99). The rs2268477 variant (MAFs 13.33% vs. 6.29%) was associated with 2.29-fold (95% CI 1.20–4.37) increased risk for CH (Table 2).

TPO variants

Among the eight *TPO* variants analyzed, only two showed statistically significant association with CH. The rs2175977 showed association with goiter (MAF: 57.14% vs. 9.38%, p = 0.002). The rs867983 variant showed association with agenesis (MAF: 71.43% vs. 21.88%, p = 0.004) (Table 1).

TG variants

Among the four variants of *TG* analyzed, only rs2076740 showed association with agenesis (MAF: 64.29% vs. 3.13%,

Table 1 Association of genetic variants based on CH phenotypes

Genetic variants	Agenesis MAF	Goiter MAF	Hypoplasia MAF	Normal MAF	Agenesis M	Agenesis W	Goiter M	Goiter W	Hypoplasia M	Hypoplasia W	Normal M	Normal W	Agenesis p	Goiter p	Hypoplasia p
TSHR variants															
rs2234919 (c.310 C>A)	14.29	7.14	20.00	0.00	2	12	1	13	2	8	0	32	0.18	0.61	0.11
rs2239610 (g.IVS 01+63 G>C)	14.29	0.00	0.00	0.00	2	12	0	14	0	10	0	32	0.18	1	1
rs2075176 (g.IVS 06–69 C>T)	35.71	7.14	10.00	6.25	5	9	1	13	1	9	2	30	0.04*	1	1
rs2241119 (g.IVS 06+13 A>G)	35.71	7.14	10.00	6.25	5	9	1	13	1	9	2	30	0.04*	1	1
rs539239352 (g.IVS 09+58 T>G)	7.14	0.00	0.00	0.00	1	13	0	14	0	10	0	32	1	1	1
rs1991517 (c.2337 C>G)	42.86	28.57	0.00	9.38	6	8	4	10	0	10	3	29	0.03*	0.23	1
rs2268477 (c.172 C>A)	28.57	14.29	50.00	0.00	4	10	2	12	5	5	0	32	0.01*	0.18	0.001*
rs373305430 (c.182 G>T)	0.00	0.00	10.00	0.00	0	14	0	14	1	9	0	32	1	1	0.48
rs7144481 (c.245 C>T)	78.57	57.14	100.00	0.00	11	3	8	6	10	0	0	32	0.0001*	0.0001*	0.0001*
rs17630128 (c.431 T>C)	35.71	28.57	30.00	0.00	5	9	4	10	3	7	0	32	0.003*	0.01*	0.02*
TPO variants															
rs2280132 (c.1208 G>T)	7.14	0.00	10.00	9.38	1	13	0	14	1	9	3	29	0.44	0.65	1
rs2175977 (c.1284 G>C)	35.71	57.14	30.00	9.38	5	9	8	6	3	7	3	29	0.09	0.002*	0.27
rs140322336 (c.1948 C>G)	7.14	0.00	0.00	0.00	1	13	0	14	0	10	0	32	1	1	1
rs1126797 (c.2089 C>T)	42.86	14.29	20.00	15.63	6	8	2	12	2	8	5	27	0.11	1	1
rs10189329 (g.IVS11+20 G>A)	0.00	0.00	0.00	6.25	0	14	0	14	0	10	2	30	0.96	0.96	1
rs867983 (g.IVS13+128 C>T)	71.43	42.86	50.00	21.88	10	4	6	8	5	5	7	25	0.004*	0.27	0.19
rs746074402 (g.IVS14-37 G>A)	0.00	14.29	0.00	0.00	0	14	2	12	0	10	0	32	1	0.18	1
Novel (g.IVS14-19 G>C)	0.00	7.14	0.00	0.00	0	14	1	13	0	10	0	32	1	1	1
TG variants															
Novel (c.1262 C>T)	7.14	0.00	0.00	0.00	1	13	0	14	0	10	0	32	1	1	1
rs2069548 (c.1999 G>A)	0.00	14.29	0.00	6.25	0	14	2	12	0	10	2	30	0.96	0.71	1
rs2069550 (c.2375T>C)	28.57	14.29	0.00	6.25	4	10	2	12	0	10	2	30	0.1	0.71	1
rs2076740 (c.6036 C>T)	64.29	35.71	30.00	3.13	9	5	5	9	3	7	1	31	0.0001*	0.014*	0.07

MAF minor allele frequencies, *M* mutant allele, *W* wild allele

*Statistically significant

Table 2 Association of *TSHR* SNPs with risk for CH in toto

rs number	Nucleotide change	CH Cases				Controls				OR	95% CI	p value
		WW	WM	MM	MAF	WW	WM	MM	MAF			
rs2234919	c.310 C>A	40	5	0	5.56	483	190	27	17.43	0.28	0.11–0.69	0.002*
rs2239610	g.IVS 01 + 63 G>C	44	0	1	2.22	482	174	44	18.71	0.1	0.02–0.40	<0.0001*
rs2075176	g.IVS 06–69 C>T	38	5	2	10.00	477	196	27	17.86	0.51	0.25–1.03	0.07
rs2241119	g.IVS 06 + 13 A > G	38	5	2	10.00	486	188	26	17.14	0.54	0.27–1.08	0.09
rs539239352	g.IVS 09 + 58 T > G	44	1	0	1.11	691	9	0	0.64	1.74	0.22–13.86	0.93
rs1991517	c.2337 C>G	31	9	5	21.11	595	92	13	8.43	**2.91**	**1.69–4.99**	<0.0001*
rs2268477	c.172 C>A	35	8	2	13.33	644	24	32	6.29	**2.29**	**1.20–4.37**	0.03*
rs373305430	c. 182 G > T	44	1	0	1.11	692	2	6	1.00	1.11	0.15–8.56	1
rs7144481	c.245 C>T	29	1	15	34.44	557	13	130	19.50	**2.17**	**1.38–3.42**	0.002*
rs17630128	c.431 T>C	37	5	3	12.22	675	9	16	2.93	**4.62**	**2.29–9.32**	<0.0001*

The bold values signify positive association with the congenital hypothyroidism

WW wild, *WM* heterozygous, *MM* homozygous, *MAF* minor allele frequency, *OR* odds ratios, *CI* confidence intervals

*Statistically significant

p=0.0001) and goiter (MAF: 35.71% vs. 3.13%, p=0.01) (Table 1).

DUOX2 **variants**

Exon 12 and 16 of the *DUOX2* gene along with splicing regions were analyzed by sequencing. The sequencing results revealed that all cases had only the wild type sequence in the *DUOX2* gene.

Discussion

The current study established Indian population-specific reference ranges for TSH in male and female neonates. Further, a slight elevation in TSH was noticed in neonates with lower or higher birth weight compared to those with optimal birth weight. Thyroid scan of 45 confirmed CH cases revealed agenesis in seven, hypoplasia in five and goiter in seven and no specific abnormality of the thyroid gland in 16 while 10 cases didn't opt for thyroid scan. All the cases were evaluated for 22 genetic variants in three candidate genes, i.e. *TSHR*, *TPO* and *TG*. Heat map analysis and Fisher exact tests were conducted to establish genotype–phenotype associations. Among the *TSHR* variants, rs7144481 and rs17630128 showed association with agenesis, hypoplasia and goiter. The rs2268477 variant showed association with hypoplasia and agenesis, while rs1991517 showed association only with agenesis. The four *TSHR* variants, i.e. rs714481, rs17630128, rs2268477, and rs1991517 showed association with CH in toto. The rs2075176 and rs2241119 variants showed borderline association with agenesis. Among the eight *TPO* variants, only rs2175977 and rs867983 showed association with goiter and agenesis,

respectively. Among the four *TG* variants, only rs2076740 showed association with agenesis and goiter. No genetic variants found in exon 12 and 16 of *DUOX2*. In silico studies revealed the deleterious nature of two variants.

The results of the current study corroborate with Grob et al., in demonstrating higher TSH levels in preterm newborns small for gestation age [19]. A targeted next generation sequencing based study of 43 Chinese CH cases demonstrated *TSHR*, *TPO*, *TG* and *DUOX2* as the most likely candidate genes affected in CH out of 29 causative genes consistent with our objective of the study [20]. The rs7144481 variant was one of the three variants identified in a genome wide association study as a genetic contributor to elite endurance performance [21]. Null association of rs7144481, rs17630128 and rs2268477 variants with papillary thyroid cancer was reported earlier [22]. The current study demonstrated the association of rs1991517 with agenesis, which in turn has lower TSH levels compared to other CH thyroid phenotypes. This phenomenon was reported earlier, even in healthy subjects with heterozygous exhibiting lower TSH values than those with wild genotype [23].

Among the *TPO* variants, rs2175977 was associated with goiter. This variant was reported earlier in five cases of CH cases in Baghdad [24]. There are no studies to corroborate our findings with rs867943. The *TG* rs2076740 polymorphism was earlier reported to be a significant genetic risk factor for autoimmune thyroid disease based on extensive meta-analysis of 3013 cases and 1812 controls [25].

In addition to the known variants, we have identified two rare genetic variants, i.e. *TPO* g.IVS14-19 G>C and *TG* c.1262 C>T.

The strengths of the current study are: (i) establishment of reference ranges for TSH in neonates by considering gender and birth weight; (ii) exploration of 22 SNVs in three

candidate genes to establish genotype–phenotype association in CH cases; (iii) the association of *TSHR* variants with CH in toto was established by analyzing 700 screen negative newborns. The limitations are: (i) all the exons and introns were not analyzed in these candidate genes and hence likely to miss rare variants; (ii) the distribution of other variants in screening negative newborns was not evaluated.

To conclude, the current study established reference ranges for TSH in neonates by segregating according to gender and birth weight. Mild elevation of TSH was observed in neonates with low or high birth weight compared to those with optimal weight. The confirmed CH cases exhibited specific genotype–phenotype associations with *TSHR*, *TPO* and *TG* SNVs. *DUOX2* genetic variants showed a null association.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval The study was performed in accordance with the ethical standards of the Institutional Ethics committee of Rainbow Hospital Institutional Ethics Committee (RCHBH/066/02-2018), Hyderabad, India and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed written consents were obtained from parents and participants of all subjects included in the study.

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ORIGINAL ARTICLE

Miniaturized electrochemical platform with ink-jetted electrodes for multiplexed and interference mitigated biochemical sensing

Jaligam Murali Mohan[1] · Khairunnisa Amreen[2] · Arshad Javed[1] · Satish Kumar Dubey[1] · Sanket Goel[2]

Abstract

Development of miniaturized devices has yielded many advantages, such as automation, portability and robustness. Out of several detection schemes, electrochemical sensing is the preferred choice due to its selectivity, accuracy and repeatability. In the present work, a miniaturized three-electrode electrochemical device has been fabricated on a glass substrate using inkjet printing of carbon conductive inks for sensing of xanthine (X), hypoxanthine (HX) and uric acid (UA). Hereby, the electroactive graphitized mesoporous carbon modified on conductive carbon paste acted as working electrode. The electrochemical behaviors of these purines were tested using cyclic voltammetry and squarewave voltammetry (SWV). The morphology and structural properties were characterized using scanning electron microscopy. Under the optimized conditions, the linear ranges for X, HX and UA were 2–12, 10–20 and 1–7 µM, respectively. The limit of detections for all these purines are 3.33, 9.99 and 0.01 µM for X, HX and UA, respectively. The developed platform was also utilized for interference with other co-existing bio-chemicals manifesting negligible interference. Further, the platform was successfully tested with human serum samples for X, HX and UA detection. Conclusively, the modified electrodes showed excellent reproducibility and specificity applicable to real samples.

Keywords Miniaturized devices · Ink-jet printing · Multiplexed electrochemical sensing · Purines

Introduction

Electrochemical sensing can definitely be benefited with the usage of 3D printing technologies due to ease of fabrication, cost-effectiveness and reproducibility. For a customized, intricate measurement systems with great versatility, 3D printing, ink-jetting which combines both ink chemistry and printers has led to a bio-electrochemical sensor fabrication approach which is rapid, simple, low cost and efficient for mass production of electrochemical devices (Komuro et al. 2013; Jeerapan and Poorahong 2020; Wang et al. 2016). Some previous works have been reported using inkjet printing on various substrates (Rosati et al. 2019). Gold nanoparticles ink electrodes printed by inkjet printing were used to sense potassium ferricyanide (Deng et al. 2014). A paper based platform for the detection of Fe^{2+} and dopamine using ink jetted carbon nano tube (CNT) electrode has been presented (Da Costa et al. 2015). An inkjet-printed silver electrode on plastic substrate for hydrogen peroxide sensing to overcome some limitations in the screen printing techniques has been reported (Shi et al. 2018). In another work, they used ink-printed three-electrode system for lactate detection with enzyme deposited over silver conductive ink electrodes using 3D ink extrusion printer on PET substrate (Dong et al. 2018).

Owing to the ease of ink jetting, we developed an ink-jetted miniaturized platform which can be used for the detection of purines by modifying the working and reference electrodes. As a proof of concept, some of the purines like xanthine (X), hypoxanthine (HX) and uric acid (UA) used in our day to day life in clinical and food applications were tested.

X, HX and UA are the purine derivatives which are produced during the degradation of ATP. Any defect in

✉ Sanket Goel
  sgoel@hyderabad.bits-pilani.ac.in

1   Department of Mechanical Engineering, Birla Institute of Technology and Science (BITS) Pilani, Hyderabad Campus, Hyderabad 500078, India

2   MEMS, Microfluidics and Nanoelectronics Laboratory, Department of Electrical and Electronics Engineering, Birla Institute of Technology and Science (BITS) Pilani, Hyderabad Campus, Hyderabad 500078, India

the degradation cycle of ATP may lead to the deviation in the levels of X, HX and UA (Mujahid et al. 2015; Murphy et al. 2011). Due to their importance in bodily activities, the interest to sense these purines had increased greatly. The accumulation of aforementioned purines in the physiological system is related to onset of disease. For instance, UA accumulation leads to tissue degradation which is a key factor in many genetic and abnormal conditions like Parkinson diseases, renal diseases, gout, myocardial ischemia, etc., (Battelli et al. 2016; Chen et al. 2016). In food quality assurance, the presence and quantification of purines in food samples like chicken, fish, meat, coffee and tea are of great importance. These could be useful to determine the freshness, and to know their time of death (Lawal and Adeloju 2012; Devi et al. 2012).Therefore, monitoring the levels of purine derivatives is of significance for both consumers and industry for the maintenance of healthy and high-quality products. The literature reports several conventional three-electrode system based on bulk analysis with different modifications of the working electrode (WE) for sensing of these purines; for example, glassy carbon electrode (GCE) modified with bromocresol purple (Wang and Tong 2010), poly/graphene (Zhang et al. 2012), poly(L-methionine) (Ojani et al. 2013), poly(amino hydroxy naphthalene sulphonic acid) (Rachel et al. 2020), graphitized mesoporous carbon (GMC) (Thangaraj and Kumar 2012) through electro-polymerization process. A new method for simultaneous detection of these purines in the human blood plasma using capillary electrophoresis ultraviolet (CE/UV) was introduced (Caussé et al. 2007). The synthesis and the usage of the CO-doped CeO_2 nanoparticles modified on GCE electrode are also used to detect X, HX and UA (Ibrahim and Temerk 2016; Lavanya et al. 2016).

Disposable pre-anodized pencil graphite electrode was used via copper complex as redox mediator for reaction with X, HX and UA (Vishnu et al. 2017; Odewunmi et al. 2018). They validated their work by real-time detection in fresh and dead fish samples.

An enzyme and pre-treated chemical-based screen-printed electrodes were also used for the surface modification of the WE. Similarly, Hasoň et al. (2020) reported pencil graphite electrode modified with graphite oxide which can sense tryptophan, UA, X and HX. Another similar work wherein graphene oxide modified with both chitosan and chromium oxide nano-elements for sensing all three analytes (Ghanbari and Nejabati 2020) has been reported. El Harrad and Amine (2016) developed a biosensor with greater storage ability whereby screen-printed electrodes (SPE) were utilized and the WE was modified with xanthine oxidase enzyme. Wang (2011) reported an electrochemical method where they used pyrocatechol carboxyl multiwalled nanocarbon tubes. Another enzyme-based work used the xanthine

oxidase on the gold nanoparticles on graphite pencil electrode (Devi et al. 2013).

The literature revealed that the reported works require larger electrodes, bulky volume and enzymatic sensing approach for these purines. To minimize the sample volume and to develop an enzyme-less point-of-care device platform, a miniaturized ink-jetted platform was designed and fabricated for the detection of X, HX and UA. The developed platform was found to be highly selective and tested several times for stability and reproducibility with relative standard deviation of 2%. The sensor exhibited selective, electro-catalytic activity towards purines oxidation at 0.52, 0.86 and 0.27 V vs Ag/AgCl with an appreciable limit of detection (LOD) as 3.33, 9.99 and 0.01 μM in comparison to the previous reports. Table 1 represents some of the existing fabricated electrochemical sensors for detection of purines these in the bulk system. Further, the platform was subjected to real sample analysis using human serum samples and quite impressive recovery values were achieved. With more fine-tuning and automation, this platform can potentially be extended for point-of-care and field analysis of X, HX and UA.

Experimental

Chemicals and apparatus

Glass slides of dimensions 76 mm (L)×26 mm (W)×1 mm (H) were purchased from Borosil. X, HX, UA and carbon mesoporous (<500 nm) were purchased from Sigma Aldrich. Sodium phosphate monobasic anhydrous (NaH_2PO_4), Sodium phosphate dibasic dihydrate ($Na_2HPO_4·2H_2O$), Potassium Ferricyanide $K_3[Fe (CN)_6]$ were procured from AVRA chemicals. The DI water, which was used throughout the experiment, was from Milli-Q (18.2) MΩ cm. The carbon conductive ink was procured from Engineered materials system, Inc (EMS) and the Ag/AgCl ink was purchased from ALS Co. Ltd., Tokyo Japan. The scanning electron microscope (SEM) was carried out using Apreo scanning electron microscope from Thermo Fisher Scientific. FTIR analysis was carried out using JASCO (FT/IR-4200) spectrometer by KBr process. The Voltera V-Oneink-jet printer was procured from Voltera, Canada, while the four-probe conductivity meter was procured from Ossila, UK. The contact profilometer was purchased from Mitutoyo (SJ-210).

Process

Fabrication of the platform

As shown in Fig. 1, a three-electrode system was designed with geometric parameters using Advanced Design System

Table 1 Previously reported chemically modified electrodes for sensing of purines in bulk system

S. no	Technique	Electrode	Analyte			Range (µM)	LOD (µM)			References
							X	HX	UA	
1	CV	Nontronite screen-printed electrode (NSPE)	X	HX	UA	4–30 2–40 2–40	0.07	0.34	0.42	Zen et al. (2002)
4	CV, DPV	GCE/β-cyclodextrin	X	HX	UA	5–105 10–170 10–225	1.25	5	5	Gong et al. (2012)
5	CV, DPV	GCE/Poly(xylithol)	X	HX	UA	1.3–75.3 5–55 4–59	0.75	4.5	3.75	Dou et al. (2014)
6	CV, DPV	GCE/Trp-GR	–	–	UA	– – 10–1000	–	–	1.24	Lian et al. (2014)
7	CV, SWV	GCE/EPPGE	X	HX	UA	0.1–50 0.1–50 0.1–25	0.06	0.08	0.03	Pierini et al. (2018)
8	CV, LSV	GCE/SWCNH	–	–	UA	0.06–10	–	–	20 nM	Zhu et al. (2009)
9	CV, DPV	GCE/CTAB-GO/MWNT	–	–	UA	30–60	–	–	1	Yang et al. (2014)
10	CV, SWV	GCE/GMC	X	HX	UA	2–12 10–20 1–7	3.33	9.99	0.01	Present work

Fig. 1 **a** Glass slide, **b** glass is placed on bed and dispenser is adjusted for uniform ink flow, **c** printed platform and **d** platform with geometrical dimensions
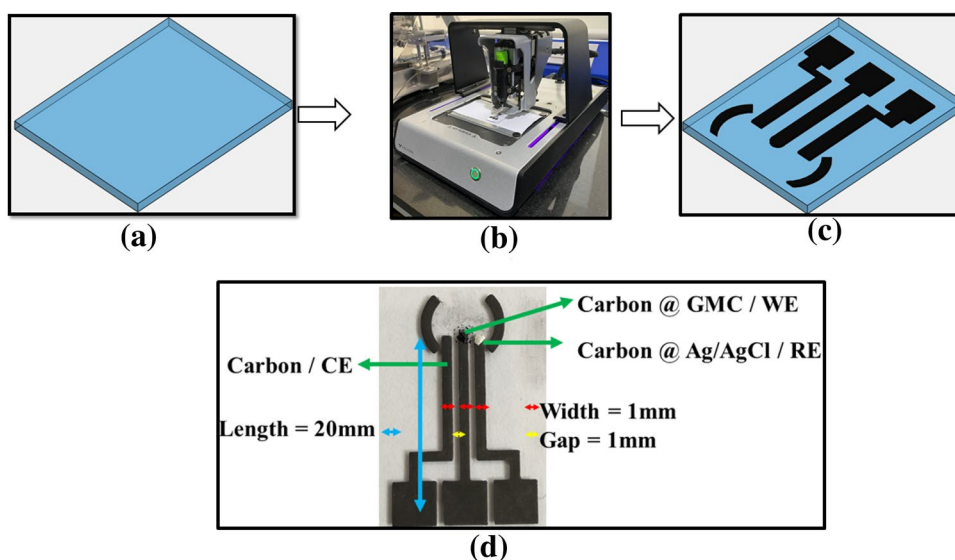
software which is mostly used for PCB designing. The designed file was saved into.gbr conversion and then file was loaded to the ink-jet printer and the carbon ink was fed into the cartridge with the optimized parameters, like 150 µm nozzle height, 0.15 mm line spacing, 50 extrusion amount, and calibrating the height between the substrate and probe (Z axis) = 0.10 mm. The design was printed on the glass slide of length = 76 mm, width = 26 mm and the thickness = 1 mm. After printing the electrodes on the glass slide, the platform was baked in an oven for 30 min at 100 °C temperature.

Subsequently, to have the central electrode as WE, it was modified with 5 µL GMC by drop casting technique. Further, one of the other electrodes was coated with Ag/AgCl ink which acted as a reference electrode (RE) and was kept for drying in the atmospheric conditions for 24 h. The final electrode was modified with the carbon paste and acted as a counter electrode (CE). To limit the volume of the sample (10 µL) for testing of X, HX, UA (purines), a convex-shaped design was printed along with the printed electrodes on both the sides. To check the electrical performance, initial

thickness of the printed carbon paste was calculated using the profilometer. The parameters like conductivity, resistivity and the sheet resistance platform were tested with Ossila four-point probe system for all the three electrodes. The values of these parameters are tabulated in Table 2.

Results and discussions

Electrochemical activity of the fabricated platform

The electrochemical behavior of the modified and bare carbon paste electrode was investigated using the redox mediator 5-mM Potassium Ferricyanide. The cyclic voltammetry (CV) was performed in the potential window from − 1 to + 1 V at a scan rate of 50 mVs^{-1}. A volume of 10 μL was used to test the electrode performance. From the Fig. 2, it is evident that the electron-transfer rate is more in case of GMC-modified electrode compared to the bare carbon electrode.

Characterization of the working electrode

To characterize the GMC-modified electrode, scanning electron microscopy (SEM) and energy-dispersive X-ray (EDX) spectroscopy were performed. In Fig. 3a–c, SEM images with different magnifications of the modified electrode depict the presence of the carbon mesoporous with good porosity on the carbon paste. Further, as summarized in Table 3, EDX as shown in Fig. 3d was performed to find the weight percentage of carbon (C) and oxygen (O). Particle size distribution plot is shown in the Fig. 3e. The mean size of the particles from the Fig. 3e was found to be 97.22 nm and the standard deviation was 56.81. To find the functional groups, FTIR spectrum analysis was performed for the bare GMC, Carbon paste along with the GMC and bare carbon. For the bare GMC, the functional group O–H was observed at 2700.2 and 1441.1 cm^{-1}. The peaks observed for the bare carbon paste are at 3441.4, 2924.5, 2861.1, 2166.6, 1637.3, 1567.8, 1410.7, 1064.5 and 616.2 cm^{-1} which correspond to the functional groups of O–H, C–H, C–H, S–C≡N, C=C, C=C, S=O, C–O, respectively. For the carbon paste along with GMC, the peaks were observed at 3438.5, 2922.6, 2170.5, 1570.7, 1414.5 and 1051 cm^{-1} belonging to O–H,
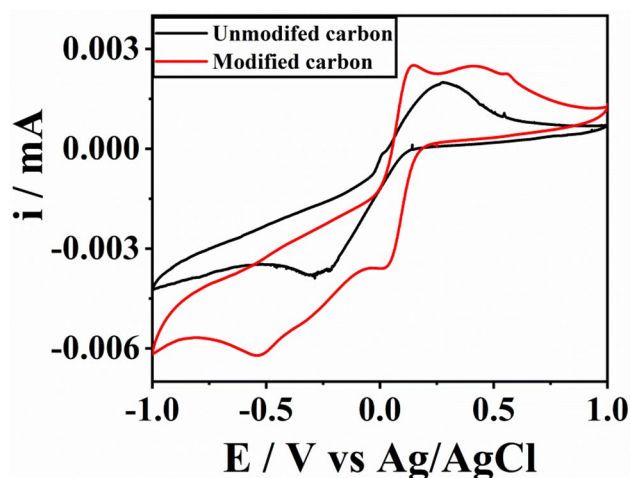
Fig. 2 Comparative CV response of bare carbon paste and GMC-modified carbon paste in 5-mM Ferricyanide in 1-mM Potassium chloride solution at a scan rate of 10 mV s^{-1}

C–H, C≡C, C=C, S=O and C–O, respectively. Therefore, post modification of carbon paste with GMC, the functional groups peak remained intact.

Electro-catalytic activity of the platform

The electro-catalytic oxidation of X, HX and UA with the fabricated platform was analyzed with their potential windows of 0.4 to 0.8, 0.4 to 1 and − 0.2 to 0.6 V, respectively, with a droplet of 10 μL in pH 7 PBS. CV was performed at 10 mVs^{-1} for 15 μM with and without the presence of purines. From the Fig. 4a–c, it is clearly observed that X, HX and UA undergo oxidation at potentials of 0.52, 0.86 and 0.27 V, respectively. The reaction mechanism for all the analytes (X, HX, UA) is shown in the Fig. 4d–f.

Effect of scan rate

Figure 5a–c is the CV responses of the modified electrode with 15 μM of the purines at 10 mVs^{-1} at various scan rates. From these figures, it can be observed that there is a linear increase in the anodic current with the increase in scan rate for all the purines. To interpret the electron-transfer mechanism of the modified electrode, effect of

Table 2 Electrical performance parameters of the three electrodes

Substrate	Electrode	Length (mm)	Width (mm)	Thickness (μm)	Sheet resistance (Ω)	Resistivity (Ω m)	Conductivity (S/m)
Glass	CE	20	1	11.35	94.69	1.070×10^{-3}	9.305×10^{2}
	WE	20	1	11.35	85.84	9.743×10^{-4}	1.026×10^{3}
	RE	20	1	11.35	82.24	9.333×10^{-4}	1.071×10^{3}
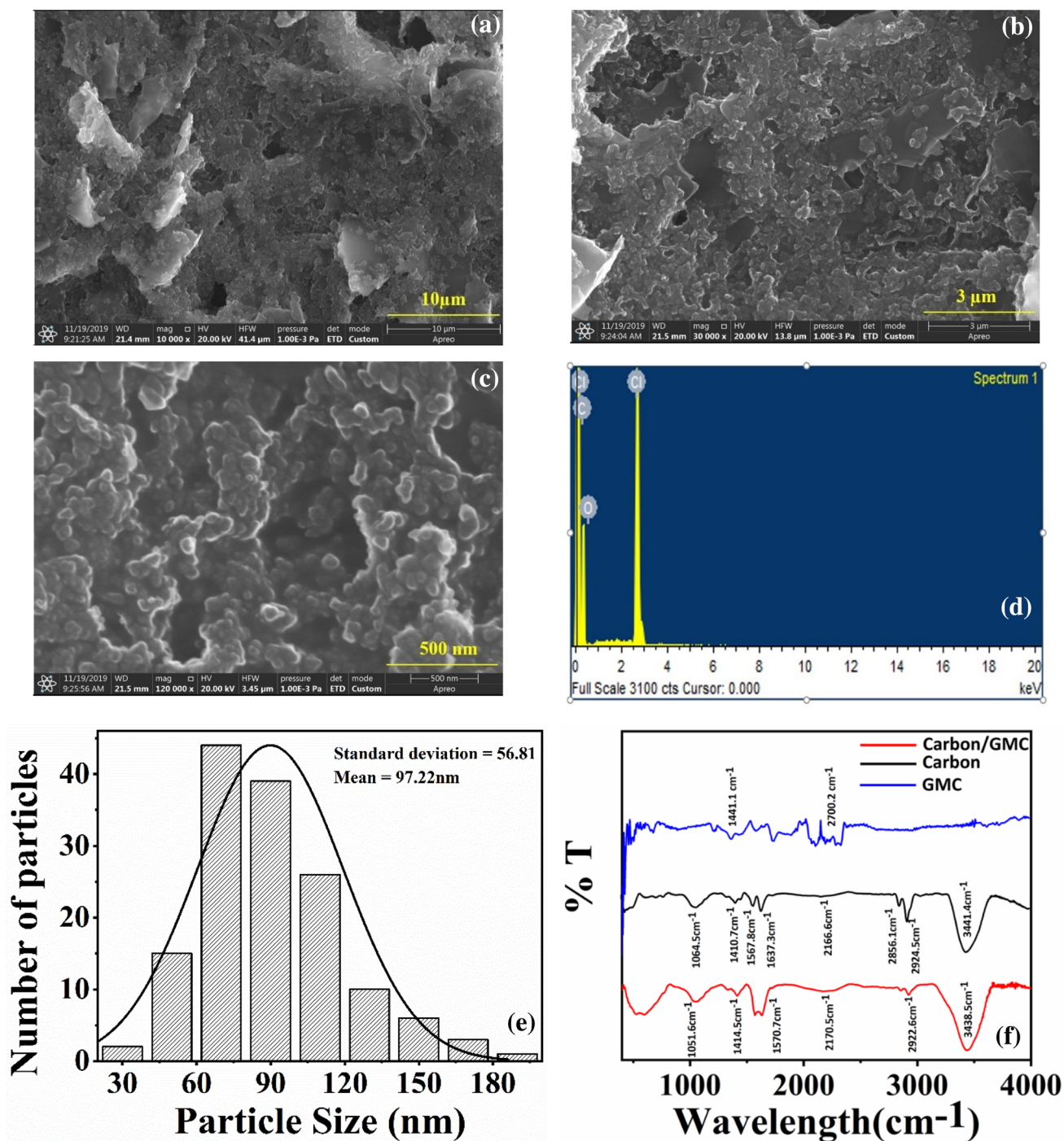
Fig. 3 **a** SEM images of the Graphitized mesoporous carbon modified electrode at different magnifications **a** 10 μm, **b** 3 μm, **c** 500 nm and **d** EDX analysis. of the modified electrode. **e** Size distribution curve plot. **f** FT-IR spectrum

varying scan rate was studied. Figure 5a, c, e is the CV responses of the carbon paste with GMC with 15 μM of X, HX, UA, respectively, at 10 mV s^{-1} with various scan rates. An orderly increase in anodic (i_{pa}) peak current was observed with increase in the scan rate. Corresponding calibration plots (Fig. 5b, d, f) with baseline-corrected

peak current for modified electrode with X, HX and UA values were plotted. An observed appreciable linearity manifests that the fabricated electrode had a surface-confined electron-transfer behavior. Usually, such type of electrochemical surface confined transfer reaction follows the equation:

Table 3 EDX data for modified electrode

Element	Modified electrode (weight %)
Carbon (C)	81.66
Oxygen (O)	1.45
Chlorine (Cl)	16.89

$$i_{pa} = 2.99 \times 10^5 n[(1 - \alpha)n_a]^{1/2} ACD^{1/2} v^{1/2},$$

wherein $n =$ total no of electrons, $A =$ electrode surface area 1 mm^2, $C =$ concentration of purines, $v =$ scan rate, $n_a =$ no of electrons involved in rate determining step, $\alpha =$ transfer coefficient. From the plot of i_{pa} vs $v^{1/2}$ (Fig. 5b, d, f), the slope values were obtained. The calculated diffusion coefficient (D) was calculated as 1.542×10^{-3}, 2.993×10^{-3} and 2.074×10^{-3} cm^{2}s^{-1} for X, HX and UA, respectively.
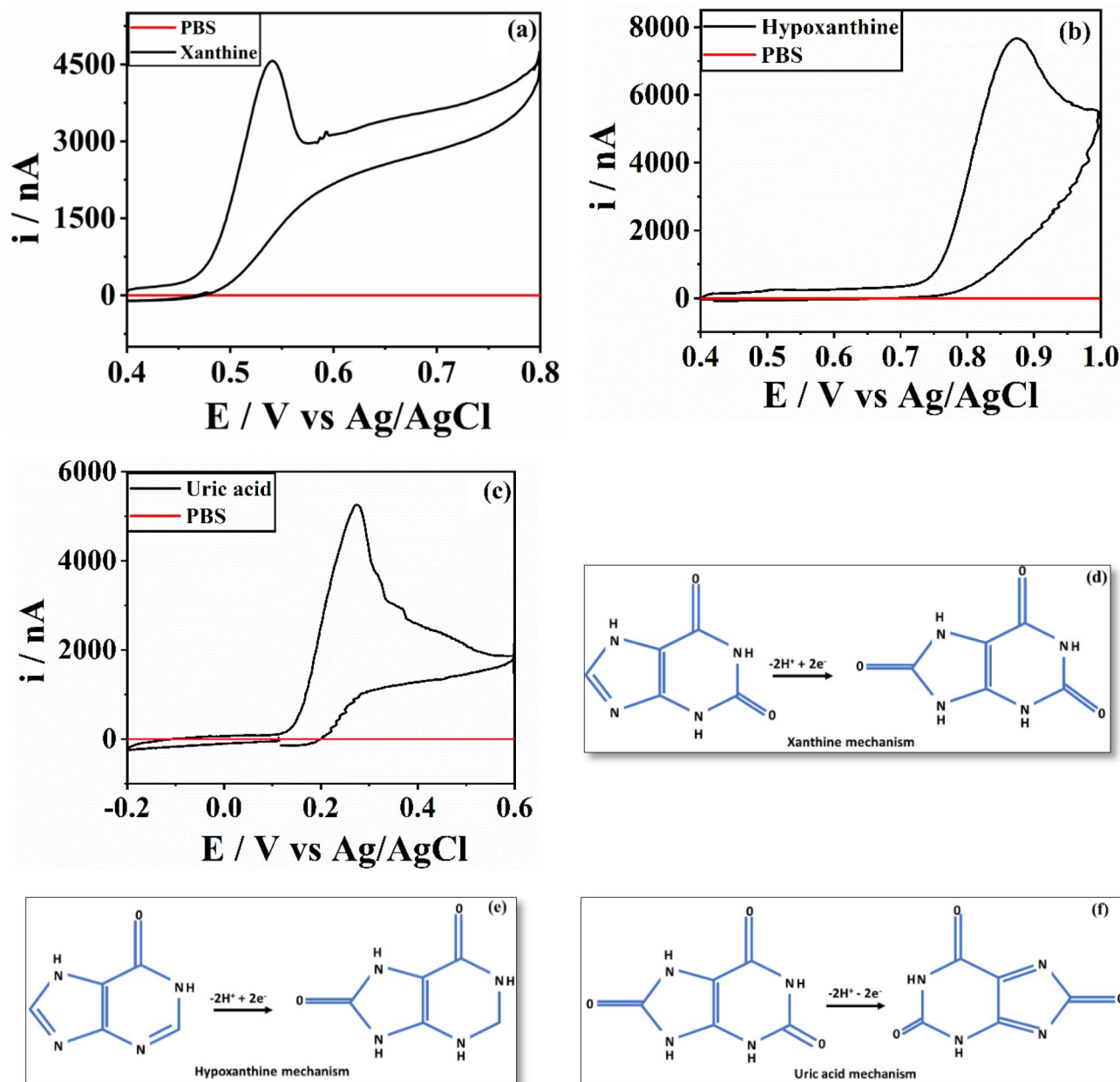
Fig. 4 **a** CV response of Xanthine in 10 μL of 0.1M pH 7 PBS, **b** CV response of Hypoxanthine in 10 μM of 0.1-M pH 7 PBS and **c** CV response of 15 μM Uric acid in of 0.1 M pH 7 PBS. **d–f** Plausible electrochemical oxidation reaction mechanism of X, HX and UA
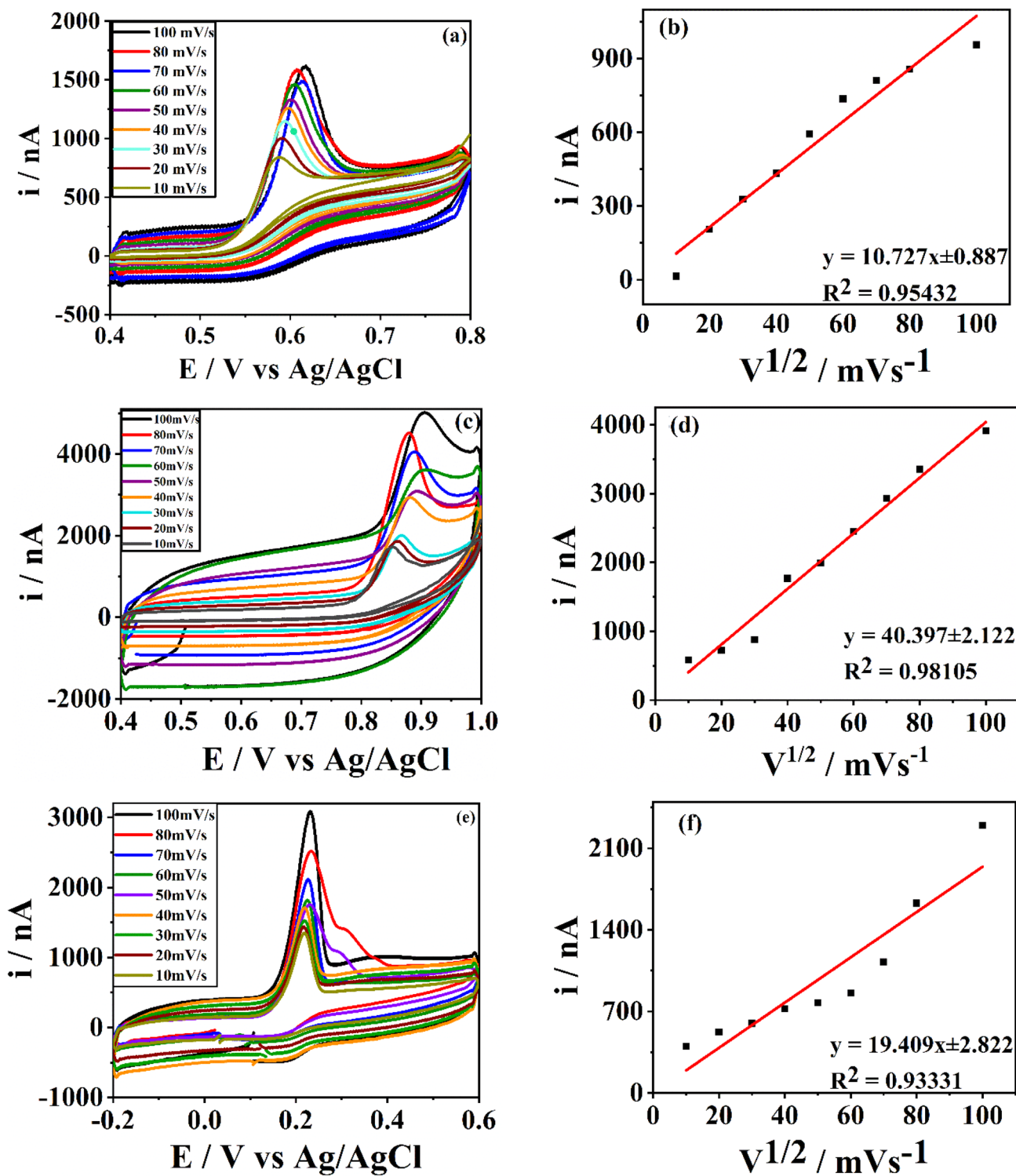
Fig. 5 **a** CV response of X at various scan rates, **c** CV response of HX at various scan rates and **e** CV response of UA at various scan rates. The baseline-corrected calibrated plots for i_{pa} vs. variable scan rates of **b** X, **d** HX and **f** UA
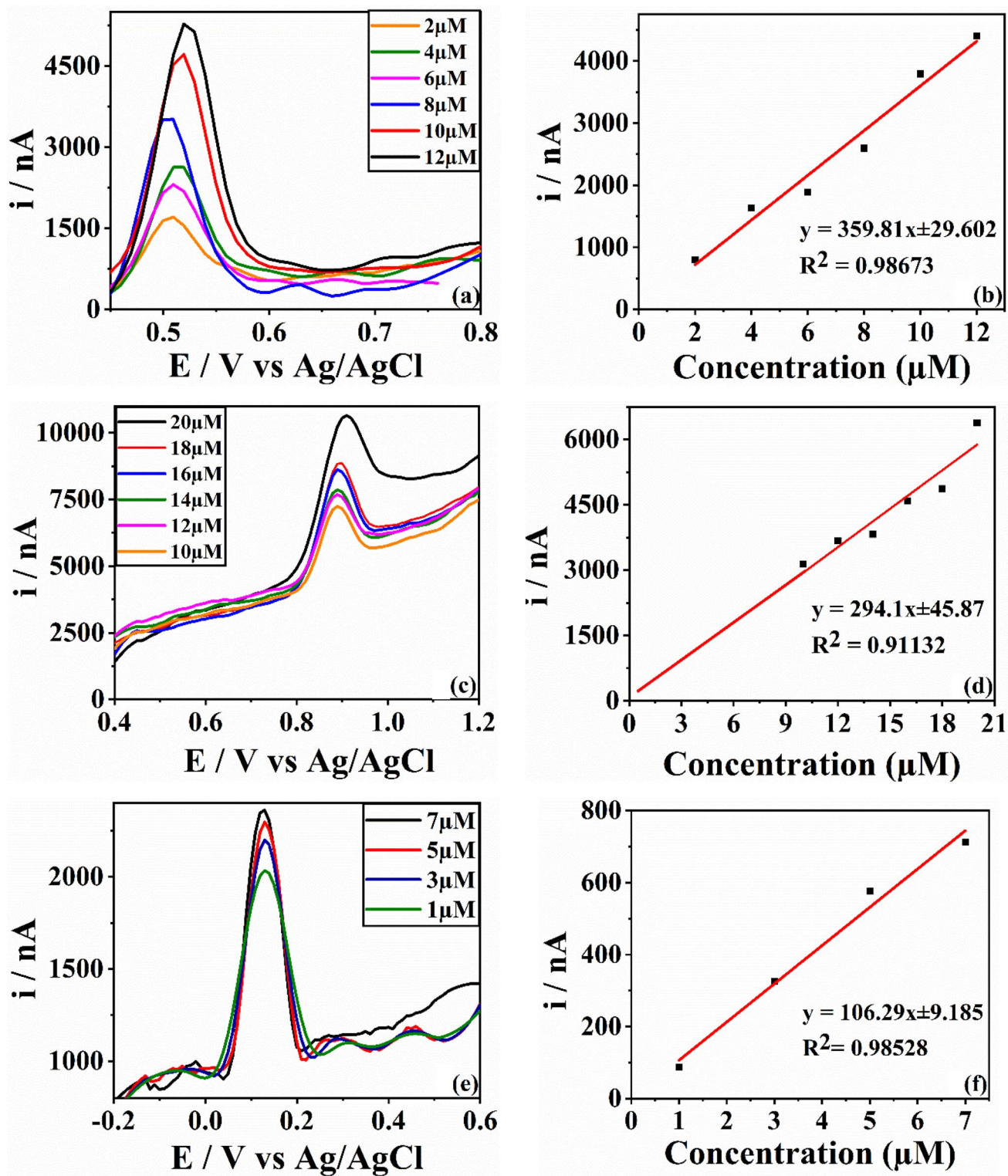
Fig. 6 SWV response of effect of various concentrations of **a** X, **c** HX and **e** UA in 0.1 M of PBS. Baseline corrected calibration plot for i_{pa} vs increasing concentration for **b** X, **d** HX and **f** UA

Effect of concentration effect

The effect of purine concentration was investigated as shown in the Fig. 6a, c, e using the squarewave voltammetry (SWV) with the optimized parameters, i.e., pulse height = 25.0 mV, pulses width = 50.0 ms, Step height = 10.0 mV, initial potential (E_i) = 0.4, 0.4, − 0.2, final potential (E_o) = 0.8, 1, 0.6, respectively. From the figures, it is clearly manifested that there is an increase in oxidation current with the increase in the concentration of purines. From the respective corresponding calibration plots from Fig. 6b, d, f the limit of detection (LOD) calculated for X, HX, UA is 3.33, 9.99 and 0.01 μM, respectively.

Interference study

Since the platform was developed with an aim to detect the aforementioned purines simultaneously, uniform concentration of X, HX, and UA was mixed in a droplet of 10 μL and dropped tested using SWV. All the three purines provided distinct peaks at different potentials proving the applicability of the present platform for simultaneous and interference

mitigated sensing (Fig. 7a). In addition, various other biochemicals like ascorbic acid, glucose, cysteine, dopamine, nitrite, nitrate and hydrogen peroxide were tested for interference. Figure 7b–d shows the bar graph for the achieved current in presence of various chemicals. Clearly, no significant interference was observed authenticating the selectivity of fabricated platform. The sensing stability of the inkjet device was also tested for all the analytes, with 15 μM concentration of the successive scans. Long-lasting stability of the platform was also investigated over a period of 5 days. Tests showed no apparent decline in the tested platform's analytical efficiency (RSD of 2 per cent).

Real sample analysis

To find the selectivity and sensitivity of the developed platform in real sample analysis, sensing of the purines in human serum samples was explored using the standard addition approach with in their respective linear ranges of X, HX and UA. The human blood was collected from the healthy volunteers from the Medical Centre BITS Pilani
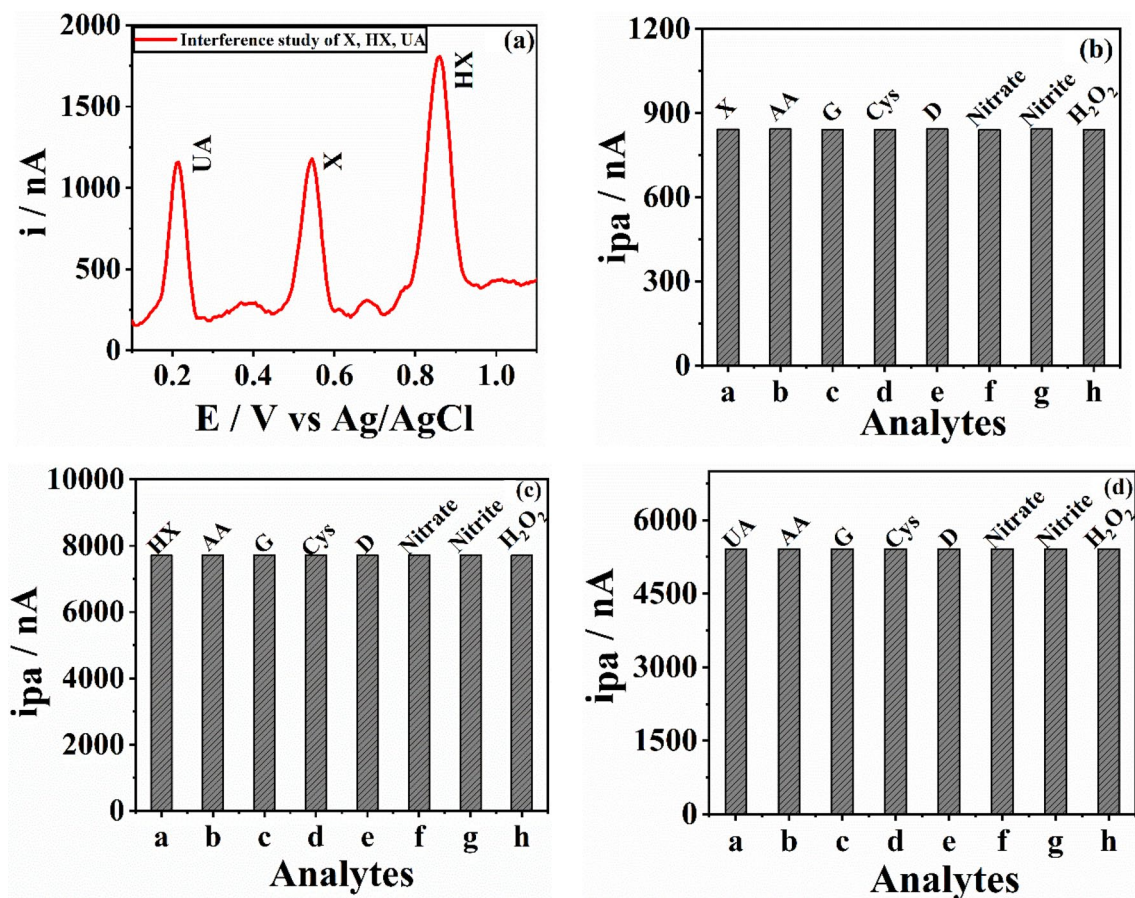
Fig. 7 **a** SWV response of effect of interference with purines in 0.1-M PBS pH 7, at 10 mVs^{-1}. **b** Xanthine interference with other biochemicals, **c** hypoxanthine interference with other biochemicals and **d** uric acid interference with other biochemicals

Table 4 Real sample analysis of human serum through standard addition approach

	Added (μM)	Found (μM)	Recovery (%)	Trail1 (nA)	Trail2 (nA)	Trail3 (nA)	Average	Standard deviation
Xanthine	2	2.5	125	1680.6	1681.7	1680.01	1680.3	0.295
	4	4.3	107.5	2533.2	2532.1	2531.7	2532.45	0.75
	6	6.8	113.3	2767.2	2770.1	2771.2	2769.2	2
Hypoxanthine	1	1.1	110	5335.1	5334.1	5334.7	5334.9	0.2
	3	3.1	103	5723.4	5726.8	5725.6	5724.5	1.1
	5	5.2	104	6178.6	6210.2	6180.3	6179.45	0.85
Uric acid	2	2.1	105	1979.8	1980.5	1975.4	1977.6	2.2
	4	4.2	105	2218.5	2310.7	2220.6	2219.55	1.05
	5	5.7	114	1979.8	1980.9	1976.8	1978.3	1.5

Hyderabad Campus. Table 4 represents the real sample of human serum with impressive recovery values.

Conclusion

In this work, an ink-jetted multi-sensing miniaturized three-electrode electrochemical sensing platform has been fabricated. Herein, carbon mesoporous modified on printed carbon paste has been utilized as a WE, conductive carbon as counter electrode and Ag/AgCl paste as reference electrode. The WE, modified with mesoporous carbon, was effectively characterized and rigorous electrochemical analyses were performed. It was observed that there was a linear increase in the anodic peak of all three purines (X, HX and UA) with the increase in their concentration in the range of 2–12, 10–20, 1–7 μM and the limit of detection 3.33, 9.99 and 0.01 μM, respectively. The developed platform was also analyzed to ensure the interference mitigation for these purines and other commonly identifiable biochemicals. Further studies were conducted with real sample of human blood serum providing excellent sensing capability of developed miniaturized sensing platform. Hence, the developed platform with ink-jetted electrodes in micro-device format provide excellent specificity and selectivity with remarkable limit-of-detection, and has a great potential for the real-time testing of purines.

Compliance with ethical standards

Conflict of interest The authors have no conflicts to declare.

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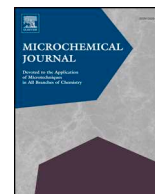
Greenly synthesized silver nanoparticles for supercapacitor and electrochemical sensing applications in a 3D printed microfluidic platform

Mary Salve, Aurnab Mandal, Khairunnisa Amreen, Prasant Kumar Pattnaik, Sanket Goel[*]

MEMS, Microfluidics and Nanoelectronics Laboratory, Department of Electrical and Electronics Engineering, Birla Institute of Technology and Science, Hyderabad 500078, India

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ABSTRACT

Herein, Silver nanoparticles (AgNPs) decorated pencil graphite electrodes (PGEs), with assimilation of Chitosan (CS), as a versatile electrode material for supercapacitor and electrochemical sensing application have been reported. The AgNPs were prepared by employing a green synthesis method using marigold flower s extract. Marigold flower contains lutein, which can be used as a reductant. The morphology and crystal structure of the prepared nanomaterial was characterized by Field emission scanning electron microscopy (FESEM), Elemental Dispersive X-Ray Spectroscopy (EDX), Fourier transform infrared spectrometer (FTIR), X-ray diffraction (XRD), UV-VIS Spectrophotometer and X-ray photoelectron spectroscopy (XPS). The prepared hybrid material, PGE/AgNPs/CS was utilized in an electrochemical 3D printed microfluidic device for supercapacitor and electrochemical sensing of H_2O_2. As a supercapacitor, the device provided a remarkable storage capacity of 367.16 mF cm^{-2} at a current density of 1 mA cm^{-2} with high cyclic stability over 1500 charge-discharge cycle. Subsequently, H_2O_2 sensing with the same electrode in a three-electrode microfluidic system gave a LOD of 0.52 μM within a linear range of 1-10 μM. In addition, the effect of common interfering species, including ascorbic acid (AA), uric acid (UA) dopamine (DA) and xanthine (XN) were thoroughly investigated. Thus, the prepared hybrid electrode material showed excellent electrochemical activity for the supercapacitor and electrochemical sensing of H_2O_2, which can be further assessed for other relevant applications.

1. Introduction

Over the past few decades, exploration of novel methods to synthesize metal nanoparticles has attracted an immense attention of researchers because of their unique optical, thermal and electronic properties, and has an extensive application in drug delivery, information storage, magnetic and optoelectronic [1,2]. There are various methods for synthesizing metal nanoparticles such as ion sputtering, chemical reduction, sol-gel [3,4]. All these methods are quite expensive and hazardous for the environment. Although nanocrystalline silver particles have great application in the field of antimicrobial, diagnostic, biomolecular detection, therapeutics and micro-electronics [5–7], yet there is a need of the commercially economical, feasible and environment-friendly method of synthesis. With this goal, green synthesis method, harnessing plant extract like roots, leaves, flower or fruit as reductants and stabilizing agents for metal nanoparticle synthesis, has received great response in diverse research fields. The green synthesis method has an advantage over other approaches as it is inexpensive, eco-friendly and reproducible [8,9]. In the present work, marigold flower extract, consisting of lutein as 95% of the component, assists the reduction of Ag$^+$ ions to give AgNPs [10].

Silver nanoparticles are extensively used in the field of electrochemical energy storage applicationas it gives high chemical stability, high electronic conductivity, surface chemical properties [11,12]. Owing to these properties, AgNPs have the potential to be used as supercapacitors and energy storage devices. Supercapacitors are considered to be one of the newest innovations in the field of electrical energy storage. With characteristics like high power density, fast charge-discharge life, low equivalent series resistance (ESR) and low-cost maintenance, there is an increase in demand of supercapacitors in electric equipment and digital communication [13–15]. Generally, the electrode materials used in supercapacitor are conducting polymer, nanocarbon and transition metal oxide. The fundamental mechanism of energy storage in a supercapacitor is achieved either by electrical double-layer capacitive (EDLC) based on nanocarbon electrode material or by pseudocapacitive based on Faradic material like transition metal oxide or conducting polymer [16–18]. Hybrid material, like carbon, while combining with conducting polymer or metal oxide, gives the

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[*] Corresponding author.
E-mail address: sgoel@hyderabad.bits-pilani.ac.in (S. Goel).

advantage of both EDLC and pseudocapacitor [19–21]. However, the disadvantage of a conducting polymer, while preparing material for supercapacitor application, is its poor stability in charge-discharge cycle due to the redox sites. Even though carbon material has low capacitance value, they have a better life cycle in comparison to the conducting polymers [22].

On the other hand, carbon materials, are often embedded with metal or metal oxide as a promising electrode material for supercapacitor applications [23]. Among various carbon materials, pencil graphite electrode (PGE) is attractive with distinctive advantages like cost-effectiveness, easy availability and reasonably good electrical conductivity. PGE surface has a rough structure as it contains a regularly ordered graphitized layer which leads to ease of modification with reproducibility [24].

The electrode material fabricated by combining PGE and AgNPs can boost the performance of the prepared composite electrode electrochemically. Reports are available on AgNPs and carbon material based composite electrode for supercapacitor application. Liu et al. have grown Ag nanoparticle directly on the porous material substrate as a composite electrode for supercapacitor application [25]. The developed electrode provided a specific capacitance of 517.5 F/g and an outstanding cycle stability of 85.6% retention after 3000 cycles. Das et al. synthesized silver polypyrrole / graphene nanocomposite as an electrode material for supercapacitor application. The developed composite gives a high specific capacitance of 472 F/g at a 0.5 A/g current density. Reports are also available on green synthesis technique for the preparation of AgNPs for supercapacitor and electrochemical application [11]. Chen et al. synthesized AgNPs by reduction of silver nitrate with vitamin C as a nature-friendly reducing agent. The synthesized nanoparticle with polyaniline nanofiber as a nano composite gave an excellent capacitive performance with 553 F/g specific capacitance [8].

Therefore, the incorporation of AgNPs processed using green synthesis techniques on PGE has a strong potential to increase the electrical conductivity and electrochemical storage capacity. Many reports indicate that hydrophilicity of an electrode surface enhances the supercapacitive properties of the active material [26]. The electrode having the property of good wettability gives better penetration of electrolyte into the active surface. Sun et al. showed that assimilation of chitosan (CS) to an electrode material led to better hydrophilic properties as it has the ability to form hydrogen bonding with inherent amine and hydroxyl groups [27]. In the present work, the aforementioned unique advantage of CS with PGE/AgNPs have been combined to investigate its supercapacitor properties and electrochemical sensing using 3DP microfluidic device.

In further, the prepared PGE/AgNPs/CS was explored for electrocatalytic detection activity towards hydrogen peroxide (H_2O_2). H_2O_2 detection has become extremely important in the past few years due to its wide application in food industries, cleaning product, cosmetics, clinic, drugs and environmental analysis [28,29]. H_2O_2 and its derivatives are powerful oxidizing agents that can be used for synthesizing organic compounds and treatment of environmental pollutant. Determining H_2O_2 concentration using electrochemistry has proven to be inexpensive and effective way to investigate the reaction of the substances. A number of electrochemical sensors with and without enzyme were developed for H_2O_2 sensing, but they are relatively costly and unstable. To avoid the drawback of enzyme-based sensing, a novel electrode based on nanomaterial gives better enzyme imitative properties.

Microfluidics offers an easy and instant sensing platform to identify particular biomolecules. From the last two decades, several microfluidic platforms has shown a great potential to meet the desire of low sample volume, cost-effective, rapid analysis and enhanced reaction reliability and reproducibility. Earlier microfluidic devices for rapid production were made using poly(dimethylsiloxane) (PDMS), but prototype development and cost were the limiting factors. 3D-printing enables rapid prototyping of single unit devices by avoiding the use of expensive masks that are necessary for the fabrication using lithography and soft-lithography. Herein, a 3D printed microfluidics platform has been developed for effective electrochemical sensing of H_2O_2 and for supercapacitor application; using the fabricated PGE/AgNPs/CS as a working, Ag/AgCl as reference and platinum wire as a counter electrode.

2. Experimental section

2.1. Chemicals, materials and instrumentation

All the chemicals were of analytical reagent grade and were used without any further purification. Hydrochloric acid (HCl) 35%, Potassium chloride (KCl), Acetic acid(CH_3COOH), Chitosan (CS), Hydrogen peroxide (H_2O_2) 30% and Silver nitrate Ag(NO)$_3$ were obtained from Sigma (St.Louis, MO, USA). Absolute ethanol was obtained from S.D Fine Chemical Limited (Mumbai, India). 0.1M KCl-HCl (pH = 2) was used as a supporting electrolyte. Deionized water (DI) collected from milli-Q was used for aqueous solution preparation. The commercially available pencil lead (2mm, 2B) obtained from Camlinwas obtained from local stationery. Electrochemical measurements were carried out with an electrochemical analyser (SP-150, Bio-Logic, electrochemical workstation (France)). The pH meter from Oakton was used for measuring the accurate pH value of the required solution. The ultra-sonication water bath was obtained from life-care. The CIC-15A hot air oven was obtained from Cintex Industrial Corporation (Mumbai, India). Remi R-4C Laboratory Centrifuge was obtained from m-LABS. ApreoLoVac Field emission scanning electron microscope (FE-SEM) was used for microscopic analysis of the developed nanoparticle. Likewise, FTIR-4200 from Jasco, Rigaku Ultima IV X-ray Diffractometer (XRD), UV-VIS Spectrophotometer V-650 JASCO, Thermo scientific K-Alpha X-ray Photoelectron Spectrometer (XPS) were used for FTIR, XRD,UV-Vis, XPS analysis.

2.2. Procedure

2.2.1. Preparation of the flower extract

Tagetes erecta (Marigold) flowers were collected from a local market in Hyderabad, India. The flower petals were washed and then dried in a hot air oven at 60 °C. 5 g of dried petals were refluxed in a 100ml of distilled water with 4–5 drops of 1 M HCl for an hour with continuous magnetic stirring at 80 °C temperature. The extract was cooled to room temperature and filtered out, giving a dark red filtrate solution (Scheme 1.).

2.2.2. One pot synthesis of silver nanoparticles

0.5 mM solution of Ag(NO)$_3$ was prepared in 50 mL DI water. The obtained solution Ag(NO)$_3$ was mixed in 1:1 ratio with the flower extract. The resulting solution was stored overnight in a dark chamber at a room temperature [5]. The Ag^+ ions were reduced to Ag nanoparticles which were observed by the change in color from dark red to orange. Following this, the solution was centrifuged (10000 RPM for 30 min), thereby the particles got settled down at the bottom, whereas the supernatant was discarded. The filtered nanoparticles were washed using 90% ethanol and deionized water for several times and then dispersed in 1ml of ethanol solution using an ultrasonicator for an hour.

2.2.3. Design and fabrication of 3D printed microfluidics platform

3D printing gives a fast and simple way to fabricate microfluidic devices directly from the computer-aided software. A commercial 3D-printer based on fused deposition modelling (Flashforge) was used to print the microfluidic-based platform with ABS filament of 1.75 mm. Using 123D design software, the computer-aided design was created, and the file was exported in .stl format, compatible with the 3D printer software. The design was printed with an optimized extruder temperature of 240 °C, bed temperature as 120°C, infill 100% and a layer

height of 100mm. Here, two 3D printed devices were fabricated that can be used for electrochemical sensing and supercapacitor applications. The fluidic device of total size 40 mm × 18 mm × 5 mm with microchannel of a rectangular reservoir of size 30mm × 4mm × 2mm with inlet holes for the electrode was realized (PGE, Pt and AgCl electrode). The channel was bonded with the glass slide to get a transparent window using a double sided tape.

2.2.4. Preparation of electrode and electrochemical measurement

The supercapacitor PGE was wrapped with non-conductive parafilm (Tarson) by leaving the disc portion (i.e. bottom part) for the electrochemical studies. The exposed surface was equalized by polishing it on Silicon Carbide Waterproof Abrasive Sandpaper Sheet 1500 Cw. On the exposed surface of PGE (2mm), AgNPs solution (3 μL) was drop-casted twice followed by a single layer of CS (2 μL). After each layer of drop-casting, it was allowed to dry at ambient temperature for 2 h.

The developed 3D printed microfluidics platform for two, and three-electrode setup was used for supercapacitor and electrochemical sensing application using 500 μL of working solution (Scheme 2.). The electrochemical storage properties of the prepared material were characterized using cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS), galvano static charge-discharge (GCD), capacitive retention in 1M HCl-KCl (pH = 2) aqueous solution. EIS measurements were carried out in the range of $0.01 \sim 10^5$ Hz with an amplitude of 10mV. The three-electrode system consisted of a modified PGE/AgNPs/CS, Pt and Ag/AgCl (1M HCl-KCl) as the working, counter and reference electrodes. In a three-electrode configuration the areal capacitance values were calculated from the GCD curves using equations:

$$C = I \, \Delta t / S \, \Delta V \tag{1}$$

Where I is discharging current in mA-cm^{-2}, Δt is the discharging time in seconds, ΔV is the potential window and S is the active area of the working electrode in cm^2.

3. Result and discussion

3.1. Characterization of the silver nanoparticles

In order to study the topographical morphology of the synthesized AgNPs, a field emission scanning electron microscope (FE-SEM) was used.The FE-SEM micrographs of the AgNPs at higher magnification suggested that the synthesized particles had a homogeneous size distribution (Fig. 1. A and B). The elemental composition of the particles, shown in the Elemental Dispersive X-Ray Spectroscopy (EDX) images (Fig. 1.C),reflects a high silver content present in the NPs study [30].

To identify the functional groups present, Fourier transform infrared spectrometer- (FTIR) was carried out (Fig. 2. A) The band at 3340 cm^{-1} was responsible for O-H stretching. The band at 1668 cm^{-1} represent the C=C stretching; furthermore the peak near 1341 cm^{-1} and 810 cm^{-1} represent C-O bending and C-H bending [6].The peaks observed corresponds to the traces of the reductant. The X-ray diffraction studies were followed up to get information about the internal lattice structure of the crystalline metallic silver nanoparticles (Fig. 2.B) Herein, the X-ray target was copper (Cu-k$^\beta$) with an operating voltage and tube current value of 40 kV, 30 mA respectively. The scanning speed was 3 ° / min covering a scan range of 5–90°. The XRD patterns reflected that the synthesized silver nanoparticles have a face centred cubic (FCC) structure that was observed from the diffraction peaks in the 2θ range of 30°–80°, that indexed to (1 1 1),(2 0 0), (2 2 0) [30].To confirm the presence of nanostructured silver particles, the ultraviolet visible spectroscopy was performed using a UV-VIS Spectrophotometer. The absorbance spectra were recorded for a wavelength range of 300–900 nm (Fig. 2.C), and it was observed that AgNPs peaks occur at 430nm with high absorbance which is specific for silver nanoparticle [6]. X-ray photoelectron spectroscopy studies (XPS) were

executed to analyse the surface composition and to examine the purity of the as synthesized silver nanoparticles extracted from the Tagetes erecta marigold petals (Fig. 2.D). The XPS spectrum was recorded in the range of 0–1200 eV. The physico-chemical characterization techniques revealed the formation of silver nanoparticles of 20 nm–50 nm The spectrum shows the presence of metallic silver because of the $3d_{5/2}$ component occurs at the binding energy of 368.3 eV, which represents the characteristic of the metallic oxidation state of Ag [31].

3.2. Electrochemical properties

For investigating the electrochemical performance of the as-prepared material, a series of electrochemical tests were performed. Fig. 3.A depicts the Cyclic Voltammetry (CV) curves of PGE and PGE/AgNPs/CS as a working electrode, platinum as a counter electrode and Ag/AgCl as a reference electrode. The CV measurements were carried out in the potential window of 0.5 to - 0.7 V at a scan rate of 50 mV/s. As can be seen, a surface-confined redox peak corresponding to AgNPs is observed at a potential 0.12V and -0.18V vs Ag/AgCl. The redox pair can be attributed to the electron transfer between silver and chitosan matrix, where AgNPs interact with active amino and hydroxyl groups in the CS matrix. The result indicates a large specific capacitance with the prepared material, which might have been obtained from the pseudo-capacitance of the electrochemically activated AgNPs/CS. Fig. 3.B shows the corresponding CV curves of PGE/AgNPs/CS at a scan rate in the range of 10–90 mVs^{-1}. Here, the results revealed that the peak current increased linearly as per Randle–Sevick equation [32].

$$i_p = 2.69 \times 10^5 A_e D^{1/2} n^{3/2} V^{1/2} C \tag{2}$$

Where i_p = peak current, n = number of electrons involved in redox reaction, D = diffusion coefficient (7.6×10^{-6} cm^2 s^{-1}), C = concentration of electrolyte, v is the scan rate, A_e is the electrochemically active area of the working electrode, which implies a diffusion-controlled electrochemical process. Upon substitution of various parameters in the above Eq. (2) active area of the working electrode (Ae) was calculated to be 0.24 cm^2 .

The stability and reproducibility of the PGE/AgNPs/CS were also examined. The stability of the PGE/AgNPs/CS was examined after storing the fabricated electrode at a room temperature for 4 weeks, and no significant changes in the current response were observed after the first five days. After 2 weeks of storage time, < 5 % loss was observed. After 4 weeks, 89% of the peak was intact. In order to check the electrode reproducibility, five different electrodes were tested. The relative standard deviation (RSD) of the current response was 1.4 %.

3.3. Supercapacitor application

The hybrid PGE/AgNPs/CS composite showed an enormous scope to be used as a standalone electrode in electrochemical sensing platforms. Meanwhile, to investigate the super capacitive performance of the fabricated PGE/AgNPs/CS electrodes, electrochemical impedance spectroscopy(EIS), galvanostatic charge-discharge(GCD) experiments were carried out using symmetric electrode. The Nyquist plots, shown in the Fig. 3.C, suggests that an equivalent series resistance (ESR) (which constitutes the resistance offered by 0.1 M HCl-KCl electrolyte, inherent resistance of the electroactive material, and the contact resistance between the electrode-electrolyte interfaces) for the hybrid PGE/AgNP/Cs composite is significantly smaller than that of bare PGE electrode, which in turn indicates a lower charge transfer resistance. From the EIS plots, the R_{ct} values were estimated to be 50 Ω for the PGE/AgNPs/CS composite as compared to 225 Ω for the bare PGE. The lower charge transfer resistance value of the PGE/AgNPs/CS can be attributed to the formation of a highly conducting membrane with nanostructured silver particles sandwiched between the PGE surface and CS polymer film which enhances the electron transport pathway
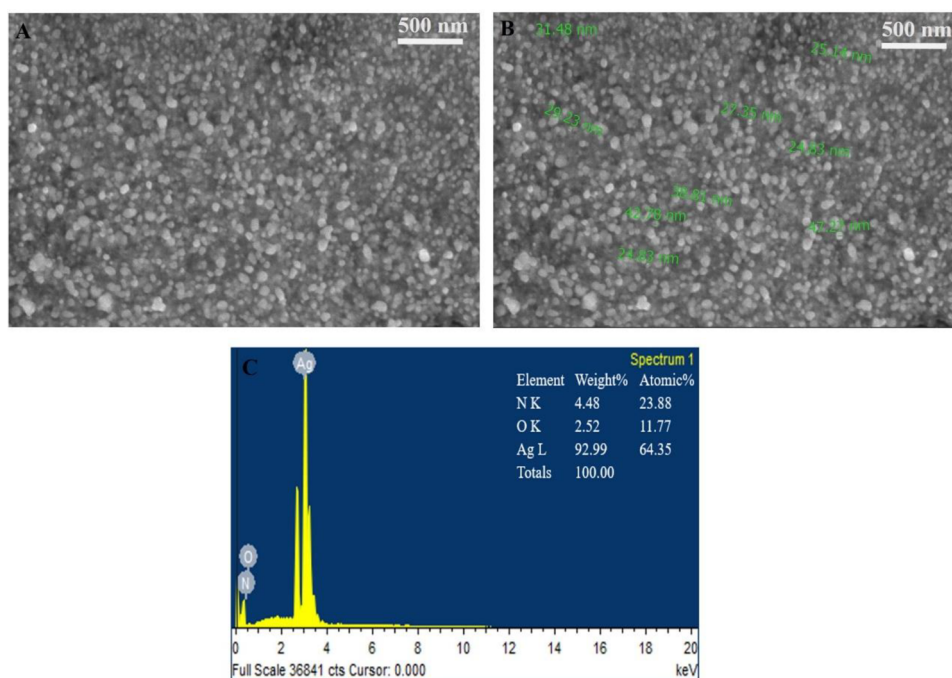
Fig. 1. (A-B) SEM images (C) EDX of AgNPs

between the electrode and the redox probe. To compare the capacitative performance, GCD experiments were carried out for a bare PGE and the hybrid PGE/AgNPs/CS composite at a higher current density of 5 mA cm^{-2}. The results presented in **Fig. 3.D** revealed that the discharge time for the hybrid PGE/AgNPs/CS composite was much higher than a bare PGE yielding a significantly higher areal capacitance value of 150.24 mF cm^{-2} which was an order of magnitude higher when compared to bare PGE (15.36 mF cm^{-2}). Some literature reports suggest, the supercapacitive behaviour of bare PGE at very low current densities, however, when evaluated at higher current densities the bare
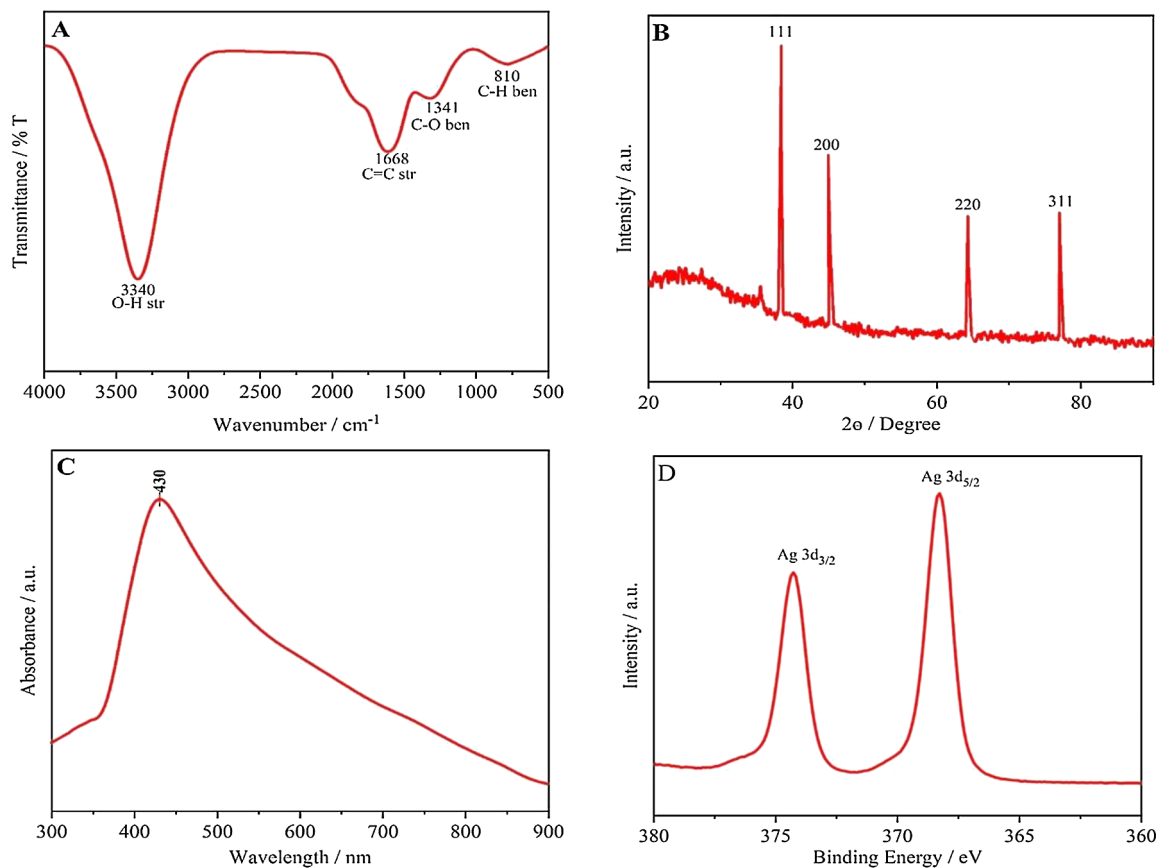
Fig. 2. Synthesized Silver Nanoparticle (A) FTIR Spectra (B) XRD (C) UV-VIS (D) XPS
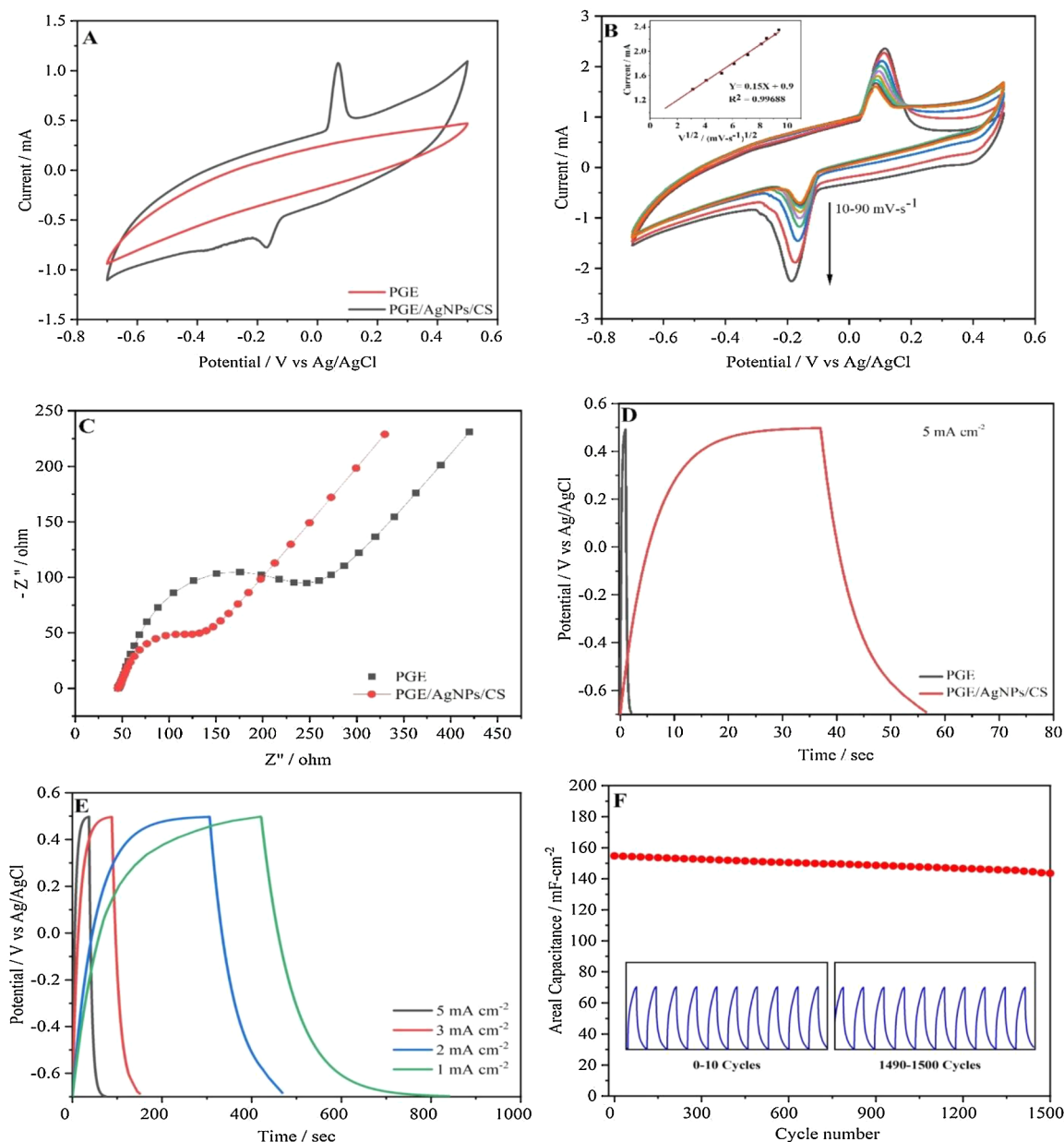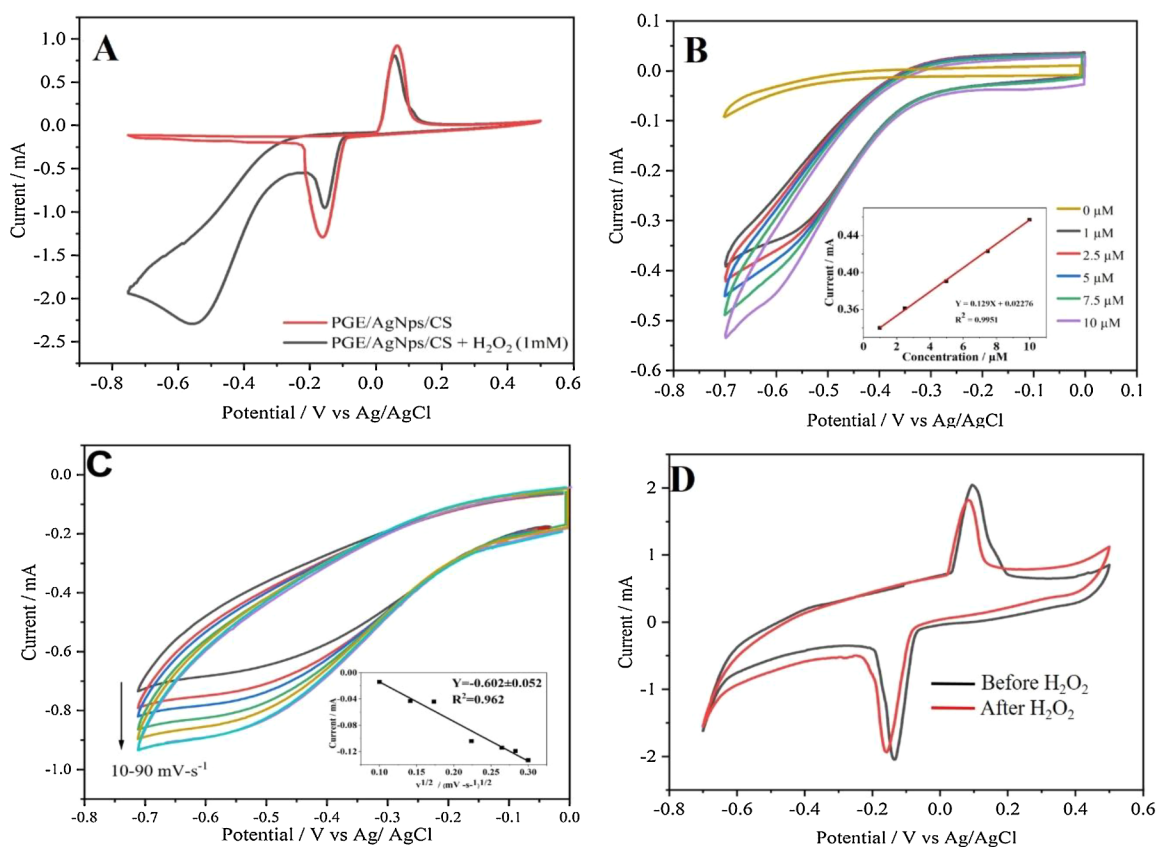
Fig. 3. Effect of potential on CV response in 0.1M HCl/KCl (pH = 2) solution at 10 mV s^{-1} (A) PGE,PGE/AgNPs/CS at 10 mV s^{-1}, (B) Potential cycling experiment within a fixed potential of -0.6 V – 0.4 V with inset plot are respectively of i_{pa} value Vs variable potential, (C) EIS responses of PGE, PGE/AgNPs/CS, (D)GCD curves of a PGE, PGE/AgNPs/CS at 5 mA cm^{-1} (E) GCD curves of a PGE/AgNPs/CS at different current densities, (F) Cycling stability of the PGE/AgNPs/CS electrode. Inset: GCD curves of first and last 10 cycles.

PGE exhibited very low areal capacitance as compared to the hybrid PGE/AgNps/CS composite [33].

The GCD plots of the hybrid PGE/AgNPs/CS electrodes at different current densities of 1,2,3 and 5 mA cm^{-2} are shown in Fig. 3E. The areal capacitance for the modified PGE electrode was estimated to be 154.67, 200.68. 283.33 and 367.16 mF cm^{-2} at the current densities of 5, 3, 2 and 1 mA cm^{-2}, respectively.The plot depicts that as the current density increases the discharge time of the PGE/AgNPs/CS electrodes decreases. The non-linear shapes of all the curves depict the faradaic battery like capacity shown by the PGE/AgNPs/CS composite. The superior supercapacitative behaviour of hybrid PGE/AgNPs/CS composite and the large operational voltage of 1.2V can be attributed to the rapid surface redox reactions of the silver nanoparticles which has been verified by the CV curves.

Long cycling life is an important criterion in the characterization of supercapacitor electrodes. As shown in **Fig. 3.F** to examine the capacity

retention the fabricated PGE/AgNps/CS electrodes, they were cycled upto 1500 cycles. Capacitance system showed minimal deterioration in the charge-discharge rate and capacitance after 1500 cycles with high capacitance retention of 92.8%.The fabrication of the PGE surface with chitosan matrix in the presence of silver nanoparticles provided a better pathway for electron transfer between the bulk electrolyte and the electrode thereby enhancing the storage capacity of the supercapacitor.

To further evaluate the charge and energy storage performance of the PGE/AgNPs/CS a symmetrical supercapacitor device was fabricated by leveragingtwo identical PGE/AgNPs/CS electrodes with pH = 2 HCl-KCl as the liquid electrolyte. Two similar devices were connected in series and were utilized to power a 3V LED for nearly a minute, which shows that the fabricated electrodes have immense potential in portable energy storage applications. The optical micrograph of the glowing LED is shown in **Fig. 4.**
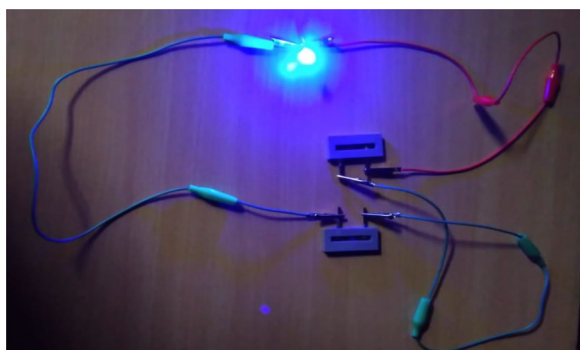
Fig. 4. LED powered by two symmetric electrodes connected in series in a 3D printed microfluidic channel.

3.4. PGE/AgNPs/CS as a H_2O_2 sensor

The prepared hybrid material, PGE/AgNPs/CS, was further explored for H_2O_2 sensing as a model system to investigate the electro catalytic activity of the prepared Ag particles. Prior to the experimental sensing, the test solutions were deaerated with high-purity nitrogen to remove any unwanted peaks due to the presence of diffused oxygen. The three-electrode system consisted of a prepared hybrid material as PGE/AgNPs/CS, Pt and Ag/AgCl as the working, counter and reference electrodes respectively.

As shown in **Fig. 5.A**, in comparison to bare PGE, the modified PGE/AgNPs/CS electrode provided considerable cathodic reduction peak for H_2O_2 reduction. The possible mechanism of H_2O_2 reduction with modified PGE/AgNPs/CS electrode is shown in Scheme 3. Here, AgNPs enhanced the electron transfer activity remarkably leading to H_2O_2

electro-catalysis. Based on previous studies, the mechanism for the electrocatalytic reduction of H_2O_2 in the presence of silver nanoparticles can be described as [34]

$$H_2O_2 \xrightarrow{Ag} \frac{1}{2}O_2 + H_2O$$

$$O_2 + 2_{e^-} + 2H^+ \xrightarrow{Electode} H_2O_2$$
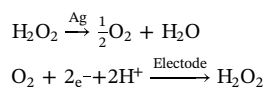
As a result of the above experiments, quantitative analysis of H_2O_2 concentration were performed using cyclic voltammograms (CV) technique in the range of -0.7 to 0.2 V. The solutions were prepared for H_2O_2 in pH 2 HCl-KCl, and a calibration graph was plotted. A linear graph was plotted (**Fig. 5.B**) for concentration over the range of 1 µM -10 µM H_2O_2. As can be seen, the detection limit was found to be 0.51 µM with the prepared hybrid material gives the better catalytic effect of AgNPs in the presence of H_2O_2.

In addition, the effect of variable scan rate on the electro-catalytic reduction of H_2O_2 with PGE/AgNPs/CS was carried out to comprehend the electron transfer mechanism. The peak current increases with the increase in the scan rate within the range of 10-90 mV s^{-1} as shown in **Fig. 5.C** in correspondence with Randle–Sevcikequation [32]. As shown in the inset of **Fig. 5.C**, corresponding calibration plot is linear with slope value -0.602 indicating a diffusion controlled reaction. Further the stability of the electrode was tested after H_2O_2sensing and it was found the peak current sustained post H_2O_2 exposure with <5% reduction (**Fig. 5.D**).

For examining the sensitivity of the modified PGE sensor towards H_2O_2 in a sample containing other interfering species such as ascorbic acid (1mM), uric acid (1mM) dopamine (1mM) and xanthine (1mM), cyclic voltammetry studies were carried out. The results revealed that the interfering species did not produce any observable peaks, therefore, negligible interference from other bio-chemicals is observed. It can be

Fig. 5. Effect of potential on CV response in 0.1M HCl/KCl (pH = 2) solution at 10 mV s^{-1} (A) PGE/AgNPs/CS with and without H_2O_2(1 mM) (B) Concentration effect of H_2O_2 on PGE/AgNPs/CS (C) Scan rate effect of PGE/AgNPs/CS in 10 µM H_2O_2 (pH = 2). Inset C, corresponding calibration plot of scan rate vs. v$^{1/2}$. (D) CV response of PGE/AgNPs/CS before and after exposure to 10 µM H_2O_2 (pH = 2) at 50 mV s^{-1}.
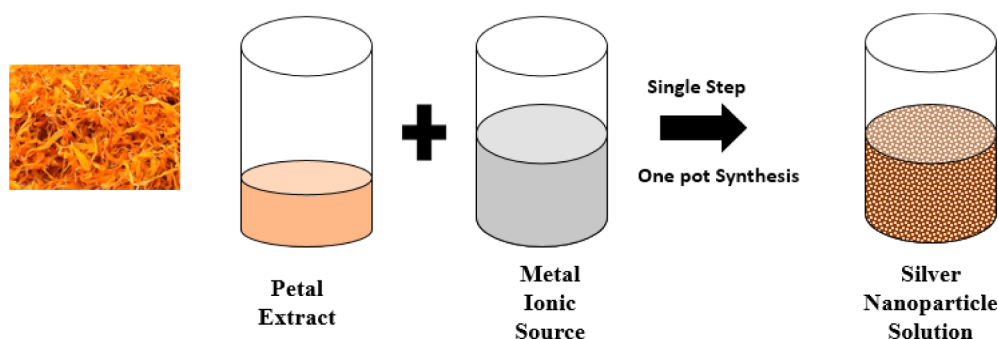
Scheme. 1. Schematic representation of Tagetes based Green Synthesis method for Silver nanoparticle synthesis.
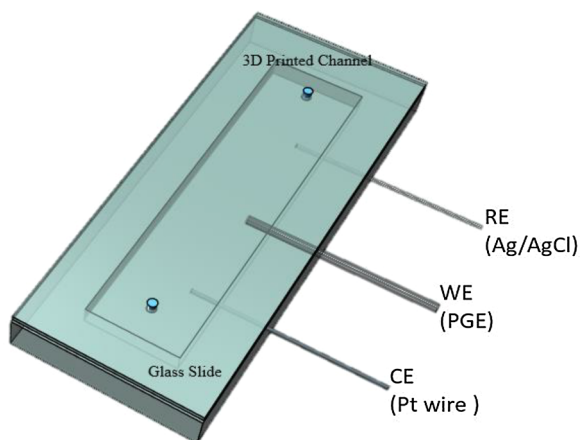
Scheme. 2. Schematic Representation of 3D Microfluidic Channel for Supercapacitor and Electrochemical sensing.
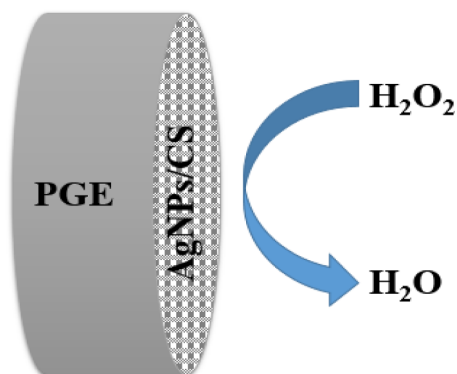
Scheme. 3. Schematic representation of the electrode and overall detection of H_2O_2 by AgNPs.

seen that the PGE/AgNPs/CS sensor is highly selective towards hydrogen peroxide sensing even in the presence of interfering species.

3.5. Real sample analysis

For testing the applicability of the fabricated the PGE/AgNPs/CS electrochemical sensor, three commercially available real samples were tested for H_2O_2 detection in FEM-bleach solution (a), hair dye sample (b) and a medicated H_2O_2 solution (c).

The samples (a) and (b) were prepared by mixing 10 mg of the bleach and 100 mg of the hair dye developer kit in 10 ml of pH 2 HCl-KCl separately, and subsequently sonicated for 15 min, filtered and used. For sample (c) preparation about 10 μM of medicated solution was diluted with 990 μL of pH 2 HCl-KCl. The detected levels and the

Table 1
Real sample analysis.

Samples	Found in original sample(μM)	Spiked (μM)	Detected (μM)	Recovery (%)
Bleach	2.34	5	5.21	104.2
Hair Dye	4.2	5	4.9	98
Medicated H_2O_2	4.56	5	5.02	100.4

calculated recovery values in the test samples are presented in the Table 1. From the obtained result, it is suggested that this method can be used for traces analysis of H_2O_2 in cosmetic products in real-time.

4. Conclusion

In summary, the Silver nanoparticles (AgNPs) have been successfully synthesized by using green synthesis technique that delivers large electrochemically active sites and electrical conductivity. A 3DP microfluidic platform was integrated with the electrode. The incorporation of AgNPs on pencil graphite electrodes (PGEs) with chitosan (CS) matrix, was of great benefit for supercapacitor and electrochemical sensing applications. The 3D printed microfluidics platform was used for supercapacitor and electrochemical sensing application with two or three-electrode arrangement. The prepared hybrid material PGE/AgNPs/CS symmetric supercapacitor exhibited electrochemical performance, along with the rapid charge-discharge rate, high specific areal capacitance of 367.16 mF-cm^{-2} at 1 mA-cm^{-2} and exceptional stability of 1500 cyclic lifetime with capacitance retention of92.8%. Also, the PGE/AgNPs/CS provided remarkable electro-catalytic activity towards electrochemical detection of H_2O_2 with LOD of 0.52 μM as compared to other AgNPs based electrodes. The proposed sensor was used to detect H_2O_2 in cosmetic as well as medical samples with high accuracy and selectivity, making it an ideal choice in the development of disposable, low-cost device for hydrogen peroxide detection. The result obtained gave appreciable recovery value suggesting high sensitivity of the electrode towards H_2O_2.

CRediT authorship contribution statement

Mary Salve: Methodology, Data curation, Writing - review & editing. **Aurnab Mandal:** Methodology, Data curation. **Khairunnisa Amreen:** Conceptualization, Writing - review & editing. **Prasant Kumar Pattnaik:** Supervision, Writing - review & editing. **Sanket Goel:** Conceptualization, Investigation, Writing - review & editing.

Declaration of Competing Interest

Acknowledgment

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.microc.2020.104973.

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Miniaturized Platform with Nanocomposite Optimized Pencil Electrodes for Selective Non-Interfering Electrochemical Sensing

Mary Salve, Khairunnisa Amreen, Prasant Pattnaik, and Sanket Goel *Senior Member, IEEE*

Abstract— Herein, a simple, miniaturized and low-cost electrochemical sensing platform integrated with three-electrode system has been presented. A two-layer microfluidic device has been fabricated entirely on a PMMA sheet using a CO_2 laser. Pencil graphite electrode (PGE), chemically modified electrode with a nanocomposite of MWCNT@polysterene-chitosan (PGE/MWCNT@PS-CS) as a working electrode (WE), PGE coated with Ag/AgCl ink as a reference electrode (RE) and a plain PGE as a counter electrode (CE) were inserted into the microreactor with reagent consumption of 2 mL. As a proof of concept, the analytical performance of the developed microfluidic electrochemical sensor was investigated for non-enzymatic glucose sensing. Highly selective, non-interfering sensing of glucose in presence of various bioanalytes, was obtained. The nanocomposite lead to an exceptional limit of detection as 0.309 nM. With fine-tuning and electrode surface modification, the designed miniaturized platform could be used for diverse electrochemical biosensing application.

Index Terms— Miniaturized electrochemical platform, CO_2 laser, three electrodes, polystyrene, non-enzymatic.

I. INTRODUCTION

MICRO total-analytical system (µTAS) or microfluidic lab-on-chip (LOC) devices are of great importance in the fields of food engineering, medical science, forensic science, etc. [1]. The advantages of µTAS are portability, low sample volume, rapid analysis and reduced waste generation. For the fabrication of polymer-based microfluidic devices, poly(methyl methacrylate) (PMMA) polymer has become the preferred material for due to its advantages such as cost-effectiveness, rapid and bulk fabrication properties [2]. PMMA microfluidic devices are prepared using a variety of manufacturing techniques such as embossing, injection molding, computer numerical controlled (CNC) micromachining and laser micromachining [3]. CO_2 Laser micromachining offers several advantages in contrast to alternative fabrication approach such as extreme accuracy, rapid prototyping and cost-effectiveness. Herein, the CO_2 laser controls the relevant laser power to process a variety of complex microchannel design on a PMMA sheet, which can be adhesively bonded by another PMMA sheet. The quality, length, and width of the channel are distinctly affected by the laser power parameter [3].

Among different techniques utilized for detection in µTAS, electrochemical method stands out due to the unique characteristics, such as high limit-of-detection, specificity, minimal power requirement and environment friendliness [4]. For electrochemical detection, various electrode materials have been reported, however, carbon stands out due to its properties such as high chemical stability, low over potential and wide working potential range [5]. In the present context, pencil graphite electrode act as an attractive alternative as they are, inexpensive and easily available. The adequacy of pencil graphite electrode in electrochemical analysis and microfluidic platform has been reported [6]. The nanocomposites of polymer are prone to low-conductivity, however, with the usage of nanomaterial such as carbon, gold, silver enhances electrical conductivity and surface area appropriate for sensing [7].

In this work, the fabrication of microfluidic device for electrochemical biosensing has been reported. The platform consists of a microfluidic device prepared using PMMA sheet integrated with sensing electrodes, pencil graphite electrode (PGE) coated Ag/AgCl ink as a reference electrode (RE) and PGE as a counter electrode (CE). The sensing PGE electrode was coated with multiwalled carbon nanotube (MWCNT)-decorated with polystyrene beads (PS), using Chitosan (CS) for bonding, realizing a thin layer of composite for impeccable electrochemical activity. Subsequently, to check the applicability of the electrochemical sensing platform, non-enzymatic glucose detection has been demonstrated with an exceptional detection limit of 0.309 nM as a proof of concept. The modified electrode showed highly selective response towards glucose with no interference from other biochemicals. By changing the modification of the WE, the same platform can be easily utilized for a variety of sensing applications.

II. EXPERIMENTAL

A. Chemicals, Materials and Instrumentation

All reagents were used as received. Polystyrene (PS) (10 µm), D(+) glucose, Hydrochloric acid (HCl) 35%, Potassium chloride (KCl), Chitosan (CS), carboxylic functionalized multi-walled carbon nanotubes (COOH-MWCNT) was

The authors are with MEMS, Microfluidics and Nanoelectronics Laboratory, Department of Electrical and Electronics Engineering, Birla Institute of Technology and Science, Hyderabad 500078, India (e-mail:

sgoel@hyderabad.bits-pilani.ac.in)

obtained from Sigma (St. Louis, MO, USA). Ag/AgCl ink obtained from ALS Co. Ltd., Tokyo Japan. 0.1M HCl/KCl (pH =2) was used as a supporting electrolyte. The commercially available pencil lead (2 mm, 2B) obtained from Camlin and PMMA sheet (4mm) was obtained from local stationery. Electrochemical measurements were performed with an electrochemical analyzer (SP-150, Bio-Logic, electrochemical workstation (France)).

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Design of two dimensional (2D) microfluidic device was created in CorelDraw software and was transferred to the CO_2 laser software. A viable laser scriber (Universal Laser System, Inc., AZ, USA) was used for producing laser engraved microfluidic channel on a PMMA substrate (5 mm thickness). The CO_2 laser operates at a wavelength of 10.6 μm with a maximum power of 30 W. The microchannel with the desired dimension was engraved and cut by moving the CO_2 laser with optimized power and speed. The microchannel was bonded to other layers of PMMA sheet (1 mm) containing inlet and outlet for the fluid using a sacrificial layer of paraffin wax and heating at a constant temperature of 70 ℃.

Figure 1. Three electrode Microfluidics Platform

As shown in Figure 1, the device contained a 40 mm long feed microchannel (5 mm wide and 2.5 mm depth) on the base substrate. Three circular ports were drilled using CNC miller of diameter 2 mm for WE, CE and RE on the base substrate, and two circular inlets/outlets port were drilled on the top substrate. The total time required for the development of microfluidic platform was approximately 10 min.

C. Preparation of sensing electrode and electrochemical measurement

The PGE was wrapped with non-conductive parafilm except the bottom disc portion for the electrochemical studies. The exposed surface was then levelled by polishing it on Silicon Carbide Waterproof Abrasive Sandpaper Sheet. On the bare surface of PGE (2 mm) of length 15mm, MWCNT solution (2 μL) was drop casted twice followed by the polystyrene layer (2 μL) with the top layer of CS (2 μL) After each layer it was allowed to dry at room temperature for 30 minutes (Figure 1).

All electrochemical measurements were carried out in three-electrode based microfluidic platform. The electrochemical behavior of the PGE/MWCNT@PS-CS was investigated by cyclic voltammetry (CV) in a 2 ml solution of 0.1 M HCl /KCl at a scan rate of 50 mV/S. Further, it was evaluated as a glucose sensor in 1 mM at desired potential using square wave voltammetry (SWV) after filling mediator in the microchannel

III. Result and Discussion

A. Electrochemical Characterization

As shown in Figure 2(A) CV was performed using 0.1M HCl/KCl for electrochemical characterization of unmodified PGE, CS/PS/MWCNT modified PGE at a scan rate of 50 mV/S. No peaks were observed on bare PGE, after being modified with polystyrene-chitosan, PGE/MWCNT@PS-CS a well-defined redox peak was observed, demonstrating that polystyrene was immobilized on PGE. To study the mass transfer process of the working electrode (PGE/MWCNT@PS-CS), the relationship between the peak current and scan rate was investigated in the range of 10 -120 mV/S.

Figure 2. (A) CV response in 0.1M HCl/KCl (pH=2) solution at 50 mV/S. (B) Scan rate effect on CV response with a fixed potential of -0.2 V to 1 V of CS/PS/MWCNT modified PGE with inset of liner fit plot are respectively of ipa and ipc value v1/2

Figure 2(B) shows that anodic and the cathodic current are directly proportional to the scan-rate $\upsilon^{1/2}$, as per Randle-Sevick equation.

$$i_p = 2.69 \times 105A_e\, D^{1/2}\, n^{3/2}\, \upsilon^{1/2}\, C$$

where i_p is peak current, A_e is electrode surface area (cm^2), n is numbered of electron transferred, υ is scan rate (v/S), C is the concentration of electroactive species (mol m^{-3}) and D is diffusion coefficient equals to 1×10^{-4} cm^2/S. The inset of Figure 2(B) is a calibration plot which gives the slope value as 0.50 authenticating a diffusion controlled reaction.

B. Characterization of the Nanocomposite film

The general morphologies of PGE, MWCNT, PS, CS and the nanocomposite film was observed using SEM as shown in Figure 3 (A-C) and the polystyrene beads were of 8- 10 μm diameter (Figure 3(B)). The UV-VIS absorption was recorded, however, the result obtained shows the inability of this technique for characterizing this system (Figure 3(D)). The FTIR spectrum in Figure 3(E) shows the bands for MWCNT, PS, CS and the composite film. The Raman spectra of MWCNT and the composite film is shown in Figure 3(F). The characterization revealed the formation of MWCNT-PS-CS nanocomposite.

C. Detection of Glucose using the microfluidic devices

The possible oxidation mechanism for non-enzymatic glucose sensing can be ascribed as MWCNT/PS/CS get electrochemically oxidized and act as an electron delivery system and further glucose get oxidized to gluconic acid as shown in Figure 4 [8-9]. Figure 5(A) shows CV curve for 1 mM glucose in
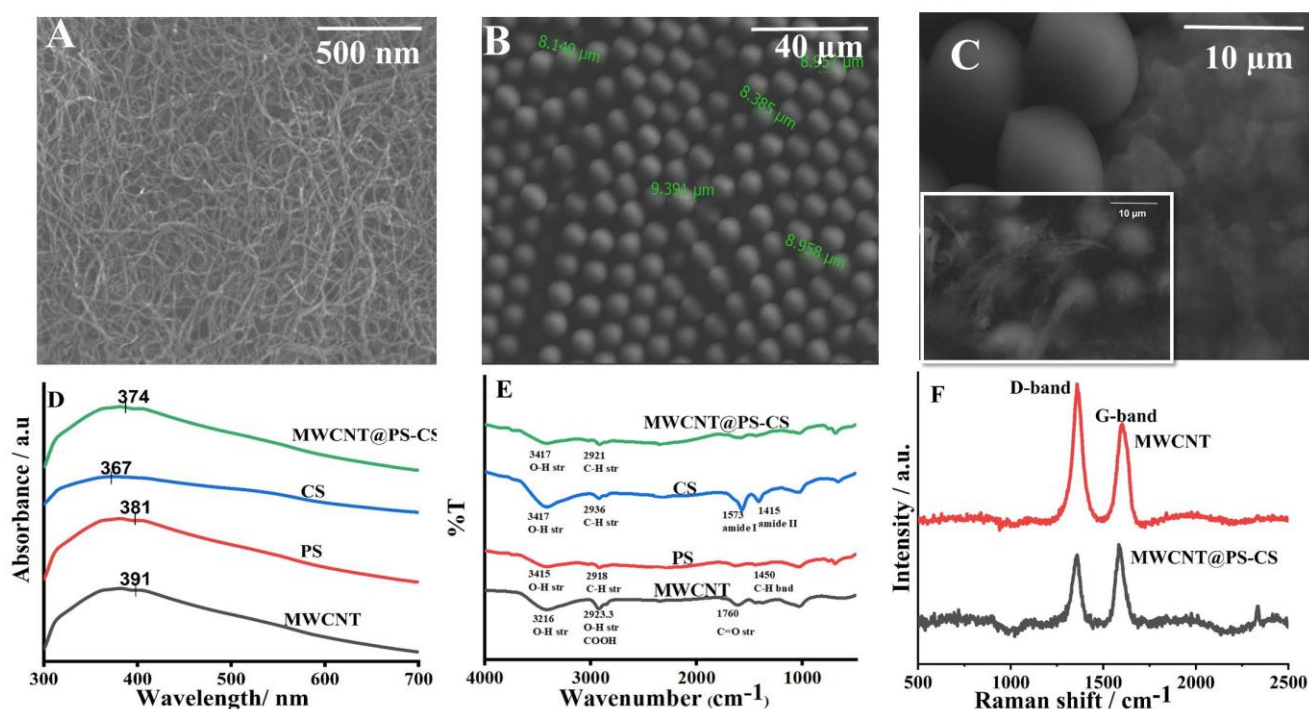
Figure 3. Characterization of modified PGE. (A-C) SEM images of MWCNT , PS and MWCNT/PS with inset of MWCNT/PS-CS (D) UV-VIS absorption spectra, (E) FTIR and (F) RAMAN of MWCNT , PS, CS, MWCNT@PS-CS

HCl/KCl (pH = 2) at a scan rate of 10 mV/S. The PGE/MWCNT@PS-CS exhibits significant oxidation current peak at 0.7 V vs Ag/AgCl corresponding to the glucose electrochemical oxidation [10].
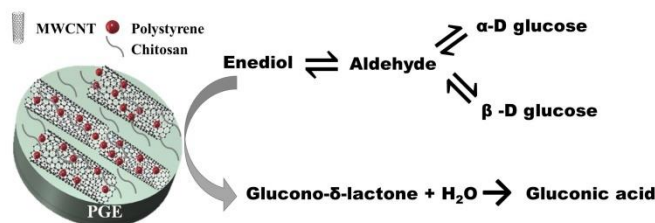

Figure 4. Schematic representation for enzymeless glucose sensing with PGE/MWCNT@PS-CS with possible reaction mechanism

Due to the high specific area of PGE/MWCNT@PS-CS, glucose penetrated through the MWCNT layer which leads to a higher sensitivity of the sensor. To evaluate the reproducibility of PGE/MWCNT@PS-CS for electro-oxidation of glucose, the experiment was carried out with three independently prepared sensing electrodes which gave a Relative Standard Deviation (RSD) of the peak current as 3.2% for 1 mM glucose.

The SWV of the sensing electrode with different concentrations of glucose (1– 25 nM/ L) was recorded (Figure 5(B)). A linear dependence of the catalytic current vs glucose concentration can be fitted on a linear equation I (μA) = 7.7525 [Glucose] (μA/μM) +0.41121 (μA) and R^2 = 0.980 with limit of detection (LOD) of 0.309 nM (Figure 5(B) inset) with a wide linear range of (1 nm - 25 nm). The LOD is compared with reported non-enzymatic glucose sensor based on PGE with gold

nanoparticle gives a LOD of 12 μM with copper nanoparticle gives a LOD of 0.44 μM [11, 12].

Figure 5. CV response in 0.1M HCl/KCl (pH=2) solution at 10 mV/S (A) PGE/MWCNT@PS-CS with and without Glucose and (B) Squarewave voltammetry (SWV) for effect of glucose concentration (inset) corresponding calibration plot.

Figure 6. Interference Study for Glucose detection on PGE/MWCNT@PS-CS with interference of biochemical (1 mM) concentration

Such microfluidic platform for the enzymeless sensing of glucose showed high stability and reproducibility. The sensing

electrode PGE/MWCNT@PS-CS was stored at room temperature and tested periodically. The SWV peaks for 1 μM glucose after 15 days maintained 93% of the initial current value.

D. Interference study

To ensure that the amenability of the platform for the interference of various biochemical, 1 mM each of uric acid, ascorbic acid, xanthine and hypoxanthine were successively added to the 1 mM glucose solution for SWV detection (Figure 6). Herein the biochemical with higher concentration were taken in comparison to concentration present in blood [12]. The PGE/MWCNT@PS-CS gives good specificity for glucose sensing with no interference.

E. Real Sample Analysis

The PGE/MWCNT@PS-CS sensor was tested to evaluate the practicability using the human serum of diabetic patients. The recovery of glucose was calculated using the standard addition approach spiking a known amount of glucose to human serum samples. The glucose concentration and recoveries were determined and summarized in Table 1, whereby the result shows that the present sensor gives the recovery in the range of 96 - 101%.

Table 1. Real Sample Analysis

Serum Sample	Added (nM)	Found (nM)	Recovery (%)
1	6	5.8	96
	12	12.11	100.9
	18	18.03	100.3

IV. CONCLUSION

Herein, a novel, cost-effective, miniaturized, electrochemical sensing platform integrated with three electrodes has been developed. The platform consisted of CO_2 laser engraved microchannel thermally bonded using a sacrificial layer of parafilm wax to the top layer for fluid inlet/outlet. The required three electrodes for electrochemical sensing were inserted in the device containing the microchannel. The analytical stability was determined using enzyme-free glucose sensing and were successfully quantified with lower limit of detection. The result obtained for the glucose in human serum sample was quite satisfactory with a relative error less than 5%. Overall, the developed miniaturized sensing platform can potentially be useful for the detection of various other analytes.

ACKNOWLEDGMENT

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Miniaturized Platform with Nanocomposite Optimized Pencil Electrodes for Selective Non-Interfering Electrochemical Sensing

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III. RESULT AND DISCUSSION

A. Electrochemical Characterization

As shown in Figure 2(A) CV was performed using 0.1M HCl/KCl for electrochemical characterization of unmodified PGE, CS/PS/MWCNT modified PGE at a scan rate of 50 mV/S. No peaks were observed on bare PGE, after being modified with polystyrene-chitosan, PGE/MWCNT@PS-CS a well-defined redox peak was observed, demonstrating that polystyrene was immobilized on PGE. To study the mass transfer process of the working electrode (PGE/MWCNT@PS-CS), the relationship between the peak current and scan rate was investigated in the range of 10 -120 mV/S.

Figure 2. (A) CV response in 0.1M HCl/KCl (pH=2) solution at 50 mV/S. (B) Scan rate effect on CV response with a fixed potential of -0.2 V to 1 V of CS/PS/MWCNT modified PGE with inset of liner fit plot are respectively of ipa and ipc value v1/2

Figure 2(B) shows that anodic and the cathodic current are directly proportional to the scan-rate $υ^{½}$, as per Randle-Sevick equation.

$$i_p = 2.69 \times 105 A_e \, D^{1/2} n^{3/2} υ^{1/2} \; C$$

where i_p is peak current, A_e is electrode surface area (cm^2), n is numbered of electron transferred, $υ$ is scan rate (v/S), C is the concentration of electroactive species (mol m^{-3}) and D is diffusion coefficient equals to 1×10^{-4} cm^2/S. The inset of Figure 2(B) is a calibration plot which gives the slope value as 0.50 authenticating a diffusion controlled reaction.

B. Characterization of the Nanocomposite film

The general morphologies of PGE, MWCNT, PS, CS and the nanocomposite film was observed using SEM as shown in Figure 3 (A-C) and the polystyrene beads were of 8- 10 μm diameter (Figure 3(B)). The UV-VIS absorption was recorded, however, the result obtained shows the inability of this technique for characterizing this system (Figure 3(D)). The FTIR spectrum in Figure 3(E) shows the bands for MWCNT, PS, CS and the composite film. The Raman spectra of MWCNT and the composite film is shown in Figure 3(F). The characterization revealed the formation of MWCNT-PS-CS nanocomposite.

C. Detection of Glucose using the microfluidic devices

The possible oxidation mechanism for non-enzymatic glucose sensing can be ascribed as MWCNT/PS/CS get electrochemically oxidized and act as an electron delivery system and further glucose get oxidized to gluconic acid as shown in Figure 4 [8-9]. Figure 5(A) shows CV curve for 1 mM glucose in
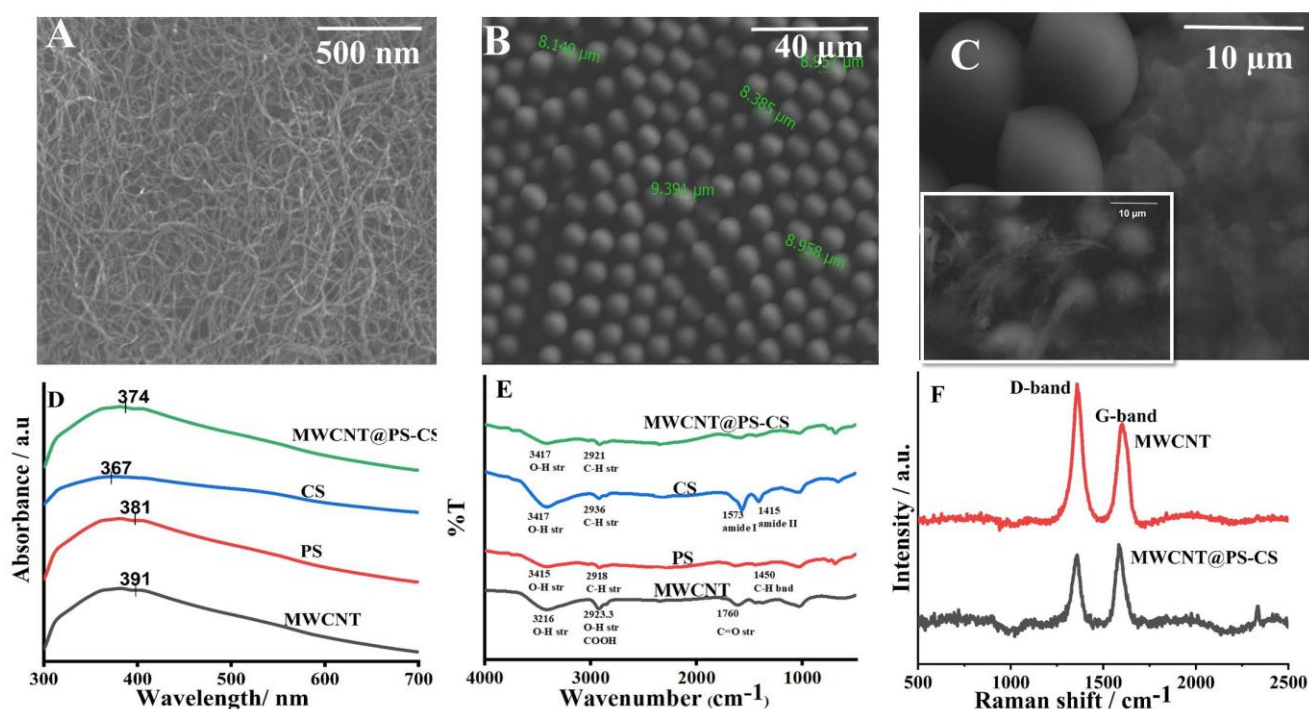
Figure 3. Characterization of modified PGE. (A-C) SEM images of MWCNT , PS and MWCNT/PS with inset of MWCNT/PS-CS (D) UV-VIS absorption spectra, (E) FTIR and (F) RAMAN of MWCNT , PS, CS, MWCNT@PS-CS

HCl/KCl (pH = 2) at a scan rate of 10 mV/S. The PGE/MWCNT@PS-CS exhibits significant oxidation current peak at 0.7 V vs Ag/AgCl corresponding to the glucose electrochemical oxidation [10].
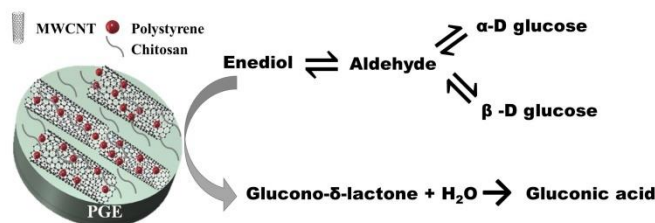

Figure 4. Schematic representation for enzymeless glucose sensing with PGE/MWCNT@PS-CS with possible reaction mechanism

Due to the high specific area of PGE/MWCNT@PS-CS, glucose penetrated through the MWCNT layer which leads to a higher sensitivity of the sensor. To evaluate the reproducibility of PGE/MWCNT@PS-CS for electro-oxidation of glucose, the experiment was carried out with three independently prepared sensing electrodes which gave a Relative Standard Deviation (RSD) of the peak current as 3.2% for 1 mM glucose.

The SWV of the sensing electrode with different concentrations of glucose (1– 25 nM/ L) was recorded (Figure 5(B)). A linear dependence of the catalytic current vs glucose concentration can be fitted on a linear equation I (μA) = 7.7525 [Glucose] (μA/μM) +0.41121 (μA) and R^2 = 0.980 with limit of detection (LOD) of 0.309 nM (Figure 5(B) inset) with a wide linear range of (1 nm - 25 nm). The LOD is compared with reported non-enzymatic glucose sensor based on PGE with gold

nanoparticle gives a LOD of 12 μM with copper nanoparticle gives a LOD of 0.44 μM [11, 12].

Figure 5. CV response in 0.1M HCl/KCl (pH=2) solution at 10 mV/S (A) PGE/MWCNT@PS-CS with and without Glucose and (B) Squarewave voltammetry (SWV) for effect of glucose concentration (inset) corresponding calibration plot.

Figure 6. Interference Study for Glucose detection on PGE/MWCNT@PS-CS with interference of biochemical (1 mM) concentration

Such microfluidic platform for the enzymeless sensing of glucose showed high stability and reproducibility. The sensing

electrode PGE/MWCNT@PS-CS was stored at room temperature and tested periodically. The SWV peaks for 1μM glucose after 15 days maintained 93% of the initial current value.

D. Interference study

To ensure that the amenability of the platform for the interference of various biochemical, 1 mM each of uric acid, ascorbic acid, xanthine and hypoxanthine were successively added to the 1 mM glucose solution for SWV detection (Figure 6). Herein the biochemical with higher concentration were taken in comparison to concentration present in blood [12]. The PGE/MWCNT@PS-CS gives good specificity for glucose sensing with no interference.

E. Real Sample Analysis

The PGE/MWCNT@PS-CS sensor was tested to evaluate the practicability using the human serum of diabetic patients. The recovery of glucose was calculated using the standard addition approach spiking a known amount of glucose to human serum samples. The glucose concentration and recoveries were determined and summarized in Table 1, whereby the result shows that the present sensor gives the recovery in the range of 96 - 101%.

Table 1. Real Sample Analysis

Serum Sample	Added (nM)	Found (nM)	Recovery (%)
1	6	5.8	96
	12	12.11	100.9
	18	18.03	100.3

IV. CONCLUSION

Herein, a novel, cost-effective, miniaturized, electrochemical sensing platform integrated with three electrodes has been developed. The platform consisted of CO_2 laser engraved microchannel thermally bonded using a sacrificial layer of parafilm wax to the top layer for fluid inlet/outlet. The required three electrodes for electrochemical sensing were inserted in the device containing the microchannel. The analytical stability was determined using enzyme-free glucose sensing and were successfully quantified with lower limit of detection. The result obtained for the glucose in human serum sample was quite satisfactory with a relative error less than 5%. Overall, the developed miniaturized sensing platform can potentially be useful for the detection of various other analytes.

ACKNOWLEDGMENT

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Electromicrofluidic Device on Multilayered Laser-Induced Polyamide Substrate for Diverse Electrochemical Applications

Avinash Kothuru, Khairunnisa Amreen, and Sanket Goel, *Senior Member, IEEE*

Abstract—**Microfluidic devices with integrated electrodes, called electromicrofluidic (EMF) device, have been reported for multiple applications. In this work, a unique approach to realizing a multilayered EMF device, with microchannel and integrated electrodes on the same polyamide (PI) substrate, has been presented. A computer-controlled CO_2 laser ablation method, with varying speeds and power values, was employed to form laser-induced graphene (LIG) on a PI substrate as per the desired design. Initially, to create a microchannel layer, the formed LIG was peeled off which left behind an etched pattern. Subsequently, to realize an electrode layer, the PI substrate with microchannel was further ablated to create patterned LIG. An optimal flow rate of 100 μL/min via a controlled syringe pump was established in EMF device. As a prototype, the developed platform, with microchannel and electrodes, was explored for variable electrochemical applications. First, the electrochemical sensing of uric acid displayed a limit of detection (LOD) as 0.61 μM in a linear range from 10 μM to 3 mM with significant recovery values. Furthermore, the polarization performance for fuel cell application was evaluated on the developed EMF platform using the chronoamperometry (CA) method with a stable open-circuit potential (OCP), harnessing the maximum power density obtained was 3.027 nW/cm^2. Finally, an array of interdigitated microelectrodes (IDEs) was realized to examine impedance-based nitrite detection. Overall, the presented multilayered EMF devices with microchannel and electrodes on the single sheet authenticate the applicability of the designed platform for a variety of sensing and energy harvesting applications.**

Index Terms—**CO_2 laser, electrochemical sensing, interdigitated microelectrodes (IDEs), laser-induced graphene (LIG), microfluidic channel, uric acid (UA).**

I. INTRODUCTION

REALIZATION of microfluidics-based platforms has gained massive attention due to umpteen number of

advantages, such as modularity, amenability with various sensing methods, robustness, possibility of multiplexed detection, and cost-effectiveness [1], [2]. Generally, these microfluidic devices can be designed to control the fluids in microliters to picoliters leading to the shortening of the time of the experiment and reducing the cost of the application. Overall, accurate control, behavior, and manipulation of fluids, at micro- and nanoscale can be accomplished by utilizing such devices.

One of the unique advantages of microfluidic devices is their adaptability and compatibility to be harnessed for diverse sensing applications based on various factors such as sensing mechanism of the sample, detection-limit, place of use (laboratory-based or point-of-use), cost, material properties, and so on. Out of these factors, the choice of sensing mechanism, like amperometric, colorimetric, electrical, electrochemical, luminescence, optical, and so on, plays a crucial role as it manifests the basic property of the sample, and directly or indirectly governs other aforementioned factors as well [3]. Microfluidic devices utilized for several sensing approaches, with electrical readout, have the inherent requirement of integrated electrodes along with microchannel(s) and are named as EMF devices [4]. Such devices are gaining importance to realize a genuinely point-of-care platform due to their added benefits such as excellent detection limit, smaller size, and the possibility to develop integrated data capturing and analysis system.

The EMF devices have a huge scope of improvement, particularly on realizing a reproducible and straightforward fabrication method to integrate microchannel(s) and electrodes, and optimization of the electrodes to enhance the sensing parameters [5]–[7]. Enormous work has been reported on both aspects to realize microfluidic devices for the electrochemical three-electrode system, interdigitated electrodes [6], biofuel cells [8], and impedimetric system [9]. However, in most of the cases, complicated fabrication approaches, such as photolithography, soft-lithography, paper-based, and injection-molding [10]–[12], have been reported. In such procedures, either the microchannel and electrode layers are fabricated separately and integrated, or the fabrication is accomplished using several layers of tedious processes. Recently, some work has been reported whereby a benchtop dual-extruder 3-D printer has been used to create an EMF device [13], [14]. However, such devices have limited applications owing to the low sensing output due to poor conductivity of the electrode zones, which is tedious to be enhanced due to the critical
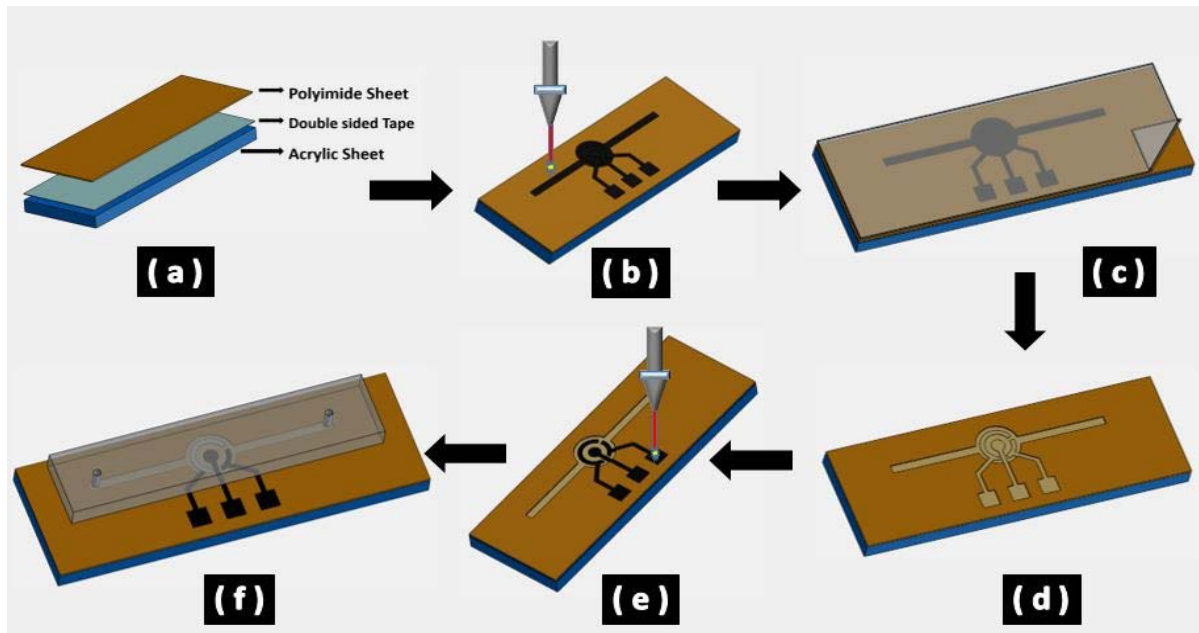
Schematic. 1.   (a) Bonding PI and (b) acrylic with tape. (c) Ablating laser on PI sheet. (d) Peeling graphene from PI sheet using tape. (e) Traces of formed graphene on PI sheet with depth after peeling. Ablating three electrodes of LIG on PI. (f) Microfluidic channel enclosed with PDMS.

thermal and mechanical requirements of the filament of the 3-D printers.

The integration of the microfluidic device with a nanomaterial-based electrode further increases its applicability as sensing and energy harvesting platforms [15]. Recently, nanomaterials based on carbon allotropes captured a great interest among researchers from chemistry, physics, and many engineering domains [16]. Graphene is a carbon allotrope, which is usually in the form of a single layer of atoms in a 2-D hexagonal lattice whereby each vertex is formed by one atom. Laser-assisted techniques for the formation of graphene/graphene oxide have been identified as an effective tool in multiple applications from the manufacturing of materials in various biochemical, biomedical, energy, and surgical pathology [17]. Recent literature reports use of polyamide (PI) sheet as a base material to generate graphene known as laser-induced graphene (LIG) [18].

The utilization of PI sheets has been increasing rapidly due to their amenability to create conductive zones of graphene using the CO_2 laser [18]–[20]. Such method has been further used for supercapacitors, biosensing, electro-mechanical sensing, and microfluidics applications [21]. In 2020, Kothuru *et al.* [22] reported that the formed LIG was a graphene oxide, characterized by Raman spectroscopy and X-ray photoelectron spectroscopy (XPS), and shown its application in resistive, capacitive, electrochemical sensing [22]. In the LIG process, by varying the power and speed of the laser system, optimized graphene, both in terms of several layers and level of defects, can be obtained [23].

In this work, a unique method has been used to create the EMF device, with microfluidic channel and electrodes on different layers of the same PI sheet by harnessing the optimized speed and power of the laser. The EMF device

TABLE I
COST ESTIMATION FOR MICROFLUIDIC CHANNEL

Material	Cost ($)
Polyamide sheet (10mil)	0.07
PDMS	0.14
Silver ink (69.82$ / 250gm)	3.58

was enclosed with polydimethylsiloxane (PDMS) and can be obtained in three steps in 10 min for less than US$4.

Using a CO_2 laser machine, various numbers, shapes, and sizes of the electrode system based on LIG have been designed over the engraved microchannel. For electrochemical sensing, a layer of three-electrodes was realized where one of the electrodes was coated with Ag/AgCl paste to form a reference electrode [24]. To enclose the device, a PDMS layer was bonded on the PI sheet, and a continuous flow of an electrolyte, phosphate buffer solution (PBS) of neutral pH was maintained using an external pump. In such an electrochemical sensing system, electrochemical detection of uric acid (UA), a physiological and metabolic by-product, has been demonstrated. In addition to this, an EMF platform, with microchannel and two integrated electrodes (cathode and anode), was realized for fuel cell application. Finally, another EMF platform with microchannel and interdigitated microelectrodes (IDEs), (with ten pairs) was created. Such a platform, bonded with an acrylic sheet, has led to a relative increase in the sensing area, maximizing reaction sites enabling better sensitivity [25] and was tested for nitrite sensing.

Overall, a simple microfluidic sensing platform based on LIG, integrated with electrode system, without any surface

TABLE II
COMPARISON RESULTS FOR THE MICROFLUIDIC CHANNEL FOR SENSING UA

Reference	Sensing	Materials	Fabrication/Process	Sample	Detection Limit
[29]	Uric Acid	Glass, PDMS	0.07	Human Urine	8 μM
[26]	Uric Acid	PET, Polyamide film (75μM), Screen Printed Electrode	0.14	Sweat	0.74 μM
This paper	Uric Acid	Acrylic Sheet, PDMS, Polyamide Sheet (10 mil)	3.58	Urine	0.6 μM

modifications, has been fabricated. Using significantly low sample volume, such EMF devices can be utilized for real-time analysis enabling the point of care capability.

II. EXPERIMENTAL SECTION

A. Materials and Apparatus

The PI sheet of 10 mil (25.4 μm) thickness was procured from Dali Electronics, India, and a CO_2 laser (VLS 3.60 from Universal Laser System) was used to obtain graphene on top of the PI sheet. UA (99% crystalline), sodium nitrite ($NaNO_2$), formic acid (HCOOH), and sulfuric acid (H_2SO_4) were obtained from Sigma Aldrich. Electrochemical experiments were carried out using Origaflex potentiostat. A syringe pump was acquired from the Holmarc Pvt. Ltd. for the continuous flow of the fluids. All the other necessary chemicals used for the electrolyte were of analytical grade. Mitutoyo SJ-210 surface profilometer instrument, which is used to find the depth of LIG microchannel.

B. Design and Fabrication of Microfluidic Channel

Herein, a microfluidic device was designed in CAD software and converted into .dxf format. The converted file was then opened in CorelDraw, and the alignment of the microchannel was carried out according to the settings of the CO_2 laser machine. An acrylic sheet of 76 mm × 26 mm dimension (similar to a microscope slide) was considered for the design layout. Then, the PI sheet and the acrylic were bonded using double-sided tape, as shown in Schematic 1(a). Once the bonding was done, the PI sheet was mounted in a laser machine, and by varying speed and power of the laser machine, graphene was formed as per the design shown in Schematic 1(b). The obtained graphene material was peeled off using a cello tape from the PI sheet, as shown in Schematic 1(c). As shown in Schematic 1(d), during the process of peeling off graphene from the PI sheet, left the traces of the design with a certain depth. Furthermore, the laser was ablated so that the microchannel design was formed again in the same place with graphene material on top of the PI sheet. The process of peeling off the graphene was repeated using the tape. This process was repeated for few times until the desired depth was obtained. Using Mitutoyo SJ-210, the depth obtained for the present microfluidic channel was approximately 84 μm. As shown in Schematic 1(e), only the places where electrodes were required (like three electrodes) were laser ablated by aligning the design in the CorelDraw on PI sheet.

For the fabrication of the cover of the microfluidic device, PDMS-based Sylgard 184 Silicone Elastomer kit was used by mixing the polymer base (Part A) and curing agent (Part B) in 10:1 proportionate. Subsequently, it was kept in a hot air oven at 70 °C for 30 min to harden up the material. The obtained PDMS layer was bonded with the microfluidic device on the PI sheet by plasma treatment of the surface for 5 min and again keeping it in a hot air oven at 50 °C for few minutes to make the best possible bonding. Schematic 2 shows the complete prototype of the LIG-based microfluidic device with electrodes for electrochemical sensing (Supplementary Video). Table I depicts the material cost estimation for the development of the LIG-based EMF device with a microfluidic channel and integrated electrodes.

C. Electrochemical Sensing

In 2019, Yang *et al.* [26] reported a simple fabrication method to make a microfluidic channel for UA sensing, where a three-electrode system over PI film was formed with laser ablation, and one of the electrodes was coated with the silver paste. To make a microfluidic channel, a polyethylene terephthalate (PET) was cut through in the shape of a microchannel for the flow of fluid. PI film and PET sheets were bonded with an adhesive layer [26].

In this work, the PI microfluidic device with LIG ablated microchannel and three-electrodes was utilized for UA sensing. Many studies have been reported for UA sensing since their concentration levels can be a biomarker for broad-scale diseases and disorders such as gout and renal disorders [17]. Although several researchers reported the UA detection technique using various complicated modifications requiring bulk volumes for UA detection, negligible studies are stated for the fabrication of compact and miniaturized microfluidic platforms. The fabricated platform here provided an excellent electrocatalytic activity toward the oxidation of UA at $E_0 = 0.35$ V. Furthermore, the effect of the scan rate revealed it being a surface-confined reaction. For exploring the practical application, UA detection in real urine samples has been demonstrated. One millimolar of UA solution was prepared in 10 mL of 0.1 M PBS. To perform UA detection, cyclic voltammetry (CV) was executed in the potential window from −1 to +1 V at a scan rate of 10 mV/s. Ag/AgCl-coated LIG was used as a reference electrode and the other two bare LIG were used as counter and working electrode. Table II shows the existing literature for microfluidic electrochemical sensing platforms for UA sensing, which clearly manifests that the current device outperforms the report ones.

D. Fuel Cell

In 2019, Rao *et al.* [27] reported a fabrication method to create a microfluidic paper-based fuel cell using various grades of cellulose letter as absorbent pads. Herein, formic acid acted as a fuel and the sulfuric acid acted as an electrolyte, and atmospheric air acted as an oxidant [27]. On the same lines, in this work, LIG-based EMF devices on PI sheet, with microchannel and two-layers of electrodes, each working as cathode and anode, were used as a fuel cell. Initially, the open circuit potential (OCP) was observed. Subsequently, the polarization performance of the fuel cell was evaluated using chronoamperometry (CA) method with stable OCP in steps from maximum to zero. All chemical concentrations were prepared by deionized (DI) water with a resistivity of 18.2 MΩ at room temperature. 0.5 M of formic acid and 0.5 M of sulfuric acid was used as a fuel and electrolyte, respectively, and oxygen acted as an oxidant in the fuel cell. For the electrochemical reaction, two reagents were continuously fed into the EMF-based fuel cell with the help of syringe pumps at a flow rate of 100 μL/min.

E. Interdigitated Microelectrodes

In 2019, Han *et al.* [28] reported a fabrication method using a CO_2 laser to form the interdigitated electrodes on the PI film, where the formed electrodes (six pairs) on the PI sheet were peeled off using a Kapton tape [28]. Then, the peeled off interdigitated electrodes were used to monitor sulfate between 1 to 10 000 ppm in the frequency swept from 10 to 4000 Hz. In this work, a 25-mg solution of sodium nitrite was prepared in 250 mL of DI water to make 100 parts per million (ppm) sodium nitrite. By following the serial dilution method, the sodium nitrite solutions 10, 20, and 30 ppm were made for detecting nitrite.

III. RESULTS AND DISCUSSION

A. Sensing of UA

1) Electrocatalytic Oxidation of UA: The electrocatalytic oxidation of UA with the fabricated platform was analyzed within the potential window of -1 to $+1$ V in a continuous flow of 10 μM UA in pH 7 PBS. CV was performed at 10 mV/s for two cycles. Fig. 1(a) shows that the designed platform gave highly sensitive oxidation of UA at an over potential 0.3 V versus Ag/AgCl. Fig. 1(b) shows the reproducibility of the platform wherein, two different fabricated platforms with the same parameters gave an equivalent response of UA oxidation with nil current difference. Furthermore, Fig. 1(c) shows the plausible oxidation mechanism of UA over the modified platform. As per the reported literature, electrocatalytic oxidation occurs via two electrons and two protons mechanism [29], [30].

2) Effect of Concentration: The effect of increasing UA concentration was examined at a scan rate of 10 mV/s versus Ag/AgCl within a linear range of 10 μM to 3 mM [Fig. 2(a) and (b)]. A systematic growth in the anodic peak current with the increase in concentration was observed in two different concentration ranges (low concentration range:
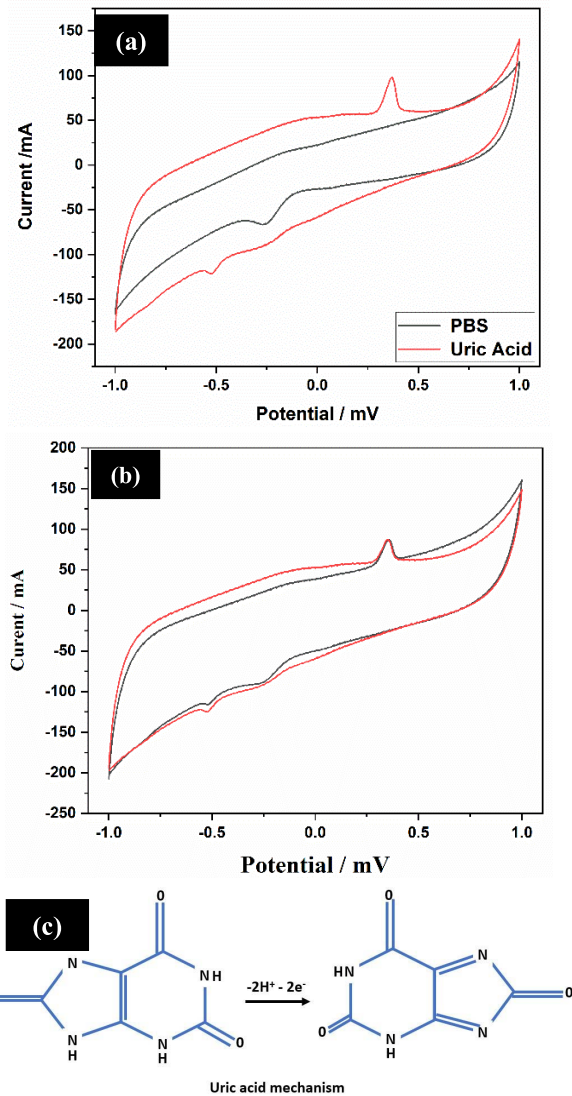
Fig. 1. (a) Comparative CV response with and without UA in pH PBS at 10 mV/s. (b) CV for reproducibility with two different platforms. (c) UA plausible oxidation mechanism.

10–90 μM, high concentration range: 100 μM to 3 mM). The corresponding baseline-corrected calibration plots [see Fig. 2(c) and (d)] portray a linearity within a limit of detection (LOD) of 0.61 μM.

3) Effect of Varying Scan Rate: For comprehending the electron-transfer mechanism, the effect of various scan rate over UA electrochemical oxidation was also studied. From Fig. 3(a), it was discovered that there was a well-ordered growth of anodic peak current as the scan rate was increased. Fig. 3(b) shows the baseline-corrected peak current calibration plot versus v1/2 wherein, the surface-confined electrode mechanism was observed as per Randle–Sevcik equation

$$i_{\mathrm{pa}} = 2.69 \times 105 A_e D^{1/2} n^{3/2} v^{1/2} C \tag{1}$$

herein, i_{pa} is the peak current, A is the electrode surface area 0.7 mm^2, C is the concentration of UA, v is the scan rate, and na is the number of electron involved. From the plot, the slope values were obtained. For UA, the calculated diffusion coefficient (D) was calculated as 2.239×10^{-3} cm^2/s.
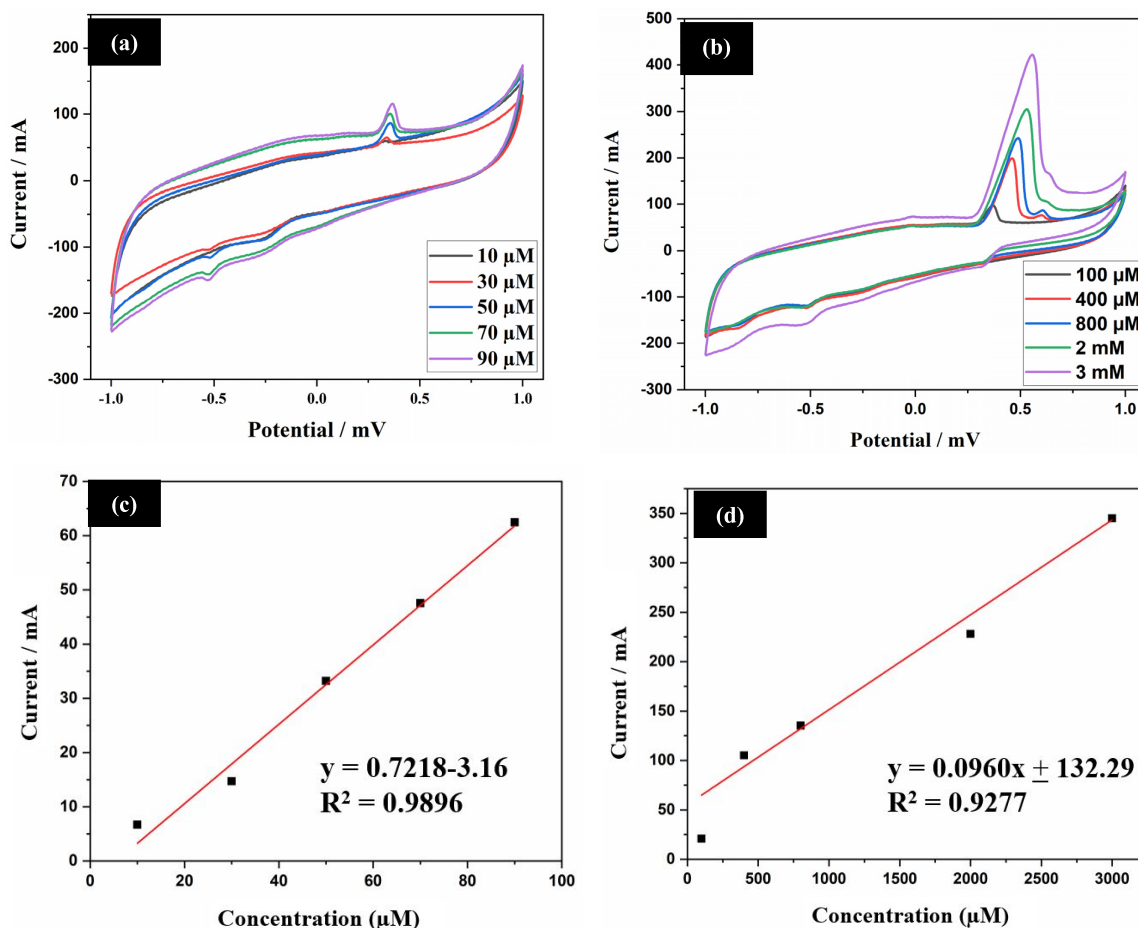
Fig. 2.   (a) CV response of UA of 10–90 μM. (b) CV response of UA of 100 μM to 3 mM. (c) and (d) Baseline corrected calibrated plots of UA for i_{pa} versus concentration.

TABLE III
REAL SAMPLE ANALYSIS OF URINE THROUGH A STANDARD APPROACH

Detected	UA Added(μM)	Found in sample(μM)	Recovery(%)
6	10	16.3	101.875
6	20	27.1	104.25
6	30	35.8	99.44

4) Real Sample Analysis: To study the practicality of the platform in nonideal real-time analysis, 1 mL of real urine sample was tested via standard addition approach. For the preparation of the real sample, 1 mL of pH 7 PBS was mixed with 1 mL of urine sample. Table III represents the real sample analysis of urine with an impressive recovery values.

B. Fuel Cell

A fuel cell was designed and fabricated with individual electrodes after successful electrochemical characterization. Using the PI sheet, the LIG-based microfluidic fuel cell with Y-shaped microchannel for the fuel cell. Here, the anolyte (formic acid) and catholyte (sulfuric acid) were streamed continuously using the syringe pumps.

For polarization, the cathode was connected to the working terminal, while the anode was connected to the reference and counter terminals of the potentiostat. Fig. 4(a) shows the schematic of LIG-based microfluidic fuel cell. The characterizations were carried out on the basis of maximum current and power density with the measured OCP in volts. Fig. 4(b) shows the polarization performance, the maximum power density and current were detected to be 3.027 nW/cm^2 and 1185 nA/cm^2.

C. Interdigitated Microelectrodes

During interdigitations, the sensing region is comparatively increased and the reaction sites are maximized for greater sensitivity [31]. This also makes IDEs suitable for applications even with low power. In this work, the IDEs was developed in the two-electrode configuration with an electrochemical sensor. Fig. 5(a) shows the interdigitated electrodes with dimensions of 10 mm $\times$ 4 mm. The electrode-to-electrode spacing was maintained to be 0.2 mm and the electrode width was 0.3 mm. The LIG-based IDEs were used for electrochemical detection of nitrite. The results from electrochemical impedance spectroscopy (EIS) were achieved using the developed microfluidic channel. Fig. 5(b) shows
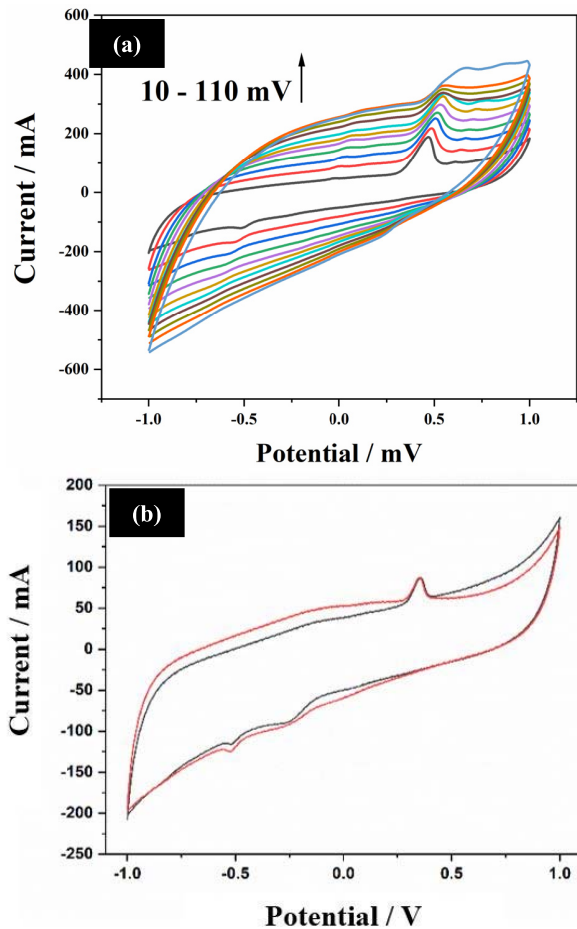
Fig. 3. (a) CV response of UA at various scan rates. (b) Baseline corrected calibrated plots for i_{pa} versus $v^{1/2}$.
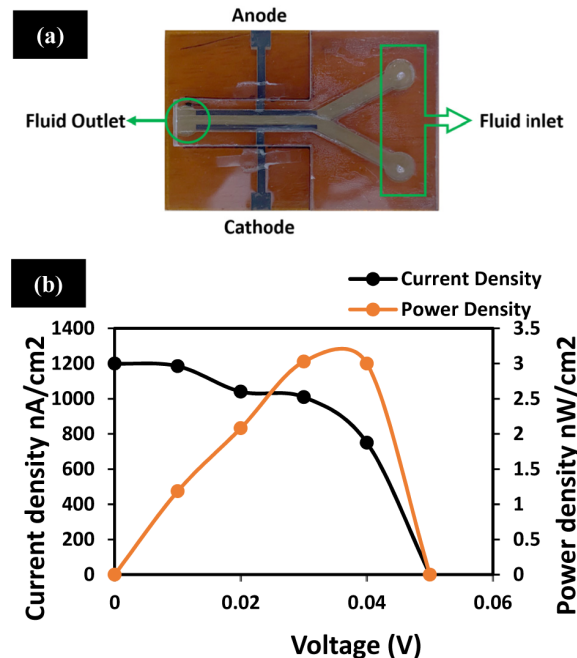
Fig. 4. (a) Schematic of LIG-based microfluidic-based fuel cell. (b) Polarization performance plot.

the result for 10–30 ppm of sodium nitrite and reveals that the resistance decreases with the increase in the concentration increase in the low-frequency region. The graphs
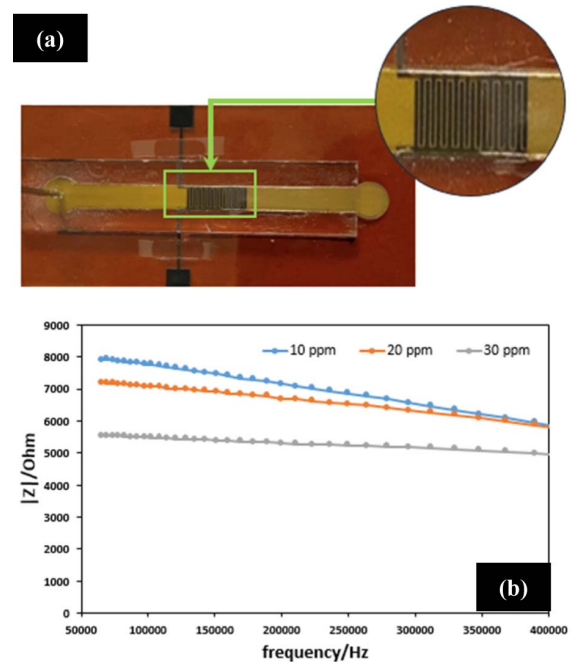
Fig. 5. (a) Schematic of LIG-based IDEs. (b) EIS result for the detection of nitrite.

were plotted between frequency and $|z|$/ohm using the EIS technique.

IV. CONCLUSION

Herein, a unique CO_2 LIG-based multilayered EMF device with microchannel and integrated electrodes has been developed. The EMF device was fabricated using a multilayered CO_2 laser and the device was bonded with PDMS with an inlet and outlet. The fabrication process has been harnessed to realize different EMF devices for variable applications like electrochemical sensing, fuel cell, and impedance-based IDEs. The electrochemical sensing of UA was carried out which provided a lower LOD of 0.61 μM. The maximum power density obtained by LIG-based microfluidic fuel cell was 3.027 nW/cm^2. The fabricated LIG IDEs also showed convincing results for different proportionate of nitrite. Overall, such a microfluidic device, with a simple, robust, and reproducible fabrication method to achieve smooth microchannel and highly conductive electrodes has strong potential to be utilized for a variety of sensing and energy harvesting applications.

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ACCEPTED MANUSCRIPT

IoT Enabled Portable Thermal Management System with Microfluidic Platform to Synthesize MnO$_2$ Nanoparticles for Electrochemical Sensing

IoT Enabled Portable Thermal Management System with Microfluidic Platform to Synthesize MnO₂ Nanoparticles for Electrochemical Sensing

Madhusudan B Kulkarni, Yashas, Prasanth K. Enaganti, Khairunnisa Amreen and Sanket Goel

MEMS, Microfluidics and Nanoelectronics Lab, Department of Electrical and Electronics Engineering, Birla Institute of Technology and Sciences (BITS), Pilani, Hyderabad Campus, 500078, Telangana, India

E-mail: sgoel@hyderabad.bits-pilani.ac.in; sanketgoel@gmail.com;

Abstract

Evidently, microfluidic devices are proven to be one of the most effective and powerful tools for manipulating, preparing, functionalizing, and producing new generation nanoparticles and nanocomposites. Their benefits include low solution/sample feeding, excellent handling of reagents, exceptional control of size and composition, compactness, easy to process with rapid thermal management, and cost-effectiveness. Such advantages have led to endorse nano-microscale fabrication methods to develop highly controllable and reproducible minuscule devices. This work aims to design and develop a microscale-based temperature controller device with added features like low-cost, portability, miniaturized, easy-to-use, minuscule reaction volume, and point-of-source system for the synthesis of nanoparticles. The device incorporates many features such as real-time data access with a GUI interface with a smartphone open-source app for Bluetooth and Database cloud for IoT module and the portable thermal device is calibrated and capable of achieving a maximum temperature of 250°C in 25 minutes. The fabricated device is harnessed for the synthesis of manganese oxide (MnO₂) nanoparticles. The synthesized nanoparticles were subjected to various characterization techniques like SEM and XPS to analyze the surface morphology. To test the applicability, as a proof of concept, the synthesized nanoparticles were tested for electrochemical sensing of hydrogen peroxide and dopamine. Overall, the portable device can be utilized for carrying out diverse temperature-controlled reactions in a microfluidic system in a user-friendly and automated manner.

1. INTRODUCTION

Nanoparticles (NPs) have drawn intense attention and have been utilized in many applications from energy to agriculture [1][2]. In recent times, Manganese dioxide (MnO₂) nanoparticles with heterogeneous crystalline morphological structure lead to unique advantages due to their unique chemical and physical properties. Also, the MnO₂ nanoparticles have numerous applications in batteries [3], water treatment, catalysis [4], biosensors [5], imaging contrast agents [6]. To have more efficient and effective performance, the principle parameters of MnO₂ particles depend on size, shape, inner structure, and surface characteristics, while remaining on environmental benignity, high theoretical capacity, and low cost [7]. So far, MnO₂ nanoparticles with distinct morphologies such as nano-tube [8], nano-rod, nano-flakes [9], nano-sphere, urchin-like [10], nano-flower, nano-wire, nanocube [11] have been synthesized, analyzed and used specially for electrochemical applications. For instance, Rutz et al. prepared and synthesized MnO₂ crystals with the biomineralization approach used for investigating the antimicrobial effects [12]. It is known that sizes, morphologies, and phases of MnO₂ nanoparticles have a great impact on properties and applications. Several effective and novel routes have been deployed to prepare and synthesize the MnO₂ nanoparticles with different shapes and exceptional properties such as sol-gel synthesis [13], precursor technique [9], pulsed laser deposition method, wet chemical route [14] and hydrothermal method [15].

The conventional based synthesis of the nanoparticle method suffers delayed response and uncontrolled time for mixing. Moreover, conventional processing requires preparation of

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samples in bulk leading to the enormous requirement of the precious and costly materials, further tends to render the process unprofitable for smaller volume. Table I summarizes the comparison between the hydrothermal conventional method and the proposed portable thermal management system for the synthesis of the nanoparticles.

TABLE I. Comparison of conventional method and proposed method for synthesis of nanoparticles

Parameters	Conventional method	Proposed method	References
Thermal aspects	High pressure and reaction temperature	Low-temperature requirement depending on microchannel	[16]
Processing time	More time required for NPs synthesis	NPs synthesis can be achieved in lesser time	[17]
Space Occupancy	Bulky Instrument, Laborious	Portable device, Mobile NPs synthesis	[18]
Volume	Higher volume	Minuscule volume	[19]
Reaction chamber	Usage of Autoclave and tedious heating chamber	Microchannel reservoir with µl to few ml usage	[20]

Microfluidics allows for controlled sample transfer on the microscale volume, and this technology has recently emerged as a valuable mechanism in the field of synthesis of nanoparticles [21]. Microfluidic device has notable and impressive results with a smaller volume of nanoparticle synthesization and find applications in various areas and sub-areas such as screening of drugs, diagnostics of various diseases, drug delivery, electrochemical and biomedical imaging, tissue engineering, and droplet-based approach for biosensing [22], [23].

The temperature controller is very essential due to its growing applications in a household appliance for heating and cooling, industrial automation, process control stations, and biomedical applications [24]. PID controller is more effective and consistent in maintaining the desired inner temperature despite rapid changes in outer atmospheric temperature, providing accuracy and reliability, that of conventional on/off controller [25]. Arduino based printed circuit board was designed on a double-sided platform, components were mounted both the sides that are compatible with hardware and software [26]. In the microfluidic platform, thermal management is imperative and enables for integrated cooling of electronic devices [27]. The portable management system with certain modifications can be used as a miniaturized mobile thermocycler for nucleic acid amplification tests which can be further used to detect pathogens and virulent [28], [29] and to determine specific heat many automated low-cost calorimeter devices have been

developed [30]. Silver nano prisms generated at room temperature by a highly reproducible and rapid synthesis [31].

In the present work, an automated, portable, easy-to-use, and inexpensive integrated device has been realized with real-time data logging thermal management system. Further, the manganese oxide (MnO_2) nanoparticles have been synthesized in a microfluidic platform with the integrated and automated thermal management system. Also, the MWCNT-MnO_2 composite was prepared using a simple sonication method. Various characterization techniques, like Field Emission Scanning Electron Microscope (FESEM), X-Ray Photoelectron Spectroscopy (XPS), and Energy-dispersive X-ray spectroscopy (EDX), were performed to study the morphology, elemental composition, binding energy, and chemical states. In addition to that, the electrochemical characterization was performed for sensing of Hydrogen peroxide (H_2O_2) and dopamine. The real advantage of the proposed portable thermal management system over conventional instruments was the system eliminates the requirement of conventional bulk processing, heavy autoclaves, and expensive tools for heating of nanoparticle during the synthesis process.

2. EXPERIMENTAL:

A. Microfluidic Device Automation:

1) Thermal Management System:

Thermal management plays a significant role in the microfluidic platform for the synthesis of nanoparticles. An automated, integrated temperature controller device has been designed and developed. It consists of a microcontroller, DC-DC converter, cartridge heaters, and feedback thermocouple sensors for precise control of the desired temperature by adopting the Proportional-Derivative-Integral (PID) based controller method which provides stability, accuracy, and reliability. PID controller comes with an inbuilt library facility and features for Arduino based microcontroller devices. This makes the use of the PID controller along with arduino easy.
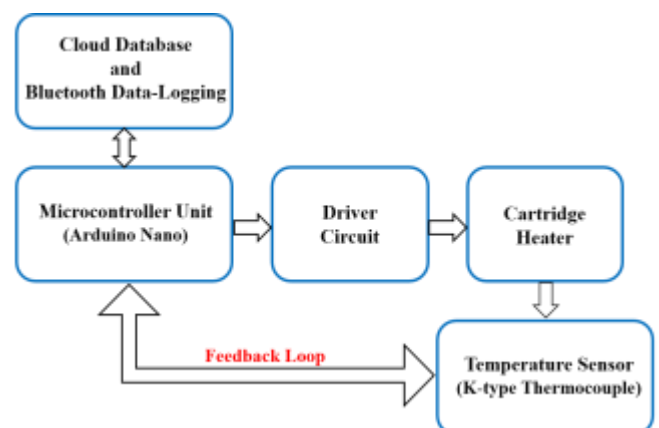

Fig. 1: Block diagram representation of the thermal management system

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1.1) Microcontroller Unit:

An arduino based microcontroller acts as the heart of the circuit to control, coordinate, and monitor the real-time temperature values. The driver circuit is designed by using BJT (BC547) and MOSFET (IRF540N), and limiting resistors were used to limit the excess flow of the current in the circuit to drive the required amount of current for the cartridge heater to be triggered for the set temperatures. Fig. 1 illustrates a simple block diagram representation for the complete device.

The arduino nano is connected to a Bluetooth Module (HC-05) to communicate with the Smartphone App, enabling to store and analyze the data. The microcontroller is also connected to the IoT ESP8266 module to allow live data uploading and sharing on to the cloud with ThingSpeak channels on the Internet of things (IoT) platform. The complete electronic board is powered up using a simple 12V/5A laptop adapter. Each electronic component mounted and soldered on to the PCB for making it compact and easier to debug the circuit. The entire coding is accomplished using an open-source software compatible with Arduino IDE (www.arduino.cc). The different electronic components used to build the circuit along with the source code has been provided. (Supplementary material S1). A 3D printed casing was designed and developed for the complete integration of the system. An automated, integrated temperature controller device has been designed and developed. It consists of a microcontroller, DC-DC converter, cartridge heaters, and feedback thermocouple sensors for precise control of the desired temperature.

1.2) Driver Unit:

The microcontroller digital pin is directly connected to the base terminal of the Bipolar Junction Transistor (BC547) for triggering the high and low signal. The need for a driver circuit is to control smaller electronic devices operating on DC input. The transistor functions as a high-current switch powered by a low-current digital logic signal from the microcontroller.
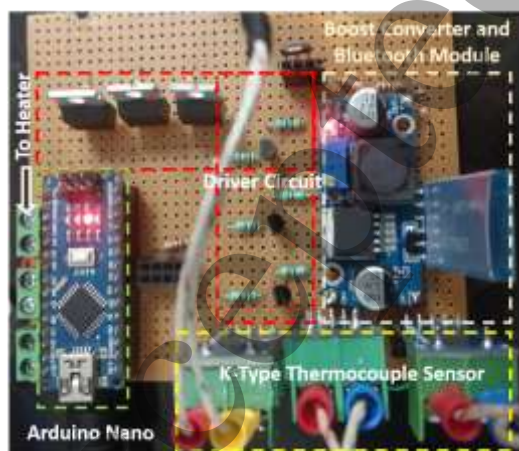

Fig. 2: Electronic components mounted and soldered on PCB

Metal oxide semiconductor field-effect transistor (IRF540N), a voltage-operated component with great efficiency, used for switching and amplifying electronic circuits along with a limiting resistor for driving the cartridge heater. Fig. 2 shows a self-developed driver circuitry designed using BJT and MOSFET mainly used for switching operation. The input signal fed to the driver circuit from the digital pin of the microcontroller unit. The digital pin is responsible for the pulse width modulation signal triggering high and low levels for controlling the current inflow towards heaters.

1.3) Heater and Sensor Unit:

Customized cartridge heaters have been employed for the heating process as it is controlled and powered by voltage and requires a very less amount of current to be driven. Fig. 3 shows the customized cartridge heater (www.ragatiyaheaters.in) with the dimensions 3mm of diameter and 25mm of length. It is made of stainless steel of 316 grade. The cartridge heater consumes low power (12V) it gets heated up to a maximum temperature of 300°C. Due to its low power consumption, it is suitable for different applications such as automotive, biomedical, and consumer electronics.

Fig. 3: A voltage-controlled cartridge heater

The calibration was carried out for a single cartridge heater which needs an input voltage of 12V DC and a current of 0.8A. When the voltage is applied across the cartridge heater it acts as resistance and drives the load. The K-type thermocouple-based temperature sensor was incorporated because of its resistance linearity. A schematic representation of the thermal management system has been provided in Supplementary material S2.

MAX6675 is a serial peripheral interface (SPI) module that ensures accuracy and compactness. The thermocouple sensor is accurate, reliable, inexpensive, and wider temperature range up to -200°C to +1260°C. A PID controller is integrated with feedback control which comes with an open-source library in arduino IDE that provides a very good stable output observed by fine-tuning $K_p=150$, $K_i=1$, and $K_d=70$ as per the requirement with very low tolerance. Herein, arduino offers an easy way of tuning of three terms such as Proportional (K_P), Integral (K_I) and Derivative (K_D). PID controller was implemented to control the temperature with a precision of ±2°C. The heaters were inserted at one end of copper blocks. The dimensions of copper heating blocks are 75mm*15mm*10mm respectively.

1.4) Real-time data logging:

The data logging was used to monitor and store the data across the device. Both Bluetooth and IoT modules were included on the same electronic circuitry platform making it easier for data accessing and data analysis. Bluetooth (HC-05) module was used for smartphone app-based for data logging. 'Bluetooth Terminal HC' open source app was installed onto the mobile phone to monitor the temperature values. The app was supported on a smartphone device with an Android OS for continuous real-time data monitoring which can be saved on the smartphone as .csv/.xls file format and additionally, ThingSpeak enables the user to retrieve the live or stored data in analog, a digital or graphical representation that easy further analysis. ESP8266-01 is a dedicated module for IoT which interacts with microcontroller and cloud acts as a medium for online communication. The source code of arduino IDE has been incorporated in the Supplementary material S3.

B) Microfluidic Device Fabrication:

2.1) Design and Fabrication of the microfluidic device:

The design of the microfluidic device was finalized after rigorous models were made with different microchannel width, length, and geometry. Autodesk Fusion 360 was used for 3D designing of microchannel for fabrication. The dimensions of the reservoir were 20mm*20mm*3mm. A design with a 3D view of the microchannel with dimensions has been provided in Supplementary material S4.

The 3D models were fabricated on Polymethyl-methacrylate (PMMA) sheets using CO_2 laser cutter /engraver (Universal Laser Systems VLS 3.6). The reservoir was engraved by 3mm of depth on a PMMA sheet with a 4 mm thickness in a desired shape of the design by varying the power and speed of the CO_2 laser machine.
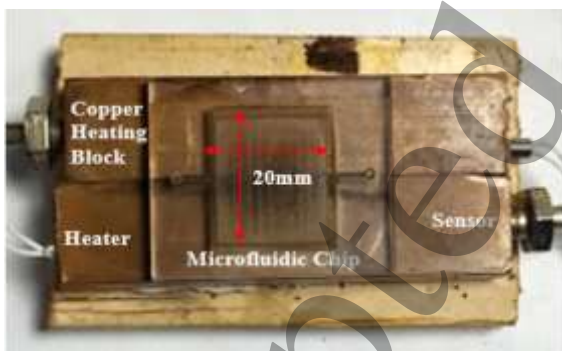

Fig. 4: Microchannel placed on a copper block

Fig. 4 illustrates the setup of microchannel on copper heating blocks. As a proof of principle here, a microchannel was designed as a reservoir for the synthesis of nanoparticle on a microfluidic platform which is mainly utilized for minuscule volume for various applications. The main reason to have a microfluidic-based reservoir is to have a smaller volume, size, easy setup, and controlled heating conditions which make it more precise for nanoparticle synthesis. The solution was prepared and poured in the microchannel reservoir for the synthesis of the nanoparticle. The microfluidic chip was covered with a glass slide that offers good optical properties, and binder clips were used to bond the microfluidic chip. The PMMA fabricated microchannel was placed on top of the copper block by applying the thermal paste for better heat transfer and conductivity.

2.2) a. Synthesis of MnO_2 Nanoparticle:

The precursor's manganese sulphate ($MnSO_4 . H_2O$) and Potassium permanganate ($KMnO_4$) were taken in the ratio of 1:2 for the preparation of the solution. The precursors were dissolved in 10 ml of DI water. The solution was well stirred on the magnetic stirrer for 20 mins for interacting with solid fragments of undissolved solution. The solution of 1ml was transferred into the microfluidic device placed on a copper heating block. The sample was heated from room temperature to 90°C for 12 hours. The brownish black-coloured powder was obtained which was filtered off by washing with DI water several times and dried at 65°C for 3 hours. This was carried out by placing the sample in the oven to gain 60 mg of filtered powder. Subsequently, this sample was tested for various characterization techniques for analysis and to observe the formed nanostructures of the synthesized MnO_2 nanoparticle.

b. Synthesis of MWCNT-MnO_2 Nanocomposite:

The MWCNT-MnO_2 composite was prepared by simple sonication method. 8 mg of MnO_2 nanoparticles, synthesized using the microfluidic device, and 2 mg of commercially purchased MWCNT were dispersed in 500 µl of ethanol and sonicated for 20 minutes to obtain a homogeneous solution. Further, this composite solution was coated on the working electrode for the electrochemical sensing application.

2.3) Electrochemical sensing:

Electrochemical sensing of dopamine and hydrogen peroxide was carried out using Cyclic Voltammetry (CV) with Bio-Logic (SP-150). Glassy carbon electrode (GCE), platinum, and Ag/AgCl electrodes were used. Multiwalled carbon nanotube (MWCNT) and dopamine were procured from Sigma Aldrich. Hydrogen peroxide (H_2O_2), 30 % was obtained from Avra synthesis private limited. All the other chemicals used for phosphate buffer (pH 7) were of analytical grade and used as received. Electrochemical sensing is used to detect cytosensors nanomaterials [18].

Electro-catalytic sensing of Hydrogen peroxide (H_2O_2) and Dopamine (DA) was carried out in a conventional three-electrode system. Herein, the GCE electrode, modified with MWCNT-MnO_2 composite, was used as a working electrode (WE), Ag/AgCl as a reference electrode (RE), and platinum as a counter electrode (CE). The GCE electrode was modified by coating the 10 µL of MWCNT-MnO_2 composite homogeneous solution to form GCE/MWCNT@MnO_2. Further, the modified working electrode

(GCE/MWCNT@Mno₂) was dried at 60°C for 2 mins. The CV was carried out at a scan rate of 10 mVs⁻¹ in 1 mM Dopamine and 1 mM H_2O_2.

III. RESULTS AND DISCUSSION

A. Automated microfluidic device for temperature management:

Elements	EDX		XPS
	Weight (%)	Atomic (%)	Atomic (%)
Manganese (Mn)	49.91	19.04	17.62
Oxygen (O)	38.58	59.28	47.77
Potassium (K)	2.35	2.06	0.23
Carbon (C)	9.16	19.62	34.38
TABLE II. Elemental analysis of MnO₂			

The automated temperature management device was designed and developed with integrating all the electronic components placed on single PCB, and a 3D printed casing was incorporated to make the device more compact and portable. The complete automated device dimensions were 10.4cm*8.5cm*3.5cm. Supplementary material S5 provides 3D printed casing of the thermal portable device.

B) Characteristics of MnO₂ Nanoparticle:

3.1) Field Emission Scanning Electron Microscope (FESEM) & X-Ray Photoelectron Spectroscopy (XPS) Analysis

The microfluidic-based temperature controller was used for heating at the desired temperature range to synthesize the MnO₂ nanoparticles. Unique nanoflakes like the structure of MnO₂ have been successfully synthesized by the microfluidic-based proposed thermal management system which was placed for 12 hours and temperature was maintained at 90°C. Fig. 5 (a), (b) shows the FESEM images of MnO₂ nanoparticles at low and high magnifications. The results obtained were analyzed to know the potential of the proposed technique, and compared with the literature which shows similar results in nanometres. The characterization results show the significant presence of key elements and these were even compared with the hydrothermal conventional methods which are being used for nanoparticle synthesis [32]. Manganese oxide nanoparticle was synthesized and the adsorption study was carried out with hydrothermal and quick precipitation based conventional methods by utilizing expensive instruments[33]. Also, Fig. 5 (c) and (d) show the morphology studies of the commercial purchased MWCNT and the composite of MWCNT-MnO₂. It was observed that the synthesized MnO₂ nanoparticles using the microfluidic device were coated on the MWCNT.

Further, these MWCNT-MnO₂ composite was used for the electrochemical sensing.

Further, the Energy-dispersive X-ray spectroscopy (EDX) was studied to analyze the elemental composition present in synthesized MnO₂ nanoparticles Fig. 6 shows the EDX spectrum for chief elements of Mn, O, and K respectively. Table II shows the statistical results of the EDX and XPS
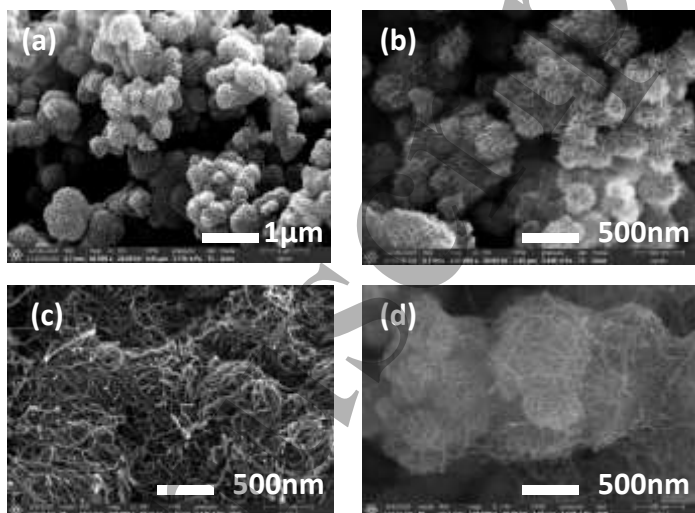

Fig. 5: Morphology studies using FESEM (a), (b) MnO₂ at low, high magnifications (c) MWCNT (d) MWCNT-MnO₂
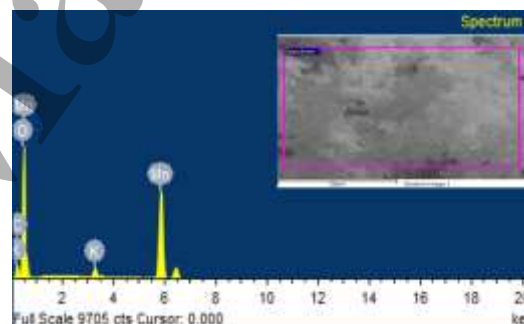

Fig. 6: Processing of the EDX spectrum for Mn, O, K, C elements

with an analysis of key elements of Mn, O, K and C. An open-source software ImageJ (www.imagej.net) was used for the image analysis of nanoparticles. The analysis of nanoparticle size distribution was carried out automatically using the plugins present in ImageJ software. However, based on the obtained results the mean size of nanoparticle (wc) and standard deviation (σ) were calculated and also a curve was plotted for nanoparticle size distribution as shown in Fig 7.
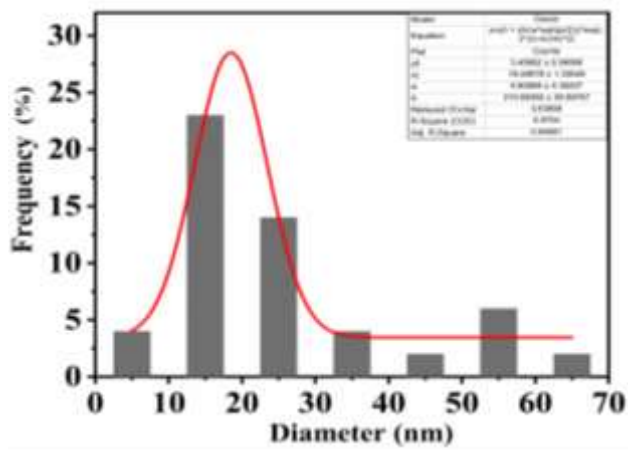
Fig. 7: Nanoparticle size distribution curve

The elementary, binding energy and chemical state of the MnO_2 nanoparticles were studied using X-ray photoelectron spectroscopy (XPS). Fig. 8(a) reveals the presence of Mn, O, C, and K elements with no noticeable supplementary hetero-elements. The analysis of Mn 2p in Fig. 8(b) manifests the emergence of peaks at 642 eV and 654 eV, with the spin-orbit splitting between the two is 12 eV, assuring the MnO_2 crystallization. Also, the peak of binding energy at 530.2 eV in Fig. 8(c) specifies the existence of O in O^{2-} a state, which in turn bound with Mn atoms. Further, the obtained XPS results were compared with the conventional hydrothermal synthesis of MnO_2 nanoparticles wherein the peak of binding energy for Mn 2p 642.5 eV and 654 eV and binding energy peak of O in 529.7 eV were observed respectively [34][35].

3.2) Electrochemical sensing of Hydrogen Peroxide and Dopamine:

In Fig. 9 and 10, it is found that the blank electrode (GCE/MWCNT), (GCE/MnO2) alone shows no oxidation peak in the presence of 1mM H_2O_2 and dopamine, whereas, GCE/MWCNT@MnO_2 gave distinct oxidation at E^0= 0.3 and -0.1 V corresponding to the oxidation of dopamine and H_2O_2. Therefore, it was discovered that the synthesized MnO_2 is electrochemically active and gave electro-catalysis by assistance with MWCNT and with fine-tuning it could be further used for the fabrication of electrochemical sensors for dopamine and H_2O_2 in real-time application.

IV. CONCLUSION

The present work encompasses the design and development of low-cost, easy-to-use, portable, automated, and point of source thermal management system using a miniaturized arduino based microcontroller. Herein, an arduino based platform provided an integrated PID controller feature, wherein it is easy-to-use, gives a faster response, accurate, with added features like real-time data access and storage on
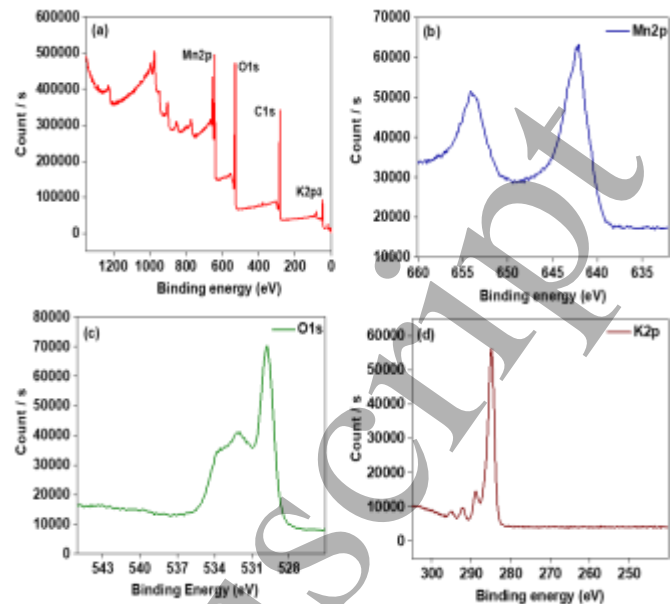
Fig. 8: XPS study of MnO_2 Nanoparticles (a) XPS survey (b) Manganese (Mn2p) (c) Oxygen (O1s) and (d) Potassium (K2p)
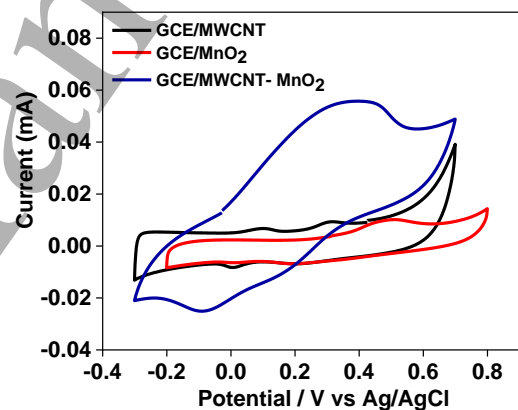
Fig.9: CV response of GCE/MWCNT and GCE/MWCNT@MnO_2 in 1 mM Dopamine (DA) solution at 10 mV s^{-1}
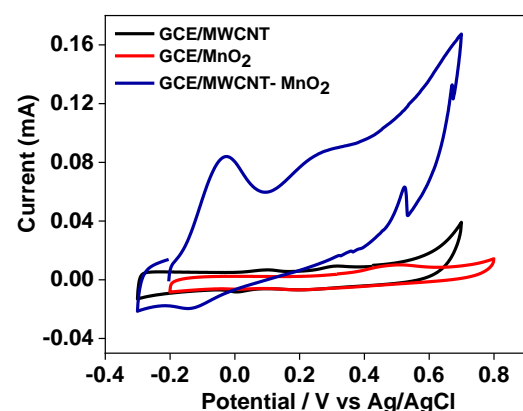
Fig.10: CV response of GCE/MWCNT and GCE/MWCNT@MnO_2 in 1 mM Hydrogen Peroxide (H_2O_2) solution at 10 mV s^{-1}

       Author *et al*

the cloud server and smartphone. The continuous data with GUI interface enabled with a smartphone application, for both bluetooth and database cloud, empowers the users to acquire geotagged information for surveillance during the long run of experiment. The fabricated PMMA microfluidic device has been used for the synthesis of nanoparticles with a variety of ravishing features. These include accurate control of reacting conditions, faster mass and heat transfer, secure operation, amenability with the environment, easy setup, and fast loading of reagents and samples with high reproducibility. Herein, MnO_2 nanoparticles have been synthesized using the portable thermal management system in microscale. SEM and XPS characterizations were carried out to analyse the MnO_2 nanoparticles. As a proof-of-principle, these nanoparticles were utilized to optimize the working GCE of the electrochemical three-electrode system for Hydrogen peroxide and Dopamine sensing. The CV method was used to determine the sensing response of the synthesized nanoparticles leading to the strong potential of the developed nanoparticles for various sensing applications.

V. ACKNOWLEDGMENT

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Laser-Induced Flexible Electronics (LIFE) for Resistive, Capacitive and Electrochemical Sensing Applications

Avinash Kothuru, C. Hanumanth Rao, Puneeth S B, Mary Salve, Khairunnisa Amreen and Sanket Goel*, *Senior Member, IEEE*

MEMS, Microfluidics and Nanoelectronics laboratory, Department of Electrical and Electronics Engineering, Birla Institute of Technology and Science, Pilani, Hyderabad Campus, Hyderabad, India.

Abstract—Engineering a cost-effective, flexible electronic device in a one-step fabrication process is quite challenging to perform. Herein, we have introduced a simple, low-cost, solid-state process for producing and printing of complex circuits using Laser-Induced Graphene (LIG). In the present work, LIG has been effectively and selectively formed from direct CO_2 laser ablation on a polyimide sheet. Varying CO_2 laser power and speed, the electrical conductivity of the LIG has shown a linear increment in the conductivity measurement. The laser-induced samples were structurally characterized using Scanning Electron Microscopy (SEM), EDX, X-ray Photoelectron Spectroscopy (XPS), Raman spectroscopy. The results show a one-step method to create Graphene-derived structures on the polyimide sheet surface. This method of generating LIG on a flexible substrate (polyimide sheet) offers an easy way to fabricate Laser-Induced Flexible electronics (LIFE) circuits. Using this, the feasibility and the realization of a capacitive touch sensor and liquid level sensor has been successfully demonstrated. Further, as a prototype system, the LIG was examined for the H_2O_2 electrochemical sensing application. It gives an appreciable response for the detection of H_2O_2 in comparison to other carbon-based electrodes with limit-of-detection (LOD) as 0.3 µM in a linear range from 1 µM to 10 µM.

Index Terms— Laser-Induced Flexible Electronics (LIFE), Polyimide, electrochemical sensing, Touchpad, water-level monitoring.
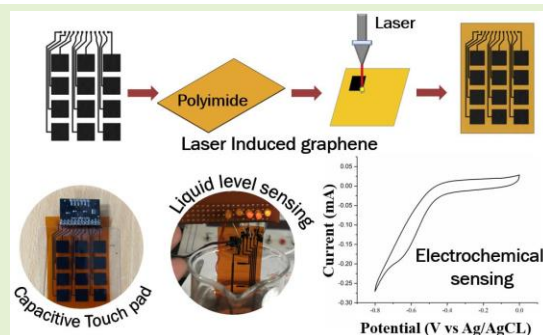
I. INTRODUCTION

Electronics has become so dominant and dependable that it is continuously harnessed in many ongoing research domains to realize cost-effective, user-friendly and robust devices with better accuracy and precision. Since their inception, the electronic circuits were bulky as they were realized on the dotted circuit boards, with a manual and time-consuming process. Later on, a thin copper sheet based Printed Circuit Boards (PCB) were developed, which has brought remarkable changes to modern electronics. On PCB, the copper conductive traces were obtained by a well-established subtractive chemical wet-etching process. Even though such a PCB fabrication process is well-established, but there is a huge scope to make it automated, additive, and cost-and-time efficient. Further, the PCB fabrication process involves complex fabrication method that includes carcinogenic chemicals, and hot soldering is necessarily leading to another significant concern. In few cases, such as packaging of the circuit, stacking of Integrated Circuits (IC), electrical connection between the stacks of circuit boards, rigid PCB have disadvantages, leading to the requirement of the flexible boards [1]–[3]. Hence, an alternative technique is necessary to create electronic circuits on a flexible surface, which reduces the area and increases the application potential of the device.

The flexible electronics has been reported for many applications in day-to-day life, includes connectors for flexible displays, contacts in keypads, keyboards, health monitoring devices and much more[4][5][6][7][8]. Such flexible electronic devices have been fabricated by popular approaches such as screen-printing and inkjet printing, where suitable conductive ink, developed using Carbon or Silver, was employed to get the conductive traces. These traces are formed on many substrates such as polymeric film, polyethylene terephthalate (PET), polyimide (PI) and can be easily connected on an onboard PCB. This approach is shown in stacking of PCBs, resulting in the reduction of the area to realize the necessary circuit. These traces can be connected to the PCB using Flexible Printed Electronic Circuit connector, or card edge connector[9]. These genres of flexible circuits play a prominent role to develop sensors, Radio-frequency

identification (RFID), flexible displays and antennas. In recent times, the compositions of conductive inks were achieved using many novel materials, such as nanoparticle, nanowires and graphene sheets, to increase the conductivity. The conductive inks were prepared by mixing conductive material with solvents to maintain the consistency based on the requirement. However, the main challenge in developing such inks is to properly synthesize the nanomaterials itself and the bonding process after solidification[10]. Other drawbacks of these methods are clogging of nozzles of the inkjet printers, preparation of stencils for screen-printing, complicated and tedious process for obtaining nanoparticles. These challenges increase the time to obtain the flexible circuits, an increase in cost and non-uniformity of the conductance of the circuit.

Various nanomaterials fabrication techniques are proposed during recent years, which includes nanoparticles, Nano-rods of materials such as copper, silver, gold, platinum that have high conductive properties. The CO_2 laser method is being used widely for obtaining the carbon on polyimide sheet[11]. An alternative process is synthesizing of Graphene, an allotrope of carbon, which has immense advantages due to its physical, chemical, mechanical and electrical properties. The graphene is a three-dimensional porous structure with very few layers (3-5 layers); this ensures flexibility, high surface area and mechanical endurance[12]. However, the major challenge exists in obtaining a few layers of graphene . As the number of layers increases, the properties change which may result in different carbon allotropes such as Graphene oxide, reduced Graphene oxide, and Graphite. Due to the advantages as mentioned above, the applications of graphene are seen in various domains including storage of electrical energy as a supercapacitor, on electrode surface reversibility of absorption and desorption of electrolytic ions. The well-established procedures to fabricate crystalline graphene structures are oxidative acid synthesis route, chemical vapour deposition on thin films of Cu and Ni at elevated temperatures leading to porous material deposition[13]. This film of graphene can be transferred from one surface to the other transfer methods involving either a wet or dry process[14]. One of the reported drawbacks with the processes of forming reduced GO is that the quality of the graphene obtained does not remain the same as of native graphene[15].

One of the innovative and straightforward process to obtain graphene or graphene oxide has been demonstrated by focusing high energy density laser beam on to the surface of the substrates. This was achieved by ablating substrates under CO_2 laser in the desired pattern, designed in software as per the requirement, and fed to the CO_2 laser equipment[16]. The CO_2 laser ablation was been reportedly performed on different non-flexible substrates such as bread slice, coconut shell, various kinds of wood and much more. A similar process has been repeated on a flexible substrate, like polyimide (PI), whereby the traces of carbon was observed [17][18]. This process is famously known as Laser-Induced Graphene (LIG)[19]. The obtained samples were subjected to physic-chemical characterization techniques, such as XPS spectra, Raman Spectroscopy[20] and Scanning Electron Microscope (SEM)[21], and provided sufficient justification to conclude that the obtained carbon traces were of graphene oxide.

Since the present approach has been carried out on a hydrophobic polyimide substrate, therefore, it will be easily compatible with subsequent material processing. In this work, an optimized process has been demonstrated to create LIGs on a polyimide sheet using a low-power CO_2 laser. Rigorous characterizations were carried out to support the feasibility to employ such LIGs to create LIFE components for sensing applications. T. han et al reported in 2019, with speed and power of CO_2 laser the polyimide sheet was ablated and used as real time strain sensing[22].   To realize the potential and scope of such LIFE devices, varied applications like capacitive touchpad, electrochemical sensing and resistive liquid level sensor is demonstrated.

II. Materials and Methods

Polyimide sheet of 125 μm thickness was purchased from Dali Electronics, and a 30 W CO_2 Laser (Universal Laser Systems, VLS 3.60) was utilized to obtain the Laser-Induced Graphene (LIG) conductive traces. Hydrogen peroxide (30%) was procured from Sigma Aldrich and other basic chemicals of analytical grade were used. The topographical morphology and elemental analysis of the obtained CO2 laser-induced graphene (LIG) were characterized by Scanning Electron Microscope (SEM) and Energy Dispersive Spectroscopy (EDS) techniques using Apreo Scanning Electron Microscope (SEM) from Thermo Fisher Scientific. Fourier transform infrared spectrometer instrument (FTIR-4200 from Jasco) was used to examine the samples for identifying the functional groups (organic or inorganic). In further, Witec Alpha 500 confocal Raman microscopy was used for the characterization. A Thermo scientific K-Alpha X-ray Photoelectron Spectrometer (XPS) instrument was utilized to examine the samples for surface analysis. For conductivity, resistance, resistivity measurement, Ossila machine, based on the Four-point probe mechanism was used. All the cyclic voltammetry (CV) measurements were performed using Bio-logic (SP-150) electrochemical workstation in a standard three-electrode configuration.

III. Exerimental Procedure

A. Formation of Laser-Induced Graphene(LIG) on Polyimide sheet

A basic design was drawn using AutoCAD Fusion 360, and the file was saved in .dxf format. The .dxf file was transferred in CorelDraw X7, and the outline width of the design was changed according to the preference of the CO_2 laser. As shown in Fig 1, by varying the speed and power, of the CO_2 laser, Laser-Induced Graphene (LIG) was obtained on polyimide (PI) [23], LIG reaches its highest crystallinity by laser power amplification[24][25].Literature reports fabrication of LIG using CO_2 laser machine by varying the power and speed on PI, wherein conductivity is obtained and calculated. For instance, Lin et. al. induced 60W CO_2 laser on PI by varying power from 2.4 W to 5.4 W, showing the high conductivity of 25 S/cm at a power of 5.4 W. In present work, for 5.4 W laser power has 91.3% of carbon, 7.7% of oxygen
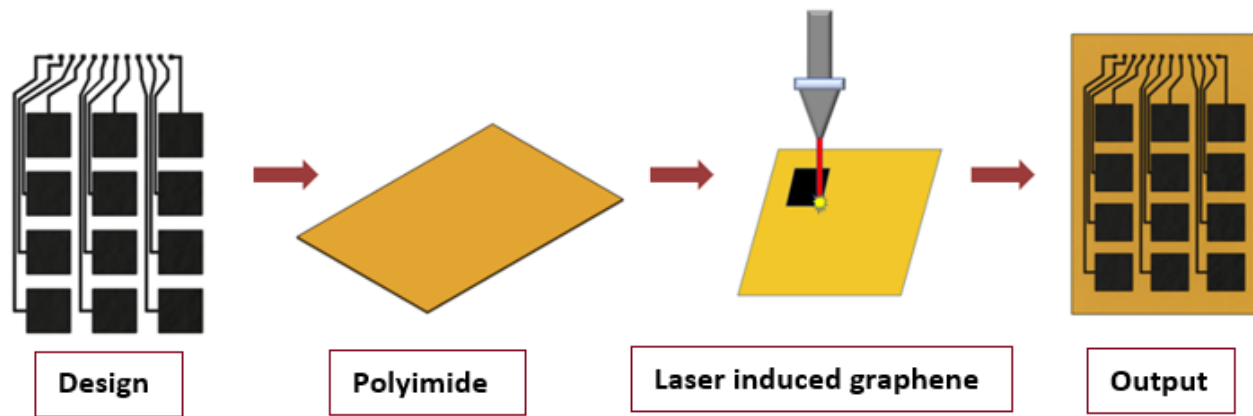
Fig 1 Schematic process to create Laser-Induced Graphene (LIG) structure on Polyimide

and 1.0% of nitrogen was observed. In the present study, above 95% of carbon was observed by varying 30 W CO_2 laser power from 1.35 W to 1.95 W as shown in Table I.

In general, by optimizing the laser power, the LIG thickness increased improving its conductivity.

B. Capacitive Touchpad

By touching a capacitive touchpad, an amount of charge is drawn to the contact point which becomes functional. The change in the electrostatic field is calculated to determine the location. In this work, touchpads were designed with different approaches such as resistive and capacitive, whereby the capacitance-based touchpad has seen the upper hand in terms of extensive usage. The electrode patterns were created to act as touchpads with a particular pre-defined pattern. These patterns obtained on the PI sheet with the help of CO_2 laser was integrated along with the ICs, meant to detect the change in the capacitance, which was used along with a microcontroller and a display unit.

C. Liquid level sensor

These sensors are used to detect liquid levels or interfaces between liquids such as water and oil. Level sensors calculate within the range. Level sensor shows whether the fluids are above or below the point of sensation. Level detection sensors can detect the fluid level in a container, and therefore, they have ample scope in real-life applications. Herein, an electronic-based approach was explored. LEDs were used to show the level, which was one of the natural and visual approaches to exhibit.

D. Electrochemical sensing

A conventional three-electrode system consisting of a working electrode, a counter electrode, and a reference electrode is used for electrochemical sensing. Herein, the laser-induced graphene (LIG) sample was used as a working electrode, Ag/AgCl as reference and platinum as a counter. Cyclic voltammetry (CV) experiments were performed using 0.2 M phosphate buffer solution (PBS) pH 7 and 500 μM H_2O_2 sample.

IV. Material characterization

In order to interpret the surface morphology and structure of the LIG, samples were exposed to various physico-chemical and microscopic characterization like SEM, EDX, XRD analysis, XPS and Raman Spectroscopy[26].

A. Scanning Electron Microscopy (SEM) and Dispersive X-ray Spectroscopy (EDX)

The Surface morphology study of the formed LIG on polyimide sheet was explored using scanning electron microscopy (SEM). Fig 2 exhibits SEM images of PI sheet before and after exposing to the CO_2 laser machine. Fig 2. (a - c) Clearly depicts the bare polyimide sheet without laser ablation, whereas, in Fig 2 (b-d), represents post exposure to CO_2 laser. An evident deposition of graphitic like framework was observed authenticating the LIG formation. Fig 2 (e) shows the EDX of a LIG sample. From the EDX, the carbon content in the LIG is above 95%, and oxygen content is near to 4%. Table 1 depicts the elemental analysis of the LIG [27].

TABLE I
CARBON & OXYGEN CONTENT IN EACH SAMPLES

Elements	Bare PI	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Carbon (%)	79.97	96.25	96.20	96.22	96.50	96.55
Oxygen (%)	20.03	3.75	3.8	3.78	3.5	3.45

For the LIG sample, the C 1s XPS spectrum peaks after the deconvolution, are at 284.67, 286.55 and 288.48 eV, similar to the reported values. The peak values represent a functional group of C-C, O-C=O, and sp2. Here, without deconvolution, some samples show a peak at 284.78 that is near to sp3 carbon. The C 1s XPS spectrum intensity peaks manifest that it contained only C and O elements indicating the absence of impurities.
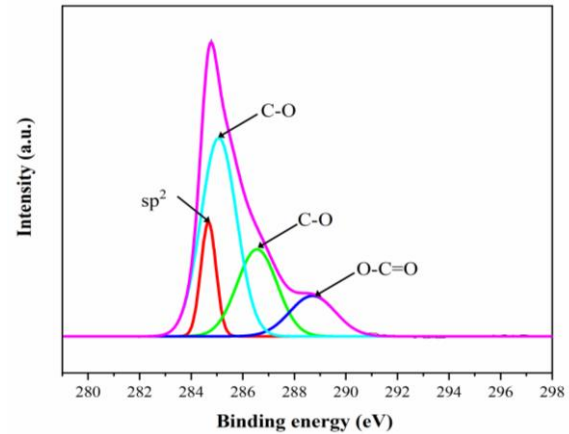

Fig 3. XPS of graphene oxide

C. Raman Spectroscopy

Investigation of the obtained LIG was carried out using Raman Spectroscopy to make sure that the material was carbon allotropes. Five LIG samples were characterized using Raman spectroscopy (varying speed and power), and the Raman peaks are illustrated in Fig 4. and Table 3. The results for the intensity of G bands, D bands and 2D bands are shown in Table 3.

Fig 2. SEM images of (a-c) bare polyimide, (b-d)LIG sample after exposing to the CO_2 laser, (e) EDX of LIG sample showing carbon and oxygen peak

B. X-ray Photoelectron Spectroscopy (XPS)

Varying the speed and power of the CO_2 laser, various samples were prepared. XPS C 1s spectrum analysis for all the samples was done. The peak values for the five samples are represented in Table 2. Fig 3. shows the XPS spectrum of the prepared Graphene Oxide (GO). From the literature [28][29], the deconvolution of GO peak happens at 284.6, 286.6 and 288.5eV. The number of counts per second depends on the concentration of the elements present in the compound. For the LIG sample, the binding energies of the corresponding functional group gives different counts per second with respect to the concentration present in the sample.

TABLE II
XPS C 1 S SPECTRUM OF LIG SAMPLE

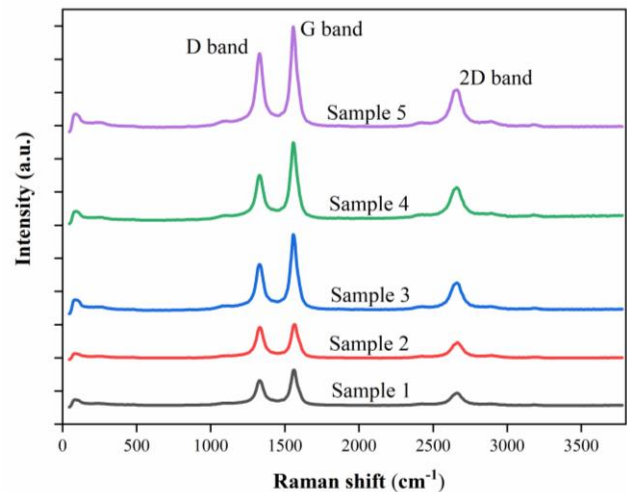| Samples	Counts/s	Binding energy (eV)
1	265713	284.38
2	138620	284.78
3	170895	284.78
4	248508	284.68
5	241663	284.58

Fig 4. Raman spectra of Graphene oxide for five samples varying speed and power

The disordered carbon structure can be represented by the intensity ratio of the G band to the D band (I_D/I_G)[23][30]. The I_D/I_G ratio of the as prepared LIG samples varied between 0.668 and 0.942, indicating the varying carbon structural defects in the lattice structure of various LIG samples. Raman results for five samples show the same D and G band wavelength, i.e. 1330.039 cm^{-1} and 1557.68 cm^{-1}. Therefore, based on the values obtained it can be concluded that the

samples had a formation of graphene oxide. As the ratio, I_{2D}/I_G, manifest the number of graphene layers, the variation of the number of layers should be one of the reasons behind the variation in electrical conductivity for various LIG samples.

TABLE III
D AND G BANDS FOR THE LIG SAMPLES

S.no	D Band	G Band	2D Band	I_D/I_G	I_{2D}/I_G
1	658.33	818.30	468.36	0.805	0.572
2	743.58	789.55	513.94	0.942	0.651
3	1007.61	1462.30	727.80	0.689	0.498
4	995.83	1490.11	810.22	0.668	0.544
5	1446.41	1849.08	898.80	0.782	0.486

D. Conductivity Test (Four-Point Probe System)

The prepared LIG samples were tested for conductivity by placing them at the center of the four-probe system. To measure the conductivity, the input of a maximum 5 V with 0.01 V increment and 25 repetitions were carried out. Fig 5. (a) shows the plot between applied voltage (V) and measured current (A), and Fig 5 (b) shows the plot between applied current (A) and measured voltage (V). As can be seen, the conductivity of the sample changes with varying speed and power of laser on the polyimide sheet.

TABLE IV
CONDUCTIVITY RESULTS FOR VARIOUS LIG SAMPLES

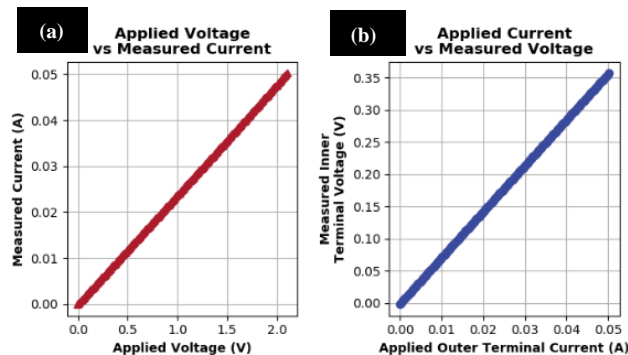| Samples	Power (W)	Speed (m/min)	Conductivity (S/m)
1	1.35	0.45	250.64
2	1.5	0.54	276.70
3	1.65	0.63	293.08
4	1.8	0.72	319.95
5	1.95	0.82	367.78

Fig 5. Measured Current vs voltage for the LIG samples

V. Results and discussion

A. Capacitive Touchpad

By using AutoCAD Fusion 360, a touchpad was designed, as shown in Fig 6. The designed file was then induced with LIG on the polyimide sheet using the CO_2 laser machine to obtain the conductive traces. The values of the capacitance and resistance have been measured using an LCR meter. The observed values for capacitance was constant i.e. 1 pF. The

resistance values for five samples are as follows 138.3 Ω, 138.7 Ω, 140.2 Ω, 520 k Ω, 490 k Ω. The obtained LIG was then used as a capacitive sensor with mounted MPR121[31], which acted as a charge transfer sensing using single wire electrodes. On touching the electrode, both the capacitance and the charge transfer time increased and the microcontroller, in turn, received the information from the MPR 121, and the result for the capacitive touch sensor was displayed on the computer.
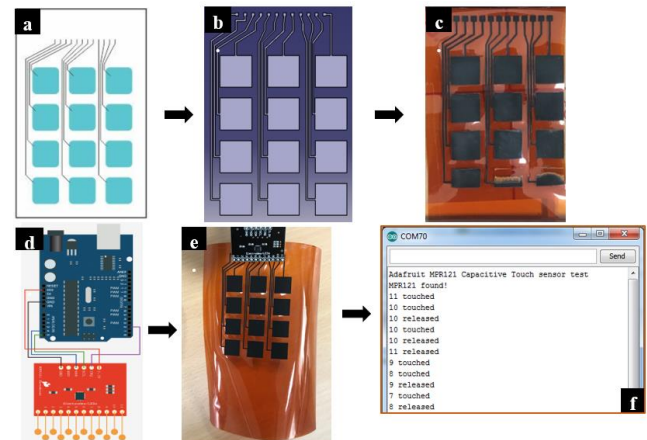

Fig 6. (a). Schematic of a touch sensor (b). CAD model of Capacitive touch sensor (c). CO_2 Laser Induced Graphene touch sensor on polyimide sheet (d). Schematic of Microcontroller integrated with MPR121 (e). MPR 121 mounted on LIG (f). Displaying results for Capacitive touch sensor.
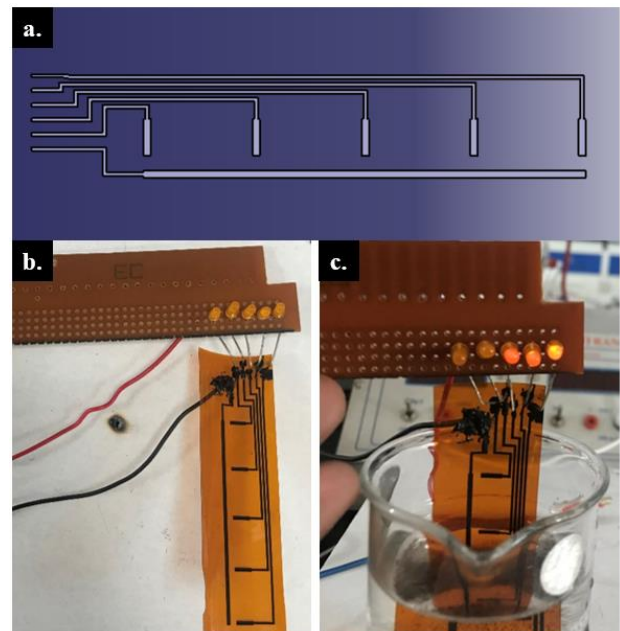
B. Liquid-Level Sensor

Fig 7. (a). CAD model of liquid level sensor (b). LIG arranged with LED lights on PCB (c). Testing of Liquid Level sensor with water.

A liquid level sensor has been designed in AutoCAD fusion 360, where the positive terminal/trace are in straight length, and the negative terminals/trace are joined with five intervals of a horizontal line as shown in Fig 7. Then after laser ablation on the polyimide sheet [32][33], the positive terminal and the negative terminals were arranged with five LED lights, and a

9 volts battery with 7805 regulator was incorporated to observe the function of the device.

C. Electrochemical Detection of Hydrogen Peroxide

For analyzing the hydrogen peroxide sensing with bare LIG, as a prototype, a 3-electrode system was created with the prepared LIG as a working electrode while Ag/AgCl and platinum wire as a reference and counter electrodes, respectively. Here, a rectangular shaped pattern with dimension 50 mm x 03 mm has been fabricated. Unmodified LIG is used for electrochemical sensing of Hydrogen peroxide. Further, 500 μM of H_2O_2 was prepared in 5 mL of pH 7 phosphate buffer solution. As stated, a three-electrode system with Ag/AgCl as working, Platinum as counter is used along with LIG as working for carrying out the cyclic voltammetry experiments. The experiments were carried out at 10 mV s^{-1} in a potential window of -1 to +1 V. Aparicio-Martinez et al. in 2019 reported silver modified Laser Scribed Graphene (LSG) for H_2O_2 sensing using a commercial GO and a 7.9 μM LOD was reported[34]. In the present case, GO was directly induced by laser and without any further modification, it was used for H_2O_2 sensing to obtain an appreciable detection limit of 0. 3 μM. Before experiments, all the solutions were purged with high-purity nitrogen. The Cyclic Voltammetry[35] measurements were carried out in 0.2 M phosphate buffer solution (PBS, pH 7.0) at the ambient temperature in the potential window 0.0 V to -1.0 V (vs. Ag/AgCl) at a scan rate of 10 mV s^{-1}. Fig 8 (a) depicts the calibration plot (b.) depicts the CV of the LIG electrode in 0.2 M PBS (pH 7.0) and with various H_2O_2 concentrations (from 1 μM to 10 μM).

With the successive addition of H_2O_2, the reduction current gradually increases, showing excellent electro-catalytic behavior of the fabricated LIG sensor towards hydrogen peroxide sensing. Based on the literature[36], the detection limit is theoretically calculated by using a standard formula, 3×Standard deviation/ Slope. Wherein, the standard deviation of the triplicated samples with the lowest concentration current was taken. The slope was derived from the calibration plot of the concentration effect. The detection limit of the sensor was estimated to be 0.3 μM within a linear range from 1 μM to 10 μM.
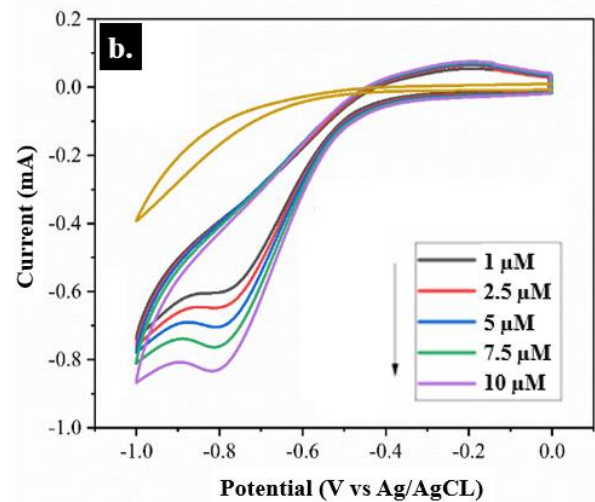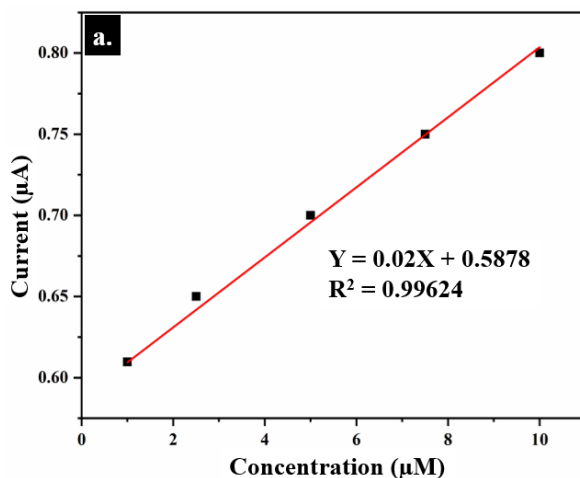

Fig 8. (a) Calibration plot of [H_2O_2] μM vs I/μA (b) Cyclic Voltammetry response for different concentrations of hydrogen peroxide (H2O2).

VI. Conclusion

Herein, a single-step, cost-effective approach to realize Laser-Induced Flexible Electronic (LIFE) circuits, using low power and user-friendly CO_2 laser, has been presented. Based on the design, graphene oxide (GO) has been observed to be formed on polyimide by CO_2 ablation. To confirm the type of carbon allotrope, various physico-chemical characterizations, such as SEM, EDX, , XPS and Raman Spectra, have been carried out. The CO_2 laser parameter, such as speed and power, has been varied to optimize the conductivity of LIFE circuits. After rigorous characterizations and optimizations, experiments were carried out to explore the feasibility to test various LIFE circuits for resistive, capacitive and electrochemical sensing. With such sensing applications, the LIFE circuits have been utilized as resistive touchpads, capacitive liquid-level sensor, and electrochemical H_2O_2 sensor. The Capacitive sensor, integrated with a mini-microprocessor, the functioning of the device, is proven by showing the result of glowing the LED and displaying the result of the touch sensor on the computer. For electrochemical H_2O_2 sensing, the device showed a good response for various concentrations and appreciable limit of detection without any further modifications, i.e. 0.3 μM. Overall, the LIFE devices have a strong potential to be harnessed for diverse sensing applications.

VII. ACKNOWLEDGMENT

VIII. References

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Avinash Kothuru received the B.Tech. degree in Mechanical Engineering in 2014 and the M.Tech. degree in Design for Manufacturing (DFM) from Osmania University, Hyderabad, India. During his M.tech., he worked on Design and Finite Element Analysis of BioMedical Scaffold for hard tissue and prototype

manufacturing using SLS Additive Manufacturing Technology. He Joined the Electrical and electronics Engineering Department, BITS-Pilani, Hyderabad Campus, Hyderabad, India in 2018 as a PhD candidate under the supervision of Dr. Sanket Goel, in developing Next Generation Circuit boards using Additive Manufacturing Technology with integrated Electronics and Microfluidics.

Hanumanth Rao C. received the M.Sc. degree in chemistry from Bangalore University, Bengaluru, India, in 1988, and the M.S. (Eng.) degree in materials science from the Indian Institute of Science, Bengaluru, in 2000.
He joined the Indian Space Research Organisation Satellite Centre, Bengaluru, in 1988, where he is currently a Manager of the High Density Interconnect (HDI) Facility. His current research interests include introduction of new technology in the field of electronic packaging miniaturization and realization of HDI and high layer count PCBs for space applications

Puneeth S. B. received the B.E. degree in electronics and communication and the M.Tech. degree in digital electronics from Visvesvaraya Technological University, Belgaum, India, in 2013 and 2015, respectively, with a focus on low area field-programmable gate array implementation of decryption unit for AES using Vedic Math. He is currently pursuing the Ph.D. degree with the Electrical and Electronics Engineering Department, Birla Institute of Technology and Science, Hyderabad, India, under the supervision of Dr. S. Goel, where he is developing the electro-microfluidic viscometer for various applications. He holds an Indian patent.

Mary Salve received the B.Tech. degree in Electronics and Telecommunication in 2016 and the M.Tech. degree in VLSI Design in 2018 from RTM University, Nagpur, India. During his M.Tech. She worked on developing PDMS based microfluidic devices for adulteration detection and cancer diagnostics. She joined the Electrical and Electronics Engineering Department, BITS-Pilani, Hyderabad Campus, Hyderabad, India in 2018 as a PhD candidate under the supervision of Dr Prasant Pattnaik and Dr. Sanket Goel, in development of Automated IoT based Microfluidic Electrochemilumines (ECL) Biosensing platform for various biomarker detection.
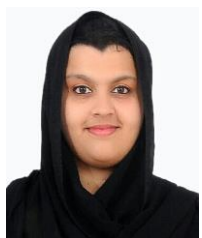
Dr. Khairunnisa Amreen is currently working as National Post Doctorate (NPDF) for DST- SERB. She has graduated B.Sc. in three majors (Chemistry, Biotechnology and Microbiology) from Osmania University-Hyderabad (2008-2011). Post which she completed her Masters in M.Sc.

Analytical Chemistry from Vellore Institute of Technology-Vellore, Tamil Nadu, India (2011-2013). Following which she continued her Ph. D. from Vellore Institute of Technology-Vellore, Tamil Nadu, India working there as a teaching cum research associate (TRA) and CSIR-SRF in the area of Electro-analytical techniques (2013-2019) under the guidance of Dr. A. Senthil Kumar, Senior professor, VIT-Vellore. The dissertation of my research work is entitled as design and development of whole blood and heme based biosensors for electroanalytical applications. During her Ph.D. tenure she published 9 international/national research articles and filed one Indian patent for the blood based biosensor developed. Presently she is working as NPDF-DST SERB under the supervision of Dr. Sanket Goel, Head & Asssociate professor, EEE, BITS-Pilani, HYD.

Sanket Goel received the B.Sc. (H-physics) degree from Ramjas College, Delhi University, the M.Sc. (physics) degree from IIT Delhi, and the Ph.D. (electrical engineering) degree from the University of Alberta, Canada, under the NSERC Fellowship. He headed the R&D Department and worked as an Associate Professor with the Electronics and Instrumentation Engineering Department, University of Petroleum and Energy Studies, Dehradun, India, from 2011 to 2015. He is currently an Associate Professor and Head, Electrical and Electronics Engineering Department, BITS–Pilani, Hyderabad Campus, Hyderabad, India. He has >60 publications, 6 patents (1 U.S. and 5 Indian) to his credits, delivered >50 invited talks, and guided 6 Ph.D. and 10 master's students. His current research interests are microfluidics and nanotechnology, materials and devices for energy (both conventional and renewable), and biomedical applications. He has won several awards during the course of his career, including the Prestigious Fulbright-Nehru Fellowship (2015), the Young Scientist Award (2013), the Best Students Paper Award (2005), and the Ph.D. Thesis Award (2005). Currently, he is the Associate Editor of IEEE Sensors Journal and IEEE Access, and holds visiting appointment with UiT, The Arctic University of Norway.

Highly Selective Electrochemical Sensing of Dopamine, Xanthine, Ascorbic acid and Uric acid using a Carbon Fiber Paper
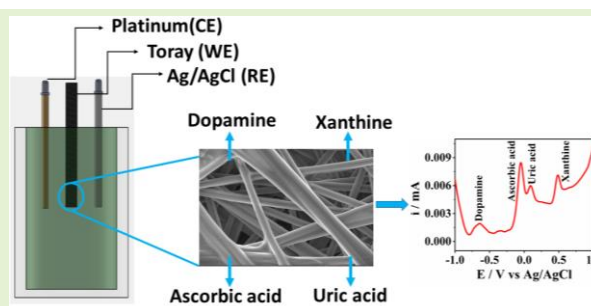
Jaligam Murali Mohan[1], Khairunnisa Amreen[2], Arshad Javed[1], Satish Kumar Dubey[1] and Sanket Goel[2*], *Senior Member, IEEE*

[1]*Department of Mechanical Engineering, Birla Institute of Technology and Science (BITS) Pilani, Hyderabad Campus, Hyderabad 500078, India*
[2]*MEMS, Microfluidics and Nanoelectronics laboratory, Department of Electrical and Electronics Engineering, Birla Institute of Technology and Science, Pilani, Hyderabad Campus, Hyderabad, India.*

Abstract—**Biological compounds like Dopamine (D), Xanthine (X), Ascorbic acid (AA) and Uric acid (UA) play a vital role in food, clinical and human metabolism. Since these compounds are present in biological fluids and have close standard potentials values, it is imperative to choose a method to sense these fluids without any electrochemical interference. Toray paper, with a porous gas diffusion layer imbibed with denser carbon fibers, is an excellent option that can be utilized as a working electrode for such sensing applications. Herein, the electroactivity of these compounds was tested using cyclic voltammetry and square wave voltammetry without any interference. The linear ranges for the compounds (D, X, AA, UA) are 10-1000 µM, 7-300 µM, 100-1000 µM and 30-1000 µM respectively, while the limits of detection are 9.67 µM, 6.54 µM, 97.12 µM and 28.74 µM respectively with S/N ratio of 1.5. Finally, the electrode was tested with human serum samples for the identification of D, X, AA and UA manifesting exceptional reproducibility.**

Index Terms—**Carbon materials; Sensors; Biological compounds; Electrochemical Sensing**

I. INTRODUCTION

Biological compounds like Dopamine (D), Xanthine (X), Ascorbic acid (AA), Uric acid (UA), play an essential role in human metabolism. Xanthine is a byproduct produced during metabolic degradation of purine where, initially, hypoxanthine gets oxidized to xanthine, which in turn gets oxidized to uric acid [1]. It is essential to monitor the levels of uric acid as its abnormality leads to several diseases like pneumonia, leukemia, etc.[2]. Ascorbic acid acts as an antioxidant in drinks, food and medicines, and is used to prevent and treat diseases like infertility, AIDS, cancer, common cold and mental illness[3]. Dopamine is a neurotransmitter in brain tissues and its monitoring helps in understanding the neurological process. Different methods, like spectrophotometry, chromatography, colorimetric and electrochemical, exists for sensing these biological compounds. Amongst all these methods, electrochemical methods are more advantageous over others.

In the recent years, sensors for detecting these analytes have been reported with different surface modifications such as reduced graphite oxide [4] and graphene using electropolymerizing [5] [6] methods. Further, multiwalled carbon nanotubes[7] [8] [9], carbon nanomaterials [10] [11] [12] modified with gold nanoparticles, multiwalled carbon nanotubes with copper polydopamine, carbon paper coated with platinum particles and modified with gold and tin, carbon nano horns have also been reported to sense these analytes [13] [14] [15]. In another work, carbon fiber electrode with the modifications of flower-shaped graphene electrode was used for the determination of uric acid, ascorbic acid and dopamine. Here, a very sharp peak was observed for these analytes, further experiments were also done on urine and human serum samples [16]. Furthermore, carbon mesoporous fibers, modified on glassy carbon electrode, were used for the simultaneous detection of purine bases like cytosine, thymine, adenine and guanine using the differential pulse voltammetry technique [17]. Also, graphene was functionalized on the surface of carbon fibers and the electrokinetics were tested with the standard mediator $[Fe(CN)_6]$. The results showed that

the electrode could be used for biofuel cells and biosensors [18].

Gas diffusion layers embedded carbon cloth consists of dense carbon fibers packed together with more porosity [19]. Owing to its porous and dense fiber properties, these carbon fibers were reported to be used in many applications like electrochemical sensing, fuel cell applications, supercapacitors etc. Polyaniline with electroetching carbon cloth was used as an electrode in a supercapacitor configuration and a capacitance of around 673F/g was achieved. It was observed that after electroetching, the surface area and hydrophilicity increases [20]. In another work, carbon fibers with a nanoscaled manganese oxide film on them were used for the electrochemical capacitors to notice enhanced electrical conductivity.

Further, the electrode was tested after modifications for mechanical bendability leading to achieve excellent results [21]. Similarly, the toray paper acted as biocathode in enzymatic biofuel cells which work using the absorption of the fuel [22]. Furthermore, Toray carbon paper with glucose dehydrogenase modified with multiwalled carbon acts as a biocathode in enzymatic flow through biofuel with maximum power and current density $705\mu A/cm^2$ and $146\mu W/cm^2$ at 1mL/min flow rate [23]. Even though a substantial amount of work has been reported for energy harvesting studies, meare reports, related to the utilization of bare toray paper as a working electrode for electrochemical sensing purposes, is available.

TABLE 1
COMPARISON TABLE WITH THE PREVIOUSLY REPORTED WORKS

Method	Electrode	Limit of Detection (μM)				Ref.
		D	X	AA	UA	
CV, DPV	GCE/L-cysteine/Au-Pt	24	---	103	21	[24]
EIS, DPV	GCE/MWCNT/Pt	0.04	---	20	0.35	[25]
DPV	Chitosan-Graphene	1	---	50	2	[26]
CV, DPV, SQW	PAAQ/Pt	3.05	---	250	115	[27]
EIS, CV, DPV	Fe_3O_4/redGo/GCE	0.08	---	20	0.50	[28]
CV, DPV	GCE/β-cyclodextrin	1.5	1.25	---	5	[29]
DPV, CV	GCE/Ni-SG	0.12	---	30	0.46	[30]
CV, DPV	GCE/HNP-Pt-Ti	3.2	---	24.2	5.3	[31]
CV,DPV	SPE/rGO/β-CD	0.01	---	67	0.02	[32]
CV, DPV	Pd/CNF's	0.2	---	15	0.7	[33]
CV, SQW	Toray Paper	9.67	6.5	97.1	28.7	This Work

In the present work, for electrochemical sensing, carbon Toray paper, with carbon fibers of an average dimension of 6-7 μm, has been utilized as working electrode (WE), platinum as counter electrode (CE) and Ag/AgCl as reference electrode (RE). Literature reports, chemically modified toray as a working electrode [22] [13]. However, to the best of our knowledge, the electrochemical activity of bare toray as an efficient and selective sensor has not been explored yet. The overall sensing mechanism was found to be very particular and tested several times for reproducibility and stability. Table 1 shows some of the existing electrochemical sensors for these bio-component sensing. Further, no interference was observed and the real sample analysis using human serum was accomplished.

II. Materials and Methods

Dopamine (D), Xanthine (X), Ascorbic acid (AA) and Uric acid (UA) were purchased from Sigma Aldrich. Sodium Phosphate monobasic anhydrous (NaH_2PO_4), Sodium Phosphate dibasic dihydrate ($Na_2HPO_4.2H_2O$) were procured from AVRA chemicals. The DI water, which was used throughout the experiment from Milli-Q (18.2) MΩ.cm. The Toray paper (TGP-H-060) was obtained from the Fuel Cell Store. The Potentiostat purchased from (SP-150, Bio-Logic, France). The Scanning Electron Microscope (SEM) was carried out using Apreo scanning Electron microscope from Thermo Fisher Scientific.

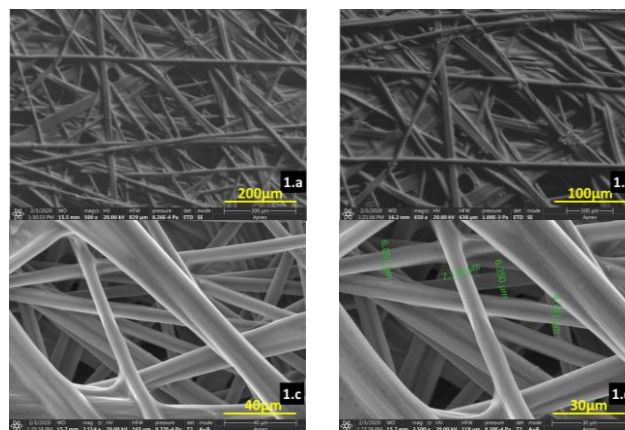## III. Experimental Procedure

A. SEM Analysis

Fig 1. SEM Analysis of toray paper with different magnifications

The SEM analysis was carried out to understand the morphology of the toray paper. As shown in Fig.1(a-d), under different magnifications, the structure revealed a fiber network of carbon fibers with an average diameter of 6-7μm.These networks helped the electron transfer mechanism to take place for the oxidation of the aforementioned biological compounds.

B. Electrocatalytic activity of the electrode

The electro-catalytic oxidation of D, X, AA and UA with the toray paper electrode with working volume of 4mL under the atmospheric conditions of 24^0C was analyzed by potential windows of -0.5 to 0.5 V, 0 to 0.8 V, -0.4 to 1V and -0.7 to 1 V, respectively using cyclic voltammetry with and without 1 mM of concentration of analytes in pH 7 PBS. At 10mV s^{-1}., it was observed that D, X, AA and UA undergoes oxidation with S/N ratio of 1.5 at potential values of 0.15 V, 0.45 V, 0.40 V and 0.2 V respectively (Fig. 2(a), 2(b), 2(c) and 2(d)). Distinctive and sharp oxidation peaks were obtained with each of the analytes.
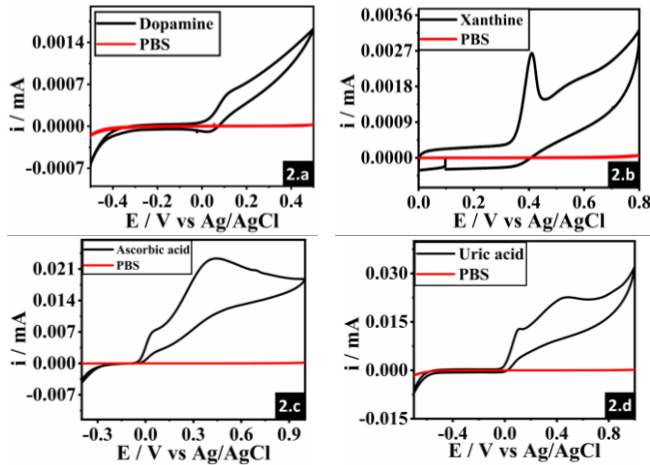

Fig 2.CV response of 1.(a) Dopamine,1.(b) Xanthine, 1(c) Ascorbic acid and 1(d) Uric acid

C. Effect of scan rate

The effect of the variable scan rate on the electrocatalytic oxidation of D, X, AA, UA via toray working electrode has been analyzed. The corresponding CV responses showed a well-ordered increment in the anodic peak current value with increasing the scan rate,as shown in Fig 3(a), 3(c), 3(e) and 3(g). This analysis helps to study the electron transfer mechanism. Here, toray paper is used with 1 mM of the analytes.

It can be further observed that there was a linear increase in the anodic current with the increase in scan rate for all the analytes (Fig. 3(a), 3(c), 3(e) and 3(g)). Corresponding calibration plots (Fig. 3(b), 3(d), 3(f) and 3(h)) with base-line corrected peak current for a modified electrode with D, X, AA and UA values were plotted. Experimental linearity manifests that the toray paper electrode had a surface-confined electron-transfer behavior. Usually, such type of electrochemical surface-confined transfer reaction follows the Randles Sevcik equation [4].

$$i_{pa} = 2.99 \times 10^5 n[(1-\alpha)\, n_a]^{1/2} ACD^{1/2} v^{1/2}$$

α = transfer coefficient
n = total no of electrons
A = electrode surface area
C = concentration of purines
υ = scan rate
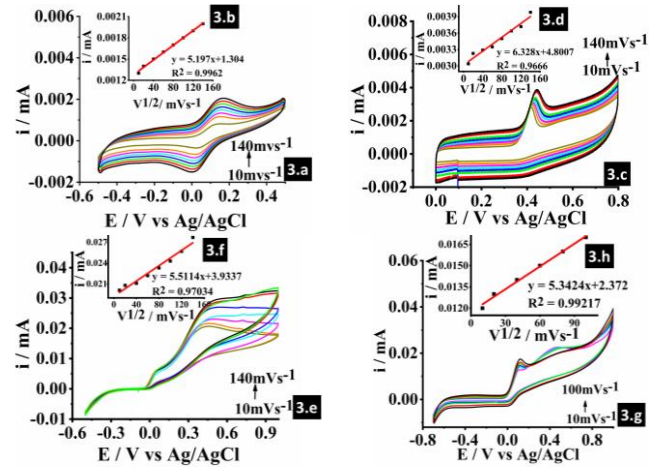n_a = no of electrons involved in rate determining step

Fig. 3.CV response of (a) Dopamine (c) Xanthine (e) Ascorbic acid (g) Uric Acid at various scan rates. Corresponding baseline calibrated plots for oxidation peak currents ipa vs v1/2 (b) D, (d) X, (f) AA and (h) UA

D. Effect of concentration

The effect of increase in concentration was studied as shown in Fig. 4(a), 4(c), 4(e) and 4(g). It was performed using square wave voltammetry (SWV) within the optimized parameter ranges i.e., Initial potential (Ei) = -0.5, 0, -0.4,-0.7, Final potential (Eo) = 0.5, 0.8, 1, 1, Pulse height 25.0 mV, Pulses width =50.0 ms, and Step height = 10.0 mV., From the figures 4(a), 4(c), 4(e) and 4(g), it is clearly manifested that, there is an increase in oxidation current with increase in the concentration of analytes.The linear ranges of concentration for different analytes are : Dopamine (10-1000µM), Xanthine (7-300 µM), Ascorbic acid (10-1000 µM) and Uric acid (30-1000 µM). From the respective inset calibration plots, (shown in 4(b), 4(d), 4(f) and 4(h)), the limit of detection (LOD) is calculated as 9.67µM, 6.54µM, 97.12 µM and 28.74 µM.
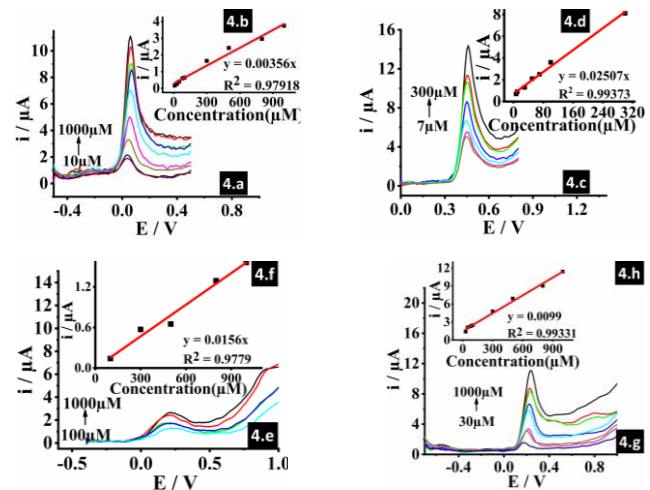

Fig. 4.Square wave voltammetry response of (a) D, (c) X, (e) AA and (g) UA in 0.1M of PBS. Baseline corrected calibration plots (b) D, (d) X, (f) AA and (h) UA.

E. Interference effect

Since all these four biochemicals are co-existing in the physiological system, it was necessary to analyze the effect of their interference with each other. Each analyte must give a distinct oxidation peak when they are present together. In order to study the effect of interference amongst the analytes SQW was performed with parameters as : Initial potential (Ei) = -1, Final potential (Eo) = 1, Pulse height 25.0 mV, Pulses width =50.0 ms, and Step height = 10.0 mV. Fig.5(a), clearly depicts four distinct oxidation peaks at -0.68V, -0.08V, 0.07V, 0.46V corresponding to D, AA, UA, X respectively.In addition to this, the interference from various other co-existing biochemicals like hypoxanthine, glucose, cysteine, nitrate, nitrite and hydrogen peroxide were tested. As can be seen in Fig. 5(b-e), there is negligible interference observed, as the current value in the presence of all the chemicals remained unchanged.
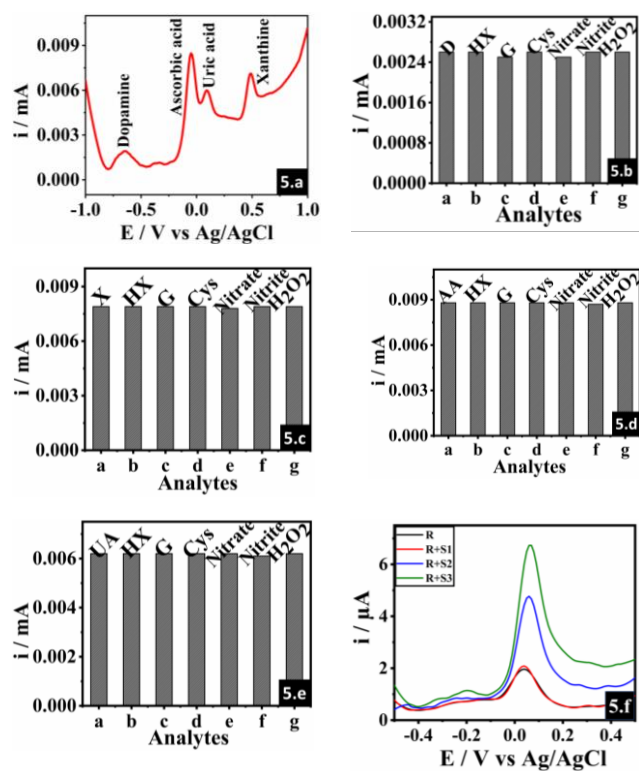

Fig.5.(a) Square wave voltammetry response of effect of interference with 0.1M PBS pH 7 (b-e): Interference with other biochemicals (f) SQW response of real sample of serum – dopamine concentration by standard addition approach with 30μM of standard concentration per every spike,S1-S3

F. Real sample study

For studying the applicability of the sensor in the real-time and to find the selectivity and sensitivity in real sample analysis, sensing of D, X, AA, UA in human serum samples was explored. Human blood serum samples were collected from the Medical center of BITS Pilani Hyderabad Campus. The standard addition approach was adopted for this, wherein the standard spiked concentration within their corresponding linear ranges was taken.The result for one of the analytes is demonstrated in Fig. 5(f), wherein, 30 μM dopamine is spiked as standard concentration thrice, (S1-S3) and R- represents the

real sample. Likewise, the other analytes were also tested via standard addition approach. The results were tabulated. Table 2 represents the observations from real sample analysis, and it is found that the added and found values gave near 100% recovery.

TABLE 2
REAL SAMPLE ANALYSIS OF HUMAN SERUM

Samples	Added(μM)	Found(μM)	Recovery(%)
	30	29.15	97.17
Dopamine	50	50.10	100.20
	80	80.02	100.03
	10	10.13	101.30
Xanthine	30	30.02	100.07
	50	49.98	99.96
	100	99.18	99.18
Absorbic aid	300	300.01	100.00
	500	501.03	100.21
	30	29.99	99.97
Uric acid	50	49.56	99.12
	80	80.12	100.15

IV. Conclusion

The present work deals with the usage of bare toray paper as a working electrode. The electrocatalytic effect of plain toray is examined using platinum as a counter electrode and Ag/AgCl as a reference electrode. Cyclic voltammetry was performed for the oxidation of biochemicals D, X, AA, UA with toray. A perfect oxidation peak at distinct non-interfering oxidation potentials was obtained. The electron transfer mechanism was analyzed using cyclic voltammetry at different scan rates. It was found that the mechanism was a surface-confined reaction. The effect of increase in the concentration of all the analytes was also examined. The linear ranges chosen were 10-1000 μM, 7-300 μM, 10-1000 μM and 30-1000 μM for D, X, AA, UA respectively. The limit of detections obtained was 9.67 μM(D), 6.54 μM(X), 97.12 μM(AA) and 28.74 μM(UA). The toray paper was subjected to interference study to ensure interference mitigation from common biochemicals like hypoxanthine, glucose, cysteine, nitrate, nitrite and hydrogen peroxide for these analytes. Further experiments were conducted with a real sample of human blood serum providing exceptional sensing capability. Hence, the bare toray paper provides excellent specificity and selectivity with an impressive limit of detection and has immense potential for usage in real-time testing.

V. ACKNOWLEDGMENT

VI. References

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Jaligam Murali Mohan has received his M.E degree in Mechanical Engineering from Osmania University and B.Tech in Mechanical Engineering. He is pursuing his Ph.D. from Mechanical Engineering, BITS Pilani, Hyderabad Campus, India, under the supervision of Satish Kumar Dubey and Sanket Goel. His research interests are Microfluidics, MEMS devices and Microfluidic electrochemical platforms integrated with IoT.

Khairunnisa Amreen is currently working as National Post Doctorate (NPDF) for DST- SERB. She has graduated B.Sc. in three majors (Chemistry, Biotechnology and Microbiology) from Osmania University-Hyderabad (2008-2011). Post which she completed her Masters in M.Sc. Analytical Chemistry from Vellore Institute of Technology- Vellore, Tamil Nadu, India (2011-2013). Following which she continued her Ph. D. from Vellore Institute of Technology- Vellore, Tamil Nadu, India she published 9 international/national research articles and filed one Indian patent for the blood-based biosensor developed. Presently she is working as NPDF-DST SERB under the supervision of Dr. Sanket Goel, Head & Associate professor, EEE, BITS-Pilani, HYD.

Arshad Javed received B.Tech degree in Mechanical Engineering from Bundelkhand Institute of Engineering and Technology, Jhansi, U.P. (2004), M. E. in Mechanical Engineering from BITS PILANI, Pilani Campus (2008) and Ph. D. in Mechanical Engineering (Topology Optimization) from BITS PILANI, Pilani Campus (2014) .He is currently working as an Assistant Professor in the Department of Mechanical Engineering BITS Pilani, Hyderabad Campus,India. His current research interest is Topology optimization, Compliant mechanisms, Micro Mechanisms, Robotics and Automation Systems, Mechatronic systems, Automation of Micro-fluidic systems.

Satish Kumar Dubey is Assistant Professor at Mechanical Engineering Department, BITS Pilani, Hyderabad Campus with qualification Ph.D. (BITS, Pilani), M. Tech. Thermal Engineering (MANIT, Bhopal). His research interest includes Computational Heat Transfer, Simulation of Thermal Systems, Microfluidics, MEMS etc.

Sanket Goel received the B.Sc. (H-physics) degree from Ramjas College, Delhi University, the M.Sc. (physics) degree from IIT Delhi, and the Ph.D. (electrical engineering) degree from the University of Alberta, Canada, under the NSERC Fellowship. He headed the R&D Department and worked as an Associate Professor with the Electronics and Instrumentation Engineering Department, University of Petroleum and Energy Studies, Dehradun, India, from 2011 to 2015. He is currently an Associate Professor and Head, Electrical and Electronics Engineering Department, BITS–Pilani, Hyderabad Campus, Hyderabad, India. He has >60 publications, 6 patents (1 U.S. and 5 Indian) to his credits, delivered >50 invited talks, and guided 6 Ph.D. and 10 master's students. His current research interests are microfluidics and nanotechnology, materials and devices for energy (both conventional and renewable), and biomedical applications. He has won several awards during the course of his career, including the Prestigious Fulbright-Nehru Fellowship (2015), the Young Scientist Award (2013), the Best Students Paper Award (2005), and the Ph.D. Thesis Award (2005).Currently, he is the Associate Editor of the IEEE Sensors Journal and IEEE Access, and holds visiting appointment with UiT, The Arctic University of Norway.

Portable 3D printed Electrochemiluminescence Platform with Pencil Graphite Electrodes for Point of Care multiplexed analysis with Smartphone based Read-out

Mary Salve, Aurnab Mandal, Khairunnisa Amreen, BVVSN Prabhaka Rao, Prasant Kumar Pattnaik, Sanket Goel*

MEMS, Microfluidics and Nanoelectronics Laboratory, Department of Electrical and Electronics Engineering, Birla Institute of Technology and Science, Hyderabad 500078, India

*Corresponding Author : sgoel@hyderabad.bits-pilani.ac.in

Abstract:

Herein, a portable 3D-printed miniaturized bipolar electrode-electrochemiluminescence (BPE-ECL) platform is presented. The platform has a smartphone enabled read-out method for ECL signal analysis, 9 V Hi-watt battery for power supply and graphtized mesoporous carbon/multiwalled carbon nanotube (GMC/MWCNT) nanomaterial modified pencil graphite electrodes (PGE) as bipolar and driving electrodes. The nanomaterial modified pencil graphite bipolar electrode (PG-BPE) showed great electro-catalytic activity towards luminol-H_2O_2, luminol-O_2 ECL reaction in neutral medium. The sensitized luminol-O_2, luminol-H_2O_2 reactions were successfully applied for sensing of H_2O_2, O_2 and CO_2. With optimize parameters determination of H_2O_2, O_2 and CO_2 can be in the linear range of 0.08 μM -5000 μM, 0.3 - 9 mg/L, 0.6 - 9 mg/L   with detection limit of 0.069 μM, 0.15 mg/L, 0.45 mg/L. As a prototype application, quantitative detection of glucose has been carried out with modified PGE anchored with GOX (GMC/MWCNT@GOX). The prepared electrode was analysed for physico-chemical and microscopic characterizations. The modified PGE showed an excellent ability to detect glucose in a linear range of 1 μM – 10 mM with a detection limit of 0.31 μM. Finally, the platform was subjected to real sample analysis of H_2O_2 in clinical H_2O_2, cosmetic bleach,

O_2 and CO_2 in lake water and tap water, and glucose in human blood serum samples. The results indicated that the proposed platform offered excellent reliability, accuracy and amenable to be used for multiple point-of-care biochemical analysis.

Keywords: 3D-printed miniaturized platform; bipolar electrodes; Electrochemiluminescence; pencil graphite; smartphone diagnostics

1. Introduction:

Development of chemiluminescence (CL)sensors is an active research area due to their proven utility to detect metal ions, antioxidants and various biochemical [1]. Amongst different CL methods, the electrochemiluminescence (ECL) detection gained more attention owing to features like absence of background signal, excellent controllability over the position or time of light emission, and possibility to enhance ECL intensity with nano-material [2]. Therefore, ECL is a highly sensitive analytical method for a wide range of applications. It involves the process of light emission at the electrode surface due to exergonic electron transfer reaction [3].

Typically, an ECL assay was accomplished by harnessing two or three electrode system [4], [5]. However, a few ECL assays are implemented using bipolar electrode (BPE) either in open or closed system. A BPE is an electronic conductor that gets exposed to an applied electric field without any direct contact with the electric circuit. A uniform electric field is applied across the solution by providing voltage to the two driving electrodes (DE). As a result, the potential difference is generated between the BPE and the electrolyte. The redox reaction is driven at the two poles of BPE due to anodic or cathodic over potentials. Such platform has advantages such as no need of direct contact, inexpensive, integration with portable system and possibility of high throughput analysis [6].

Arora et al.developed the first BPE-ECL based miniaturized sensor with U-shaped platinum film as a BPE [7]. Subsequently, several research groups have developed BPE-ECL based miniaturized devices [4], [5]. The devices developed by Crook and Wang group consists of poly(dimethylsiloxane) (PDMS) based microchannel irreversibly bonded to a glass slide fabricated using standard lithography procedure with indium tin oxide (ITO) [8] or gold as a BPE [9].There are a couple of reports wherein the BPE was placed inside the capillary tube to perform BPE-ECL.

For integrating electrode in PDMS, miniaturized devices, techniques like electrode insertion, usage of screen printed electrode (SPE) and a single step fabricated 3D printed device with electrode have been adopted to gain accuracy [10], [11], [12], [13], [14]. However, while performing biological studies, experimental replication from the multiple samples were usually required. Hence, device-to-device or electrode reproducibility became a major concern. In recent few decades, efforts have been made to create hybrid, reusable devices using standard lithography process. Nevertheless, these cells require expensive device fabrication process and electrode materials. Moreover, expensive peripheral device, such as CCD camera, Photomultiplier Tube (PMT) or ECL analyser for data collection, bulky DC power supply to provide the potential and external micro pumps for injection and extraction of solution, are generally required. Therefore, an alternative and novel BPE-ECL method to overcome these limitations is necessary.

Usually, ITO, gold, platinum and carbon based electrodes have been the conventional electrodes used in ECL sensing. In comparison to these traditional electrodes such as glassy carbon electrode(GCE) [15], [16], pencil graphite electrodes (PGE), gives an advantage of high electrochemical reactivity, mechanical rigidity, reproducibility and ease of modification that are often necessary to develop disposable electrochemical sensors [17]. Considering these advantages, PGE are often used in analytical application and can be used as BPE.

Luminol is an inexpensive and widely-reported classic reagent that can be used for both CL and ECL reactions, different mechanisms have been proposed for ECL reaction of luminol. The luminol ECL reaction happens in the presence of hydrogen peroxide (H_2O_2) or also in dissolve oxygen but higher ECL intensity is obtained with H_2O_2. Recently, various efforts are made to sensitize and use luminol ECL reaction for detection of biological compounds. The luminol ECL reaction can be sensitize by the production of reactive oxygen species when specific potential is applied. The electrogenerated reactive species present at electrode surface oxidise luminol, and the product of luminol generates the excited state 3-aminophthalate anion which emit light.

The electrode modified with functionalized carbon nanomaterial is another way to produce and enhance ECL intensity. The sensitized luminol reaction can be used for detection of superoxide dismutase which is based on the quenching effect of luminol O_2 ECL system [18]. The sensitized ECL reaction make a probability of sensing dissolve O_2 and CO_2 in food, environmental and biomedical industry. A reliable, rapid, cost-effective, portable gas sensor is still an issue. Determination of dissolved oxygen (O_2) and carbon dioxide (CO_2) in aqueous solution is of great importance many efforts have been made to detect dissolve oxygen using CL, electrochemical and optical sensor, but few papers are reported using ECL technique [19].

In the present work, a portable 3Dprinted miniaturized platform (PG-BPE-ECL), integrated with 9V Hi-watt battery and a smartphone for ECL signal analysis is proposed. The key features of PG-BPE-ECL are: (i) reusable (sample can be injected directly using a micro-pipette) (ii) the PGE is readily available and inexpensive (iii) requires low driving potential with minimum energy consumption (a 9V battery can be used for 20 hours), (iv) usage of smartphone as a read out. Henceforth, the platform is appropriate for portable biochemical analysis at a point-of-care level. For method validation and authenticate the practicability, the fabricated PG-BPE-ECL was used for determining H_2O_2. In addition, the applicability of the

proposed platform was explored for O_2, CO_2 and glucose sensing with modified and optimized PGE.

2. Experimental Section

2.1 Chemicals, Materials and Apparatus

The details of chemical, materials, instrumentation and electrode preparation used in this work are available in Supplementary Information (S1)

2.2 3D Printed Device Design and Fabrication

Detailed 3D printed miniaturized device design (**Scheme. IA**) and fabrication procedure are provided in supplementary information (S2). 3D printed device CAD model (**Scheme. IC-D**) with length as 25 mm, width as 10 mm and height as 8 mm with total volume of the reagent reservoir as 320 μL±10 μL. The open reservoir for BPE of dimension 9 mm × 4 mm × 3 mm was in the middle in contact with the DE for the fluid to flow and interact with all the three electrodes. A holder for the driving electrode (DE) was fabricated with dimensions 4 mm × 4 mm ×6 mm. The BPE and the DE were kept equidistant (~3 mm) for achieving the potential difference across electrode. The developed platform was fully reusable, after each experiment, the platform was cleaned using water gun and was dried in oven at 40°C for 30 sec.

2.3 Design and Assembly of Portable ECL Platform

Scheme. II A shows the overall design of portable ECL platform comprising a dark-box, miniaturized ECL sub-system (2.5 cm×1 cm×0.8 cm), 9V hi-watt battery (operating current 500 mA), electronic circuit module and a smartphone. The3D-printed miniaturized device (**Scheme. II B-C**) and dark-box (**Scheme. II D- E**) was prepared using a black acrylic sheet (4 mm) with dimensions 17 cm × 8 cm × 10 cm. The voltage was regulated, maintained and displayed using an electronic circuit module LM2596. From the potentiometer, the output

voltage was regulated for the electrodes, and with adequate driving potential, the ECL signal was captured using a smartphone. The smartphone camera was placed on top of the miniaturized ECL platform at a distance of 10 cm to ensure that the captured image is clearly focussed. The ECL images were captured clearly without any mechanical movement of the platform or smartphone, and were wirelessly transmitted to desktop for rapid analysis. The overall cost of the components of the portable ECL platform was less than $8 (detailed cost estimate in given in **Table S3**), excluding the cost of the smartphone.

2.4 Data acquisition and Analysis

The captured images were recorded in JPG format and were analysed using ImageJ software (openware free imaging software). An automated image processing macro program was created in ImageJ for batch image analysis. As a result, the developed program measured the relative light intensity after converting them, into 8-bit mode. This was followed by image inversion to acquire a white background for light area, with different shades of gray area to capture the ECL intensity leading to the calibration of optical density. The gray spot with 1700 pixels area on the BPE anode was analysed for each image and mean gray values were obtained. The calculated relative light intensities value was in relative light units (RLU). With this automated program, the images with maximum ECL intensity were easily obtained. The attained ECL data was used and calibration graph were plotted.

2.5 Preparation of nanomaterial modified PG-BPE

GMC decorated MWCNT-COOH composite were prepared by adding 1:1 ratio of both in ethanol and then ultra-sonicated in water bath for 15 mins. The prepared suspension of GMC and MWCNT was drop casted on the anode part of the BPE and was dried in oven at 90°C for 10 mins. Control experiments after each modification were performed (Supplementary Fig. S4) to study the role of each layer. It was discovered that the ECL intensity increased with each

modification. The prepared composite was used for H_2O_2, CO_2, O_2 sensing. For glucose sensing, GOX enzyme was anchored to the modified PGE by immersing in the GOX enzyme solution of 5 U mL^{-1} for 30 minutes followed by air drying for 30 minutes.

3. Results and Discussion:

The PG-BPE-ECL assays based on the luminol/H_2O_2 reaction system were performed to explore the applicability of the system. The luminol/ H_2O_2 reaction mechanism was proposed by Marquette and Blum [20] (supplementary information S5).The detailed principle is given in the supplementary information S6 and is shown in **Scheme. 1B**. Smartphone was used as read-out method for ECL signal, avoiding CCD camera and PMT module. Evidently, smartphone was used as an effective readout method for colorimetric detection in miniaturized devices but seldom used for ECL signal analysis [21].The smartphone was placed on blackbox and its camera was aligned with the PG-BPE-ECL platform with focus kept on BPE anode. For clear capture of ECL image, it was observed that the distance should be ~10 cm (for less than 10 cm, images were blurred whereas for more than 10 cm it was difficult to capture).

3.1 Characterization of nanocomposite modified Electrode

Modified electrode was characterized by physico-chemical and microscopic characterization. **Fig. 1** shows the SEM characterization of GOX modified and unmodified systems. **Fig. 1A** shows dark sphere like structure describing the attachment of GOx on PGE, **Fig. 1B** shows PGE modified with MWCNT+GMC where agglomerated spheres and tube type structures indicates the basic structure of MWCNT+GMC composite. **Fig. 1C** reveals that GOX has covered the entire layer of composite (GMC/MWCNT).

Fig. 1D-F depicts the physico-chemical characterizations by Raman, UV-Vis, and FT-IR spectroscopy of PGE/MWCNT + GMC and PGE/MWCNT+GMC@GOx modified electrodes. Raman Spectroscopic response (**Fig. 1D**) of PGE/MWCNT+GMC with (a)and

without (b) GOx showed D and G band at 1350.6 cm^{-1},1583.77 cm^{-1} and 1336 cm^{-1},1594 cm^{-1} respectively. The G-band, corresponding to the graphitic nature (Sp2 carbons), showed a significant decrement while the disordered graphitic structure (Sp3 carbons), due to oxygen functional groups, showed an increment post modification with GOX [22]. The I_D/I_G ratio for GOx unmodified system was 0.71 whereas, GOx modified system gave value of 1.17, which is due to π-π interaction between the GOx enzyme and graphitic units of GMC+MWCNT[23]. UV-Vis response (**Fig. 1E**) of the fabricated electrode before and after modification with GOx showed almost similar absorption peak only with a slight bathochromic shift 312 nm to 312.5 nm indicating the insensitivity if this technique for identification of GOx immobilization. The FT-IR characterization (**Fig. 1F**) showed peaks at 3146 cm^{-1}, 1640 cm^{-1}, 1540 cm^{-1} and 1110 cm^{-1} corresponding to O-H (stretch), C=N (stretch), N-O (stretch) and C-O (stretch) respectively for GOx alone. On the other hand, the composite of MWCNT+GMC showed 3129 cm^{-1} (O-H stretch), 1401 cm^{-1} (O-H bend) and 1121 cm^{-1} (C-O stretch) due to the carboxylic groups on MWCNT. Upon modification of the carbon matrix with GOX, there was a substantial increase in (O-H) stretching at 3140 cm^{-1} (plausibly due to OH groups of GOx). Similarly, peaks at 1642 cm^{-1} (C=N), 1537 cm^{-1} (N-O) and 1113 cm^{-1} (C-O) were also significantly observed indicating immobilization of GOX on MWCNT+GMC surface.

3.2 Effect of length of PGE on driving potential

Investigations were carried out on the effect of PG-BPE length utilized with respect to the driving potential keeping DE length constant as 12 mm. The reservoir was first filled with 320 μL mixture of 10 mM luminol and 1 mM H_2O_2. As the ECL intensity is related to BPE length, the study was done on 2 mm diameter PGE with length ranging from 1 mm – 7 mm. As shown in **Fig.2A & B**, the intensity on BPE is directly proportional to the length, with the maximum obtained at 7 mm. Hence, 7 mm length was chosen to achieve the strongest ECL intensity (**Fig.**

2A & B). With optimized driving potential, longer BPE goes high partial potential, which leads to lower ECL initial potential and strong ECL intensity. It was witnessed that the ECL intensity increases with the increase in driving potential from 3 V to 7 V, which can be due to the increase in EC oxidation rate of luminol to form 3-aminophthalate. With the driving voltage less than 3 V, no ECL signal was obtained, as the potential is not sufficient to oxidise the luminol. Whereas, with more than 7 V potential, the ECL signal decreased plausibly due to background reaction like oxidation of H_2O releasing oxygen. The optimized length was 7 mm as greater than this reduced the intensity.

3.3 Effect of Luminol Concentration

Herein, luminol works as a luminescence reagent, hence, its concentration plays a key role in the ECL response. As shown in **Fig. 3 A & B**, the ECL intensity grows with the increase in luminol concentration ranging from 1 mM to 7.5 mM. This is possibly due to the luminolendoperoxide formation as the concentration increases [24]. Nonetheless, the ECL intensity decreases with more than 7.5mM luminol, credibly due to the self-quenching effect [25].

3.4.1 ECL behaviour of luminol-H_2O_2 system

Under optimum conditions, PG-BPE-ECL platform was used for quantification of H_2O_2. Based on charge balance mechanism, the ECL intensity increased with gradual increase in H_2O_2 concentration over the range from 5 μM to 5000 μM as shown in **Fig.4 A & B.** It was observed that the ECL intensity was directly proportional to H_2O_2 concentration ranging from 0.08 μM to 100 μM (inset in **Fig.4B**) whereby, the limit of detection (LOD) was estimated to be 0.069 μM The measured LOD was lower than that obtained using carbon ink SPE in paper based BPE-ECL device (46.6 μM), cloth based BPE-ECL (24 μM) [26] using CCD as an imaging system and using commercial chemiluminescence imaging system with PDMS-ITO glass

microchip (60 µM) [27].The proposed method gives wide linear range, with better sensitivity and LOD. The LOD was calculated using the given formula where σ is standard deviation,

$$LOD = \frac{3\sigma}{SLOPE}$$

3.4.2 ECL behaviour of luminol-O$_2$ System

In luminol – O$_2$ ECL system, the dissolve oxygen is considered to be endogenous co- reactant that generates O$_2$ on the working electrode reacting with luminol anion that emits light. For determining the efficiency of the luminol – O$_2$ process, the experiments were carried out with GMC modified PG-BPE ina working electrolyte of luminol in neutral and alkaline aqueous medium (0.5 M phosphate buffer solution). It was found that in neutral aqueous solution, the ECL signals were greatly enhanced as compared to that of alkaline medium. This may be attributed to proton as well as hydroxide ion as a quencher in ECL reaction [28]. The ECL measurements were carried out under saturated O$_2$, N$_2$ and in air electrolyte solution. It was observed that under saturated O$_2$, strong ECL signal were obtained and in N$_2$ hardly any ECL signal were observed. When the air free solution was prone to air, ECl signals were observed and its intensity increase respect to prone time, with the result that ECL signal are dependent on O$_2$. It seems that at higher concentration of dissolved O$_2$ give higher ECL intensity as it might be able to enhance O$_2$ adsorption.

Under the optimal condition, luminol - O$_2$ system, it was observed that ECL intensity is dependent on the concentration of dissolve oxygen. As shown in F**ig.5 A & B**, the ECL intensity increases with increase in oxygen concentration. The ECL intensity was observed to be linear in the range of 0.3 - 9 mg/L with a detection limit of 0.15 mg/L. The measured LOD was lower than obtained using fluorescence probe sensor of 0.35 mg/L [29] and optical fibre sensor method giving LOD of 0.98 mg/L [30].

3.4.3 ECL behaviour of luminol-CO_2 system

In CL, it has been reported that CO_2 greatly increase the luminol CL in the presences and absence of added oxidant. But no studies are reported in ECL for CO_2 sensing with and without added oxidant. For verifying the feasibility of CO_2 gas sensing, the experiments were carried out in presence and absence of added oxidant (H_2O_2). It was observed that in the absence of added oxidant no ECL signal was obtained. The plausible principle of CO_2 ECL sensing can be CO_2 reacts with H_2O_2 to form peroxycarbonate, that decomposes to highly reactive radical anion which are capable of oxidizing luminol [31], [32].The concentration of sensing solution was optimized as luminol (7.5 mM) and H_2O_2 (100 μM).

As expected, the ECL signal increases with an increase in CO_2 concentration with the optimized PG-BPE in the linear range of 0.60 – 9 mg/L. Corresponding calibration plot (**Fig.6B**) gives a slope value of 0.666 ± 0.0295 and R^2= 0.994with an appreciable detection limit of 0.45 mg/L.

3.4.4 ECL behaviour of Luminol-glucose system

Glucose sensing was studied by anchoring Glucose oxidase enzyme (GOx) on the PGE electrode surface. The developed platform should have the potential for glucose assay, as H_2O_2 detection is usually the basis for glucose biosensing. For this, the PGE was chemically modified with MWCNT+GMC composite and GOx enzyme and has been shown in **scheme. 1C**.

Further, the optimal pH, drying time, concentration of enzyme was also optimized based on the intensities obtained (Supplementary S7).

3.4.5 Glucose detection by the proposed sensor

As a model system, glucose sensing in PBS was performed in the range 1 μM to 10000 μM. As shown in **Fig.7 A & B**, the glucose concentration increases linearly from 1 μM to 30 μM (inset in **Fig.7B**), whereby the LOD was estimated to be 0.30 μM. The obtained results

show that the PG-BPE-ECL platform has the ability to detect glucose and can provide low LOD in comparison to the previously known procedures. Even though the direct comparison at present stage is difficult as different detection methodologies have been used elsewhere, but it is significant that the present detection limit is much lower than that obtained using colorimetric, electrochemical and electrochemiluminescence methods as tabulated in **Table. S8** (supplementary information S8).

Besides, the stability, reproducibility and selectivity of the proposed ECL biosensor was also tested (supplementary information S9). It was found that the fabricated electrode is highly stable upto2 days with less than 2% error. Also, upon testing with various biochemical, the electrode showed high selectivity (supplementary information S9).

3.5 Real sample analysis

In order to find the selectivity of the prepared electrode in real sample, sensing of the analytes like clinical H_2O_2 and cosmetic bleach, lake water, tap water and human serum were tested for H_2O_2, O_2 CO_2 and glucose. Real Sample preparations are mentioned in supplementary information S10. **Table 1 and 2** shows the real sample analysis recovery values indicating the applicability of the system. Here the recovery was above 100% may be due the calculated concentration of the reaction contain more impurities which may result in greater concentration value than it actually would be if the real sample used was pure. This is considered as positive error [33-34].

4. Conclusion:

In the present work, a portable ECL platform is presented integrated with PG-BPE-ECL, 3D printed miniaturized device and a battery with smartphone imaging. With luminol/H_2O_2 as the base for ECL reaction, the developed platform gave an excellent O_2, CO_2 and glucose detection. The developed ECL system gives high throughput and sensitive biochemical analysis that can be easily performed at a POC level with corresponding limit of detection of H_2O_2, O_2, CO_2 and

glucose as 0.069 µM, 0.15 mg/L, 0.45 mg/L and 0.31 µM respectively. In future, the developed portable ECL system may find board application in environmental monitoring, bimolecular interaction and clinical diagnostics.

Acknowledgement

## Conflict of Interest

The authors have no conflict of interest

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Scheme I
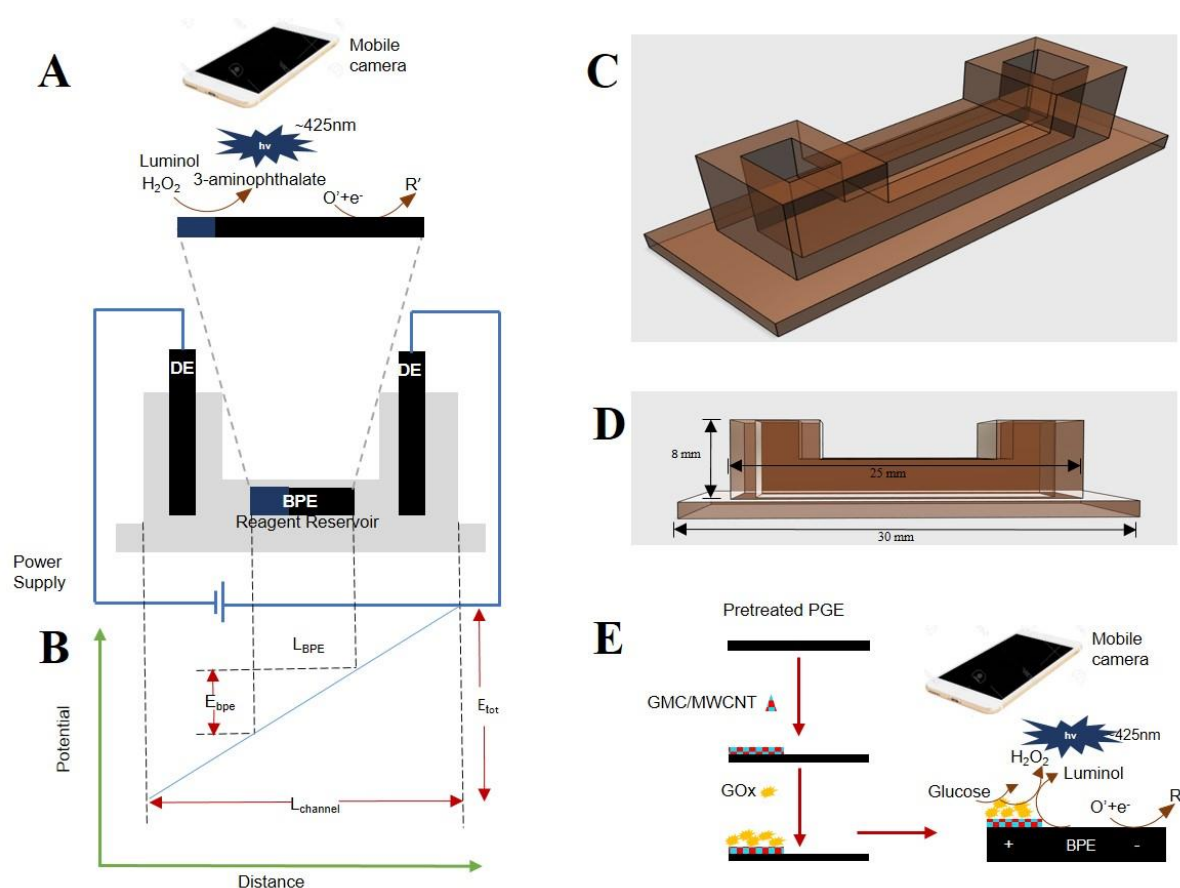
Scheme I. Schematic illustration of the proposed design and sensing principle (**A**) Proposed 3D printed miniaturized design(BPE: bipolar electrode; DE: driving electrode). (**B**) Principle of Open-BPE-ECL for biochemical analysis. (**C-D**) Side and front view of CAD model of proposed design. (**E**) PG-BPE modification process and luminol based sensing for glucose detection

Scheme II
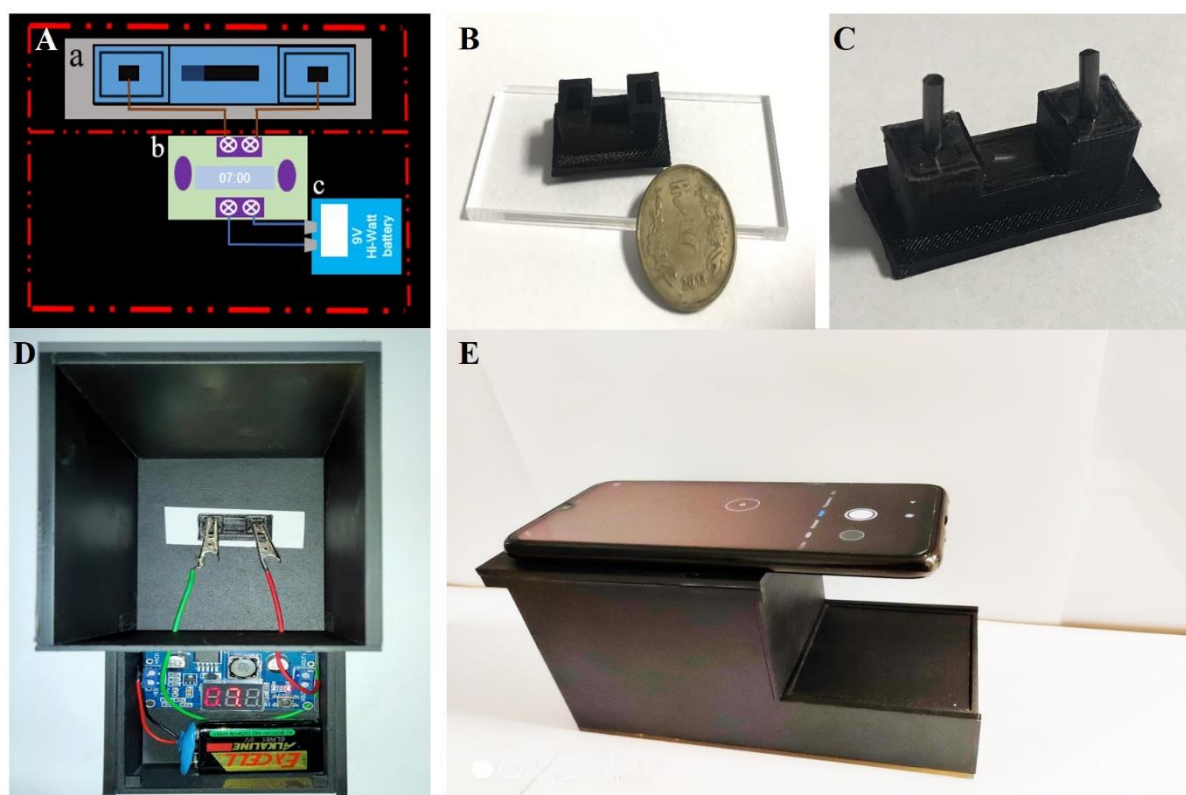
Scheme. II Schematic of the proposed platform (**A**) Illustration of the proposed platform (top-view) (a) PG-BPE-ECL 3D printed platform (b) electronic circuit module (c) 9V battery. (**B**) Picture of 3D printed miniaturized channel and (**C**) bonded with acrylic sheet for optical window containing with PG-BPE and DE. (**D-E**) Dark box open and closed view
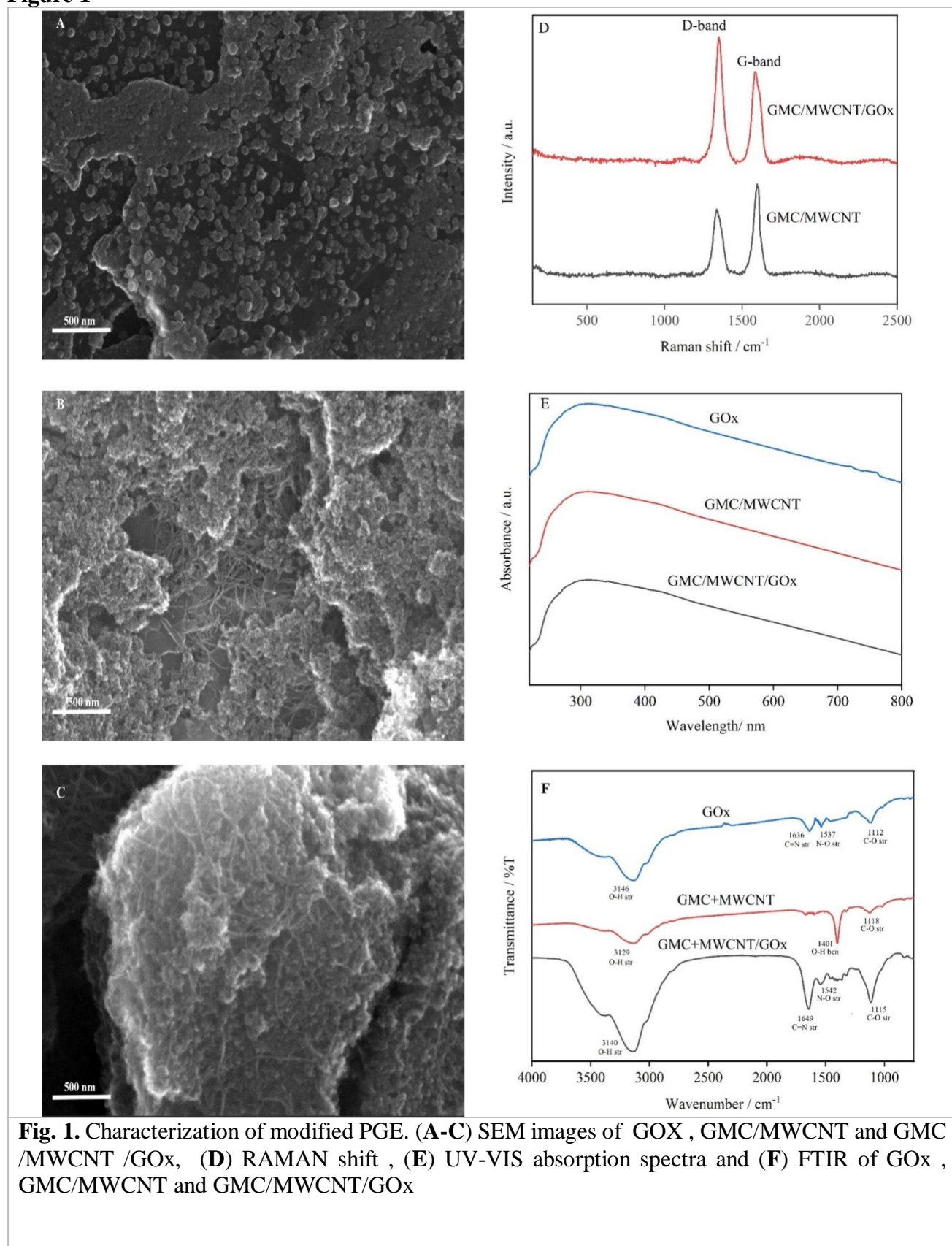
Figure 1

Fig. 1. Characterization of modified PGE. (**A-C**) SEM images of GOX , GMC/MWCNT and GMC /MWCNT /GOx, (**D**) RAMAN shift , (**E**) UV-VIS absorption spectra and (**F**) FTIR of GOx , GMC/MWCNT and GMC/MWCNT/GOx
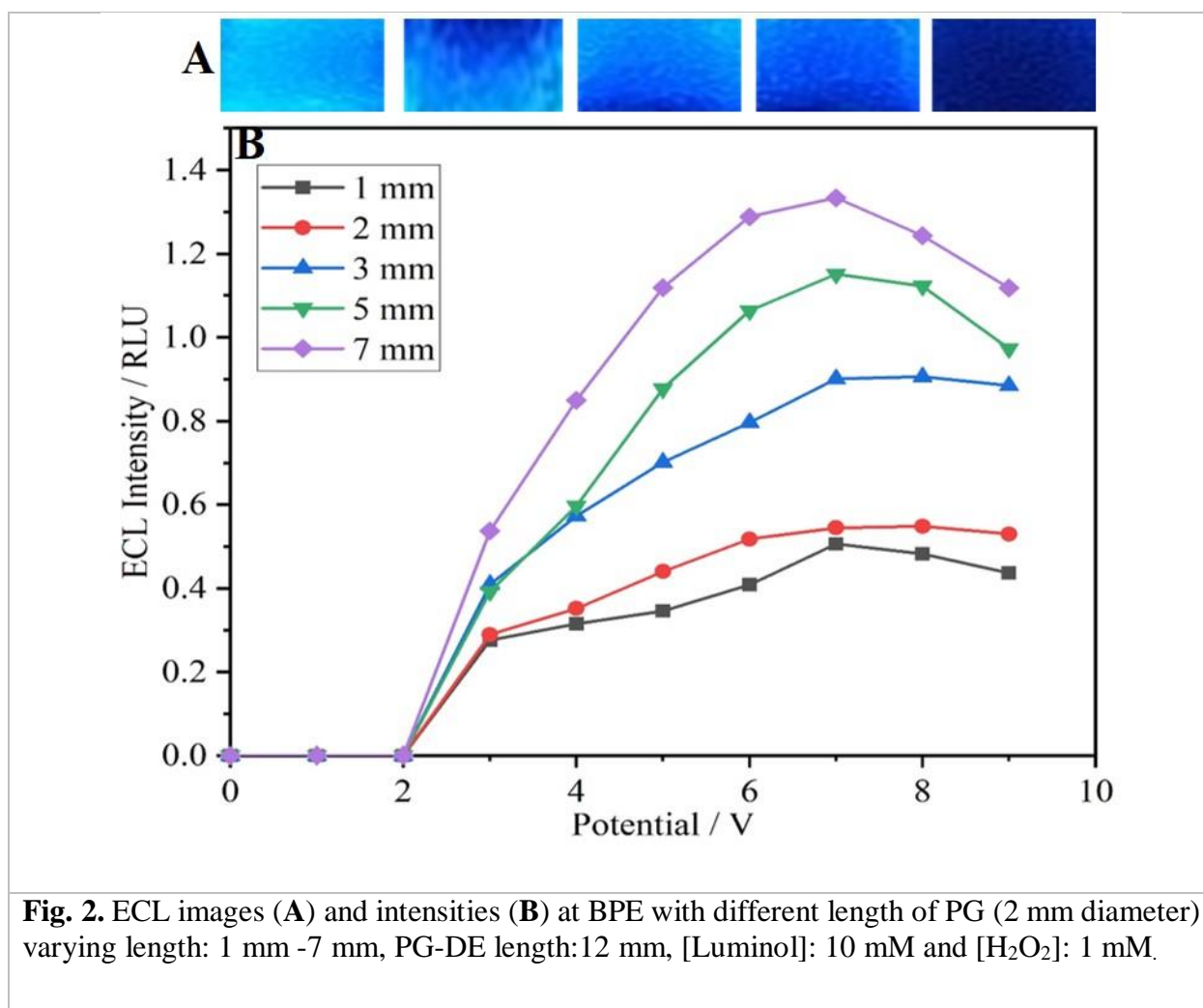
Figure 2

Fig. 2. ECL images (**A**) and intensities (**B**) at BPE with different length of PG (2 mm diameter) varying length: 1 mm -7 mm, PG-DE length:12 mm, [Luminol]: 10 mM and [H_2O_2]: 1 mM.
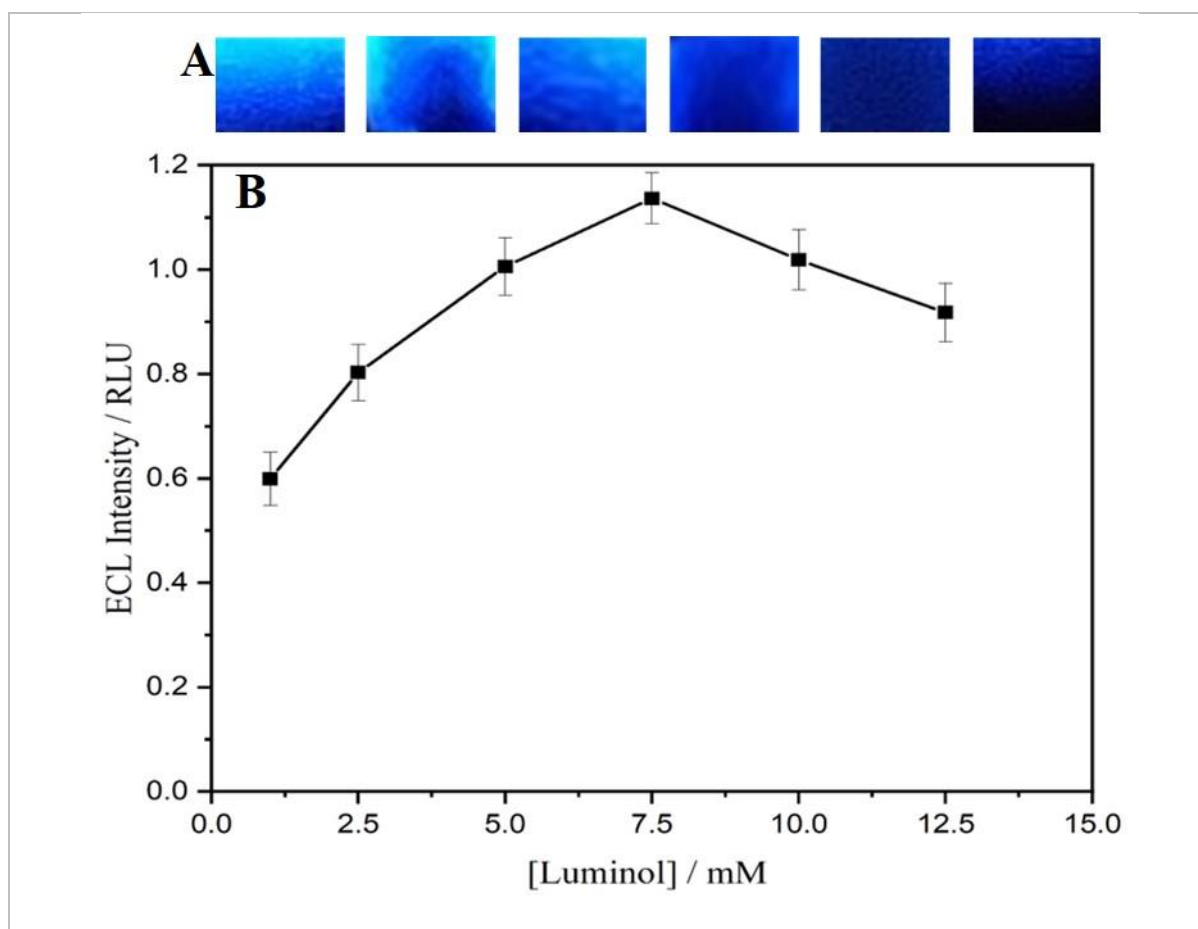
Figure 3

Fig. 3. ECL images (**A**) and intensities (**B**) Effect of [Luminol] on PG-BPE. Experimental conditions: [Luminol]:1, 2.5, 5, 7.5, 10 and12.5 mM, [H$_2$O$_2$]:1mM, PG-BPE: 7 mm, PG-DE: 12 mm, driving voltage: 7V. The error bar represent the standard deviation from five independent experiment.
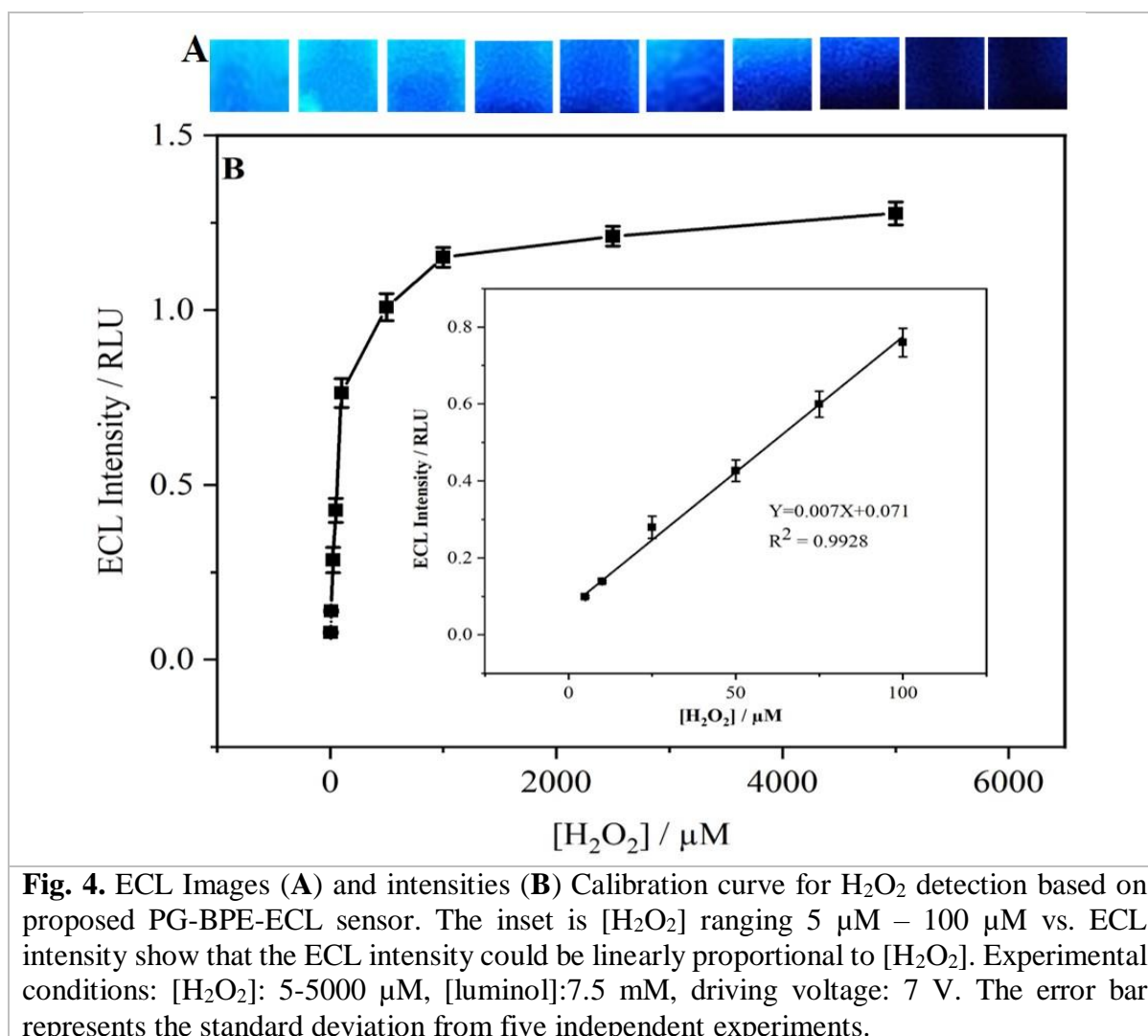
Figure 4

Fig. 4. ECL Images (**A**) and intensities (**B**) Calibration curve for H_2O_2 detection based on proposed PG-BPE-ECL sensor. The inset is [H_2O_2] ranging 5 µM – 100 µM vs. ECL intensity show that the ECL intensity could be linearly proportional to [H_2O_2]. Experimental conditions: [H_2O_2]: 5-5000 µM, [luminol]:7.5 mM, driving voltage: 7 V. The error bar represents the standard deviation from five independent experiments.
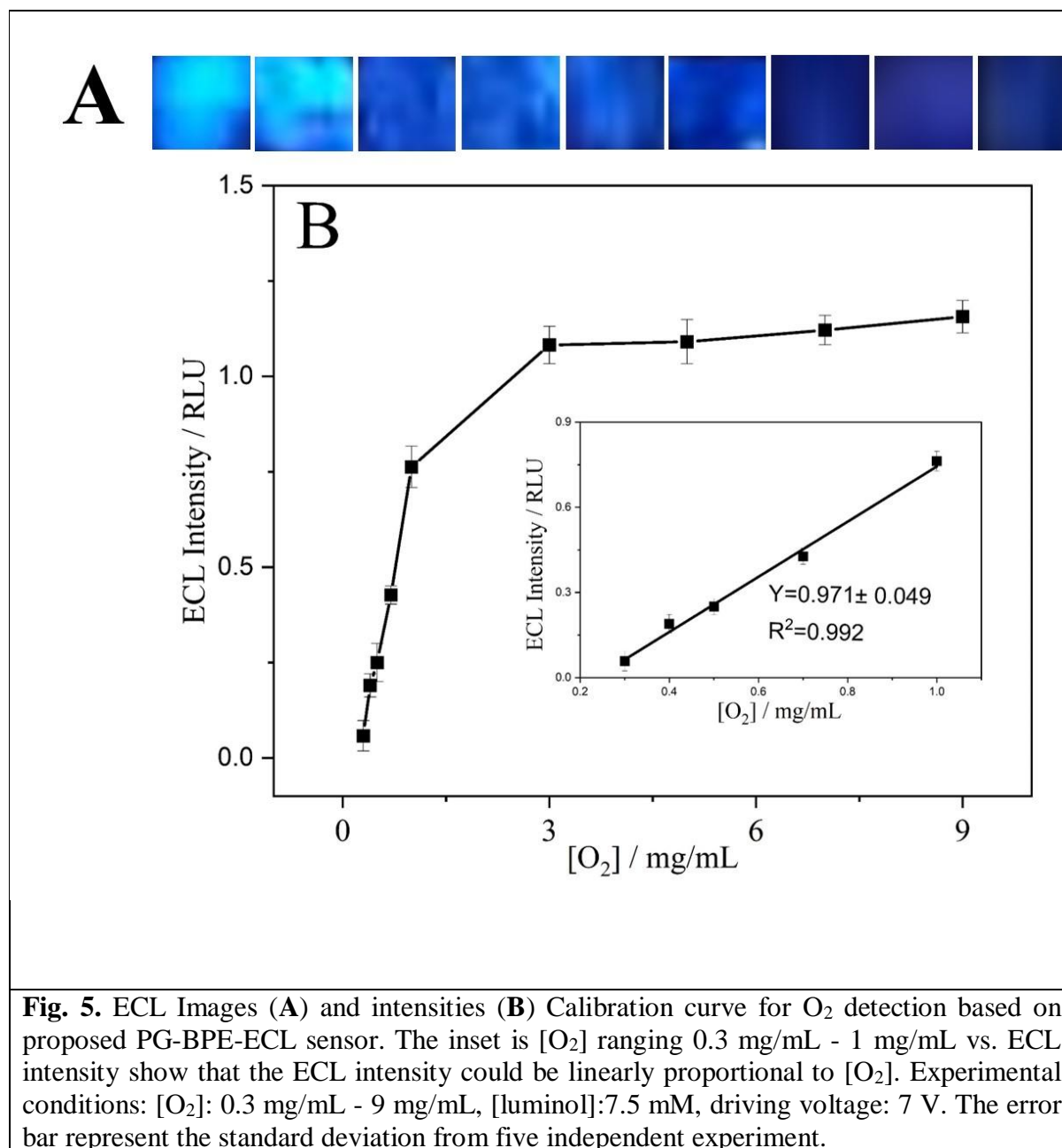
Figure 5

Fig. 5. ECL Images (**A**) and intensities (**B**) Calibration curve for O_2 detection based on proposed PG-BPE-ECL sensor. The inset is [O_2] ranging 0.3 mg/mL - 1 mg/mL vs. ECL intensity show that the ECL intensity could be linearly proportional to [O_2]. Experimental conditions: [O_2]: 0.3 mg/mL - 9 mg/mL, [luminol]:7.5 mM, driving voltage: 7 V. The error bar represent the standard deviation from five independent experiment.
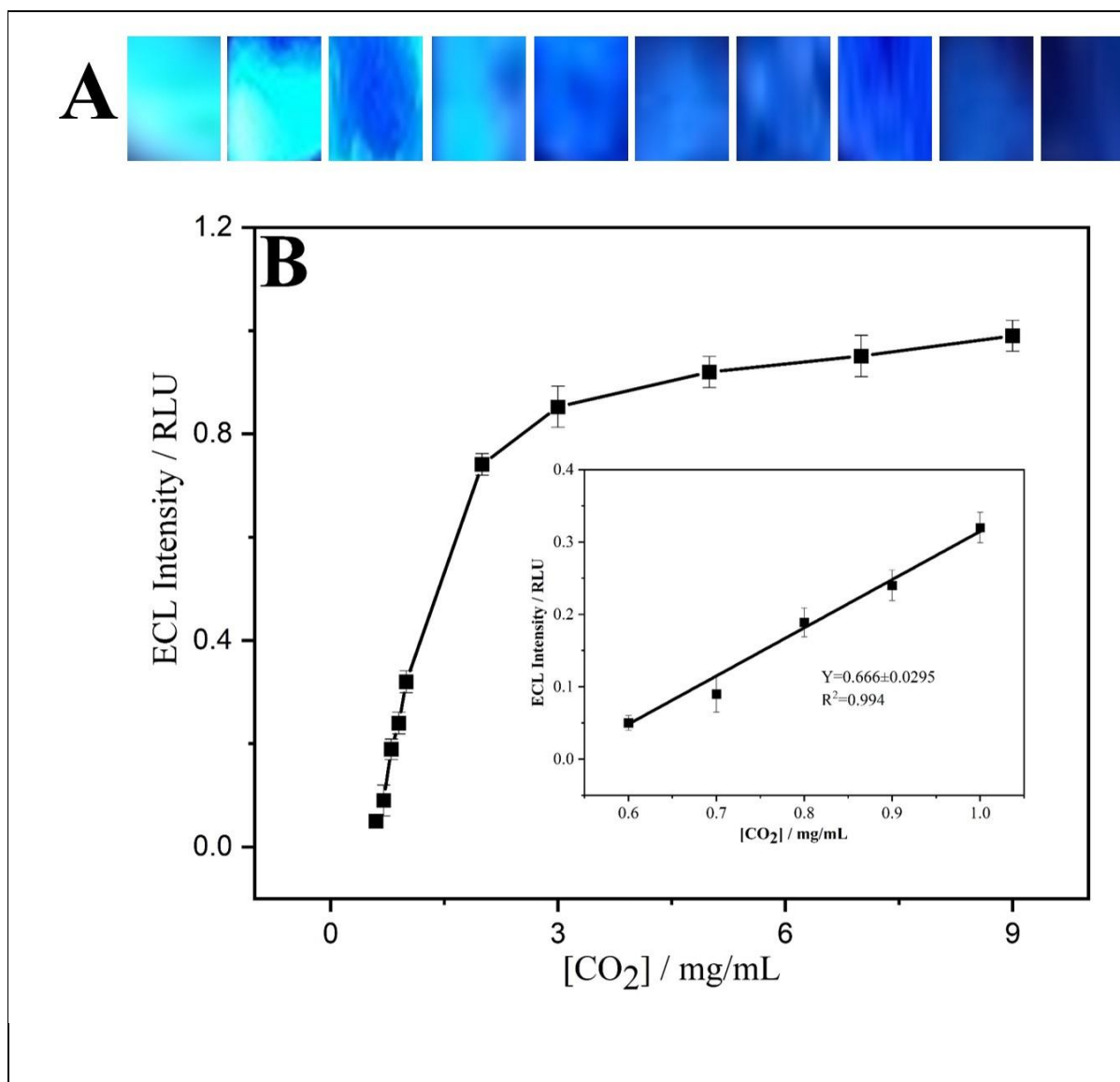
Figure 6

Fig. 6. ECL Images (**A**) and intensities (**B**) Calibration curve for CO_2 detection based on proposed PG-BPE-ECL sensor. The inset is [CO_2] ranging 0.6 mg/mL-1 mg/mL vs. ECL intensity show that the ECL intensity could be linearly proportional to [O_2]. Experimental conditions: [CO_2]: 0.6 mg/mL - 9 mg/mL, [luminol]:7.5 mM, [H_2O_2]: 1 mM, driving voltage: 7 V. The error bar represent the standard deviation from five independent experiment.
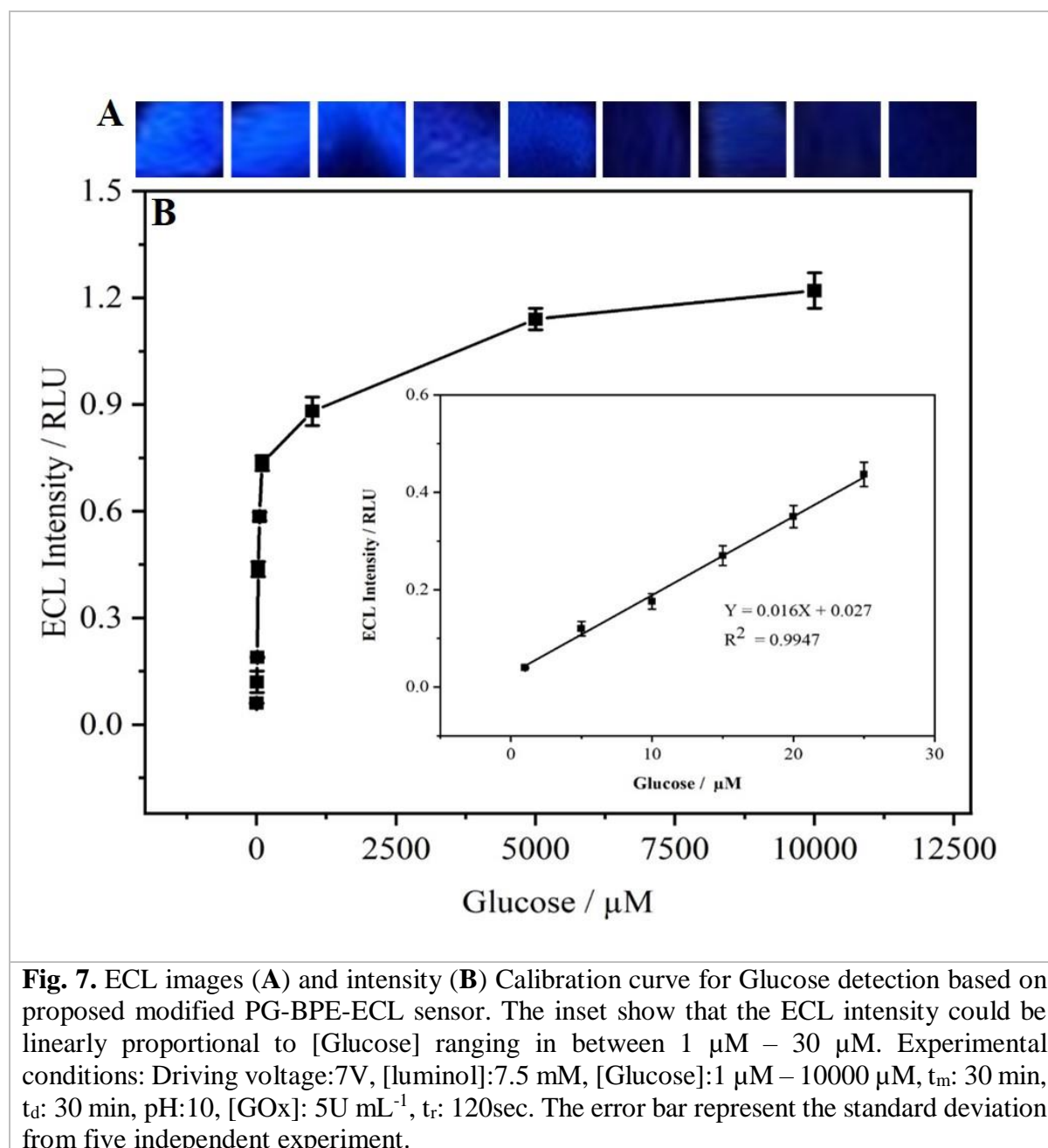
Figure 7

Fig. 7. ECL images (**A**) and intensity (**B**) Calibration curve for Glucose detection based on proposed modified PG-BPE-ECL sensor. The inset show that the ECL intensity could be linearly proportional to [Glucose] ranging in between 1 μM – 30 μM. Experimental conditions: Driving voltage:7V, [luminol]:7.5 mM, [Glucose]:1 μM – 10000 μM, t_m: 30 min, t_d: 30 min, pH:10, [GOx]: 5U mL^{-1}, t_r: 120sec. The error bar represent the standard deviation from five independent experiment.

Table 1: Real Sample Analysis

Found In sample	H_2O_2 added (μm)	Found In sample (μm)	Recovery %
Clinical H_2O_2 (3%)			
7.94	20	29.05	103.99
	40	51.11	106.61
	60	70.722	104.09
FEM Bleach (4%) H_2O_2			
9.055	20	29.05	105.16
	40	51.11	100.78
	60	70.722	100.40
Found In sample (mg/ml)	O_2 added (mg/ml)	Found In sample (mg/ml)	Recovery %
O_2 in Tap Water			
1	1	1.98	99.01
	1.5	2.6	104.32
	2	3.02	100.67
O_2 in Lake Water			
4	1	4.23	105.75
	1.5	4.64	102.22
	2	5.	101.8
Found In sample (mg/ml)	CO_2 added (mg/ml)	Found In sample (mg/ml)	Recovery %
CO_2 in Tap Water			
0.8	1	1.83	101.66
	1.5	2.51	108.69
	2	2.76	98.57
CO_2 in Lake Water			
3	1	4.17	104.25
	1.5	4.64	103.11
	2	5.13	102.66

Table 2. Real Sample Analysis in Human Serum

Clinical method (µM) (500 times diluted)	This method (µM)	Added (µM)	Found (µM)	Recovery%
16.98	17.25	5	22.16	99.59551
		10	28.16	103.3394
		15	32.42	100.5271

Research article

Functional and molecular characterization of plant growth promoting *Bacillus* isolates from tomato rhizosphere

Sadaf Kalam, Anirban Basu, Appa Rao Podile [*]

Department of Plant Sciences, School of Life Sciences, University of Hyderabad, Hyderabad, 500 046, Telangana, India

A B S T R A C T

The rhizosphere offers a quintessential habitat for the microbial communities and facilitates a variety of plant-microbe interactions. Members of the genus *Bacillus* constitute an important group of plant growth promoting rhizobacteria (PGPR), which improve growth and yield of crops. In a total of 60 bacterial isolates from the tomato rhizosphere, 7 isolates were selected based on distinct morphological characteristics and designated as tomato rhizosphere (TRS) isolates with a number suffixed *viz.*, TRS-1, 2, 3, 4, 5, 7, and TRS-8. All the seven isolates were Gram positive, with *in vitro* plant growth promoting (PGP) traits like phosphate and zinc solubilization, and also produced indoleacetic acid (IAA), phytase, siderophore, hydrogen cyanide (HCN), and 1-aminocyclopropane-1-carboxylate (ACC) deaminase, besides being antagonistic to other microbes and formed biofilm. The seven isolates belonged to the genus *Bacillus* as per the 16S rDNA sequence analysis. Phylogenetic tree grouped the isolates into four groups, while BOX-PCR fingerprinting allowed further differentiation of the seven isolates. The PGP activity of the isolates was measured on tomato seedlings in plant tissue culture and greenhouse assays. A significant increase in root colonization was observed over 15 days with all the isolates. Greenhouse experiments with these isolates indicated an overall increase in the growth of tomato plants, over 60 days. Isolates TRS-7 and TRS-8 were best plant growth promoters among the seven isolates, with a potential as inoculants to increase tomato productivity.

1. Introduction

The rhizosphere harbours a diverse group of plant beneficial microorganisms, possessing the inherent ability to improve plant growth and development, and soil health. Beneficial interactions between roots and microbes in the rhizosphere determine overall plant health and soil fertility (Parray et al., 2016; Kalam et al., 2017a). Such interactions play a vital role in regulating various biophysical and biogeochemical processes in the soil. A broad canopy of rhizosphere colonizing bacteria, referred to as plant growth promoting rhizobacteria (PGPR), produce growth promoting substances and elicit phytoprotective effects on plants through several direct and indirect mechanisms (Dutta and Podile, 2010). Inoculation with PGPR plays a significant role in facilitating plant growth and/or safeguarding crops against phytopathogens (biological control) (Parray et al., 2016; Qiao et al., 2017), thereby providing an eco-friendly alternative to chemical fertilizers and fungicides. Hence, exploring the diversity of potential PGPR strains suitable for different environmental conditions, including soil type, is relevant for sustainable agriculture.

The genus *Bacillus* represents one of the most abundant and phylogenetically diverse groups of easily cultivable PGPR (Orozco-Mosqueda et al., 2020). Bacilli, due to their avid rhizosphere colonization and PGP characteristics, offer considerable interest for improving crop productivity and yield (Zhou et al., 2016; Sansinenea, 2019). *Bacillus* spp. promote growth by increasing the bioavailability of minerals *viz.*, phosphorus and zinc, fixing atmospheric nitrogen, sequestration of iron through siderophores, and also by the production of phytohormones. In addition, biosynthesis of ethylene catabolism related 1-aminocyclopropane-1-carboxylate (ACC) deaminase, antibiosis, lytic enzyme production, detoxification and degradation of pathogens' virulence factors (Ahmad et al., 2008; Barea and Richardson, 2015) also contribute to the plant beneficial effects of Bacilli. Seed bacterization was often employed to study the effect of Bacilli or their formulations on plant growth (Kishore et al., 2005; Das et al., 2010). Beneficial *Bacillus* spp. have the potential to improve soil health and enhance crop yield as external inputs.

Tomato (*Solanum lycopersicum* L.) is one of the most commonly used vegetables all over the world. There is a need to adopt non-chemical

alternatives to increase yield, safety and quality of tomato. Biofertilizers based on *Bacillus* PGPR have been widely documented to enhance tomato yield and fruit quality. Multiple *Bacillus* species such as *B. licheniformis, B. subtilis, B. polymyxa, B. cereus, B. amyloliquefaciens, B. megaterium,* and *B. pumilus* successfully colonize the tomato rhizosphere and contribute to better growth and yield (Chen et al., 2013; Vaikuntapu et al., 2014; Zhou et al., 2016).

BOX-PCR fingerprinting, along with 16S rRNA gene sequencing, is often employed for identification and molecular typing of bacterial species. It involves amplification of the BOX-elements (interspersed repetitive DNA sequences present in bacterial genomes) with BOX-A1R primer and demonstrates intraspecies diversity (Versalovic et al., 1994). Here, we report the characterization of multifarious PGPR from the tomato rhizosphere both *in vitro* and *in planta.* We also demonstrate the potential of BOX-PCR to distinguish different *Bacillus* spp.

2. Material and methods

2.1. Seed material

Tomato (*Solanum lycopersicum* L.) seeds (var. Arka Vikas) were procured from ICAR-Indian Institute of Horticulture Research, Bangalore, India.

2.2. Microbial cultures

A commercially available strain of *Bacillus licheniformis* (C*Bli*) was procured from M/s Sri Biotech, Hyderabad, India. Fungal pathogens *Curvularia* sp. and *Fusarium* sp. were obtained from Osmania University, Hyderabad, India. Phytopathogenic bacterium *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) strain BXO43 was obtained from CSIR-Centre for Cellular and Molecular Biology, Hyderabad, India. *Xanthomonas axonopodis* pv. *citri* (*Xac*) strain was from our laboratory collection.

2.3. Rhizospheric soil sample collection

Healthy tomato plants were uprooted, and rhizospheric soil samples were collected from several tomato fields across different districts of Andhra Pradesh and Telangana, India. Sterile sample containers were used for sampling and storage of rhizospheric soil at 4 °C until further use.

2.4. Isolation of rhizobacteria

To isolate rhizobacteria, 1 g rhizospheric soil was added to 10 ml of PBS (phosphate buffer saline) [pH 7.0] followed by vortexing, serially diluting and finally plating the inoculum onto two different media *viz.*, minimal medium-1 (M1) (gL^{-1})- KH$_2$PO$_4$, 0.2; NH$_4$Cl, 0.25; KCl, 0.5; CaCl$_2$.2H$_2$O, 0.15; NaCl, 1.0; MgCl$_2$.6H$_2$O, 0.62; Na$_2$SO$_4$, 2.84; HEPES [4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid] (pH 6.8), 10 mM and minimal medium-2 (M2) (gL^{-1})- MgSO$_4$, 0.5; KNO$_3$, 0.5; K$_2$HPO$_4$, 1.3; Ca(NO$_3$)$_2$, 0.06; glucose, 0.06; casamino acids, 0.001; pH 7.5. The plates were incubated at 30 °C for 48 h. Phenotypically distinct isolates were subcultured in fresh medium and purified. The seven bacterial isolates used in the present study included TRS-1, 2, 3, 4, 5, 7 and 8, which were compared with C*Bli*.

2.5. Selection of rhizobacterial isolates based on plant growth promotion traits

In vitro PGP traits of the rhizobacterial isolates were assessed using standard protocols for production of indoleacetic acid (IAA), ACC deaminase, chitinase, phytase, siderophore and HCN, solubilization of zinc and phosphate, and biofilm formation (Saravanan et al., 2004; Demirkan et al., 2014; Vaikuntapu et al., 2014). Antagonistic activity of the test isolates was determined against two soil-borne phytopathogenic

fungi *viz., Fusarium* sp. and *Curvularia* sp. as described by Vaikuntapu et al. (2014) and also against two phytopathogenic *Xanthomonas* strains *viz., Xac* and *Xoo,* according to Sharma and Kaur (2010).

2.6. Characterization of rhizobacterial isolates

Physiological and biochemical characterization was done using standard protocols (Cappuccino and Sherman, 2014). Selected seven isolates were identified to the genus level using 16S rRNA gene sequencing. Further differentiation of the isolates to species and sub-species was done using BOX-PCR.

2.7. 16S rRNA gene-based molecular characterization of rhizobacteria

Rhizobacterial isolates were grown for 12 h in M1 and M2 broths at 30 °C with shaking at 160×*g*. Rhizobacterial genomic DNA was isolated, according to Sharma and Singh (2005). 16S rRNA gene was amplified using 100 ng of genomic DNA and employing the universal primers (Sigma-Aldrich, USA): 27F (5′-GTTTGATCCTGGCTCAG-3′) and 1494R (5′-ACGGCTACCTTGTTACGACTT-3′) as described earlier (Kalam et al., 2017a). The PCR products were electrophoresed in 1.5 % TAE-agarose gel, purified using Nucleospin® Extract II Kit (Macherey Nagel, Germany), and subjected to Sanger sequencing at First Base, Malaysia, using ABI PRISM 3730XI Genetic Analyzer (Applied Biosystems, USA). Resulting nucleotide sequences were analyzed using the BLAST® sequence analysis tool provided by the US National Center for Biotechnology Information (NCBI). Rhizobacterial isolates were identified based on the percentage of similarity with the top-hit taxon. The 16S rDNA sequences were deposited in GenBank, and accession numbers were obtained. All the sequences were aligned with MEGA6 (Molecular Evolutionary Genetics Analysis version 6.0) software (Tamura et al., 2013) for constructing a phylogenetic tree.

2.8. BOX-PCR analysis

For the genotypic fingerprinting of closely related *Bacillus* strains, BOX-PCR was performed using BOX-A1R primer (5′-CATA-CGGCAAGGCGACGCT-3′) as described by Versalovic et al. (1994). The PCR mixture contained 1μM of primer, 1X of PCR buffer with 1.5 mM MgCl$_2$ (Sigma-Aldrich, USA), 10mM of each dNTP (Fermentas, USA) and 2 U of Taq DNA polymerase (Sigma-Aldrich, USA). The PCR was carried out for 30 cycles including an initial denaturation step for 5 min at 95 °C, denaturation for 1 min at 94 °C, annealing for 1 min at 50 °C, polymerization for 1 min at 72 °C and a final extension for 10 min at 72 °C. Amplicons were separated by electrophoresing on 2% TAE-agarose gel.

2.9. Plant growth in tissue culture

Surface sterilized tomato seeds (treated with 2 % sodium hypochlorite solution) were bacterized with culture suspensions of 1×10^8 colony forming units (CFU) mL^{-1} in 1 % sterile carboxymethyl cellulose (CMC). C*Bli* and CMC were respectively used as positive and negative controls. The bacterized seeds were blot dried and transferred aseptically to plant tissue culture bottles containing half-strength Murashige and Skoog (MS) medium (Hi-media, India). The bottles were maintained in a plant growth chamber at 26 °C, 16h/8h photoperiod and 40 μmol m^{-2} s^{-1} light intensity for 15 days. The experiment was repeated four times with triplicates. After 15 days, three seedlings were randomly selected from each replication, and the root and shoot lengths were measured. The samples were dried to a constant weight in an oven to measure the dry weights.

For root colonization, 5-, 10- and 15-days old seedlings grown on MS medium were sampled. The roots were excised and serially diluted in 0.85% saline, and were grown onto M1 and M2 plates at 30 °C for 24–48 h followed by counting the colonies in the form of CFUs.

2.10. Plant growth in greenhouse

Pot experiments were conducted in the greenhouse with selected rhizobacterial isolates (TRS-2, 7, and 8) and controls (C*Bli* and CMC). Before the start of the experiment, the physicochemical properties of the greenhouse soil were determined using Soil Test Kit (Hi-Media, India). Surface sterilized tomato seeds were bacterized and sown into plastic pots filled with greenhouse soil. The plants were maintained in a greenhouse (16 h/8 h photoperiod, 30 ± 2 °C, and 70 % relative humidity). The same volume of tap water was used to water the plants daily, without applying any other nutrients or PGPR inocula. The experiment was repeated three times with triplicates. Plant growth parameters (root length, shoot length and dry weight) were assessed at 20-, 40- and 60-days post-inoculation by randomly selecting and uprooting three plants from each treatment.

2.11. Statistical analysis

Data were analyzed using GraphPad Prism statistical software (Version 6.0) for significant mean differences via either one-way or two-way Analysis of Variance (ANOVA), respectively followed by Dunnett's or Bonferroni's post-hoc test for multiple mean comparisons, as per requirement. Depending on the comparisons made, Dunnett's test was used for multiple comparisons with the control mean, while Bonferroni's test was used for pairwise comparisons. Statistical significance was determined at the critical alpha level of 0.05.

3. Results

3.1. Isolation, selection, and characterization of rhizobacteria

A total of 60 distinct bacterial colonies were isolated from tomato rhizosphere on two minimal media using standard plating methods. Seven isolates were selected based on differential colony morphology, and designated as tomato rhizosphere (TRS) isolates with a number suffixed. Three isolates, TRS-1, TRS-3, and TRS-5, were isolated on M1, and four isolates, TRS-2, TRS-4, TRS-7, and TRS-8, were isolated on M2. Physiological and biochemical characteristics of the bacterial isolates are presented in Table 1.

3.2. PGP traits of rhizobacterial isolates

All the seven isolates *viz.*, TRS-1, TRS-2, TRS-3, TRS-4, TRS-5, TRS-7, and TRS-8 were Gram positive and exhibited multiple PGP activities (Table 2). Phosphate solubilization was observed with the TRS-5 only.

Out of seven isolates, TRS-1, TRS-3, and TRS-8 exhibited good zinc solubilization capability and phytase production. Siderophore, HCN, and IAA production was common for all the test isolates, while none of the isolates hydrolyzed chitin. Isolates TRS-1 and TRS-7 formed biofilm, while TRS-2, TRS-4, TRS-7, and TRS-8 produced ACC deaminase.

All the seven rhizobacterial isolates were screened for their antagonistic ability against phytopathogenic fungi like *Fusarium* sp. and *Curvularia* sp., and phytopathogenic bacteria like *Xanthomonas axonopodis* pv. *citri* and *X. oryzae* pv. o*ryzae*. None of the seven isolates was antagonistic to *Fusarium* sp. TRS-1 inhibited the growth of *Curvularia* sp., while TRS-1 and TRS-5 showed antibacterial activity against both the Xanthomonads (*Xac* and *Xoo*).

3.3. 16S rRNA gene-based molecular characterization

Amplicons of approximately 1500 bp were obtained after PCR amplification of the 16S rDNA. NCBI-BLAST analysis of the 16S rRNA gene sequences of all the test rhizobacterial isolates (GenBank accession nos. KJ572791, KJ572792, KJ572793, KJ631602, KJ631603, KJ631604, and KJ631605) indicated that all seven isolates are *Bacillus* spp., sharing 99–100% similarity with members of the genus *Bacillus* (Table 3).

3.4. BOX-PCR analysis

BOX-PCR amplification conditions were optimized to obtain fingerprints with distinct informative bands (Figure 1). BOX-PCR analysis allowed differentiation of individual strains, resulting in 6 different electrophoretic patterns or fingerprinting profiles for the seven isolates with isolates TRS-2 and TRS-4 sharing the same pattern. A large number of polymorphic bands of variable intensity were observed in the profiles, whose size ranged from 0.2–3.0 kb and remained consistent as the experiment was done in triplicate (n = 3) to confirm the reproducibility and stability. Although all the isolates showed a banding pattern typical of genus *Bacillus*, the intensity of a few bands was high in isolates TRS-1, TRS-2, TRS-3, and TRS-4.

3.5. Phylogenetic tree

A phylogenetic tree, constructed based on the 16S rRNA gene sequences, indicated a considerable genetic homogeneity among the seven *Bacillus* isolates (Figure 2). The isolates could be divided into four groups, with the first, second, third, and fourth groups having 2, 2, 1, and 2 isolates, respectively.

Table 1. Physiological and biochemical characterization of rhizobacterial isolates.

Characteristics	TRS-1	TRS-3	TRS-5	TRS-2	TRS-4	TRS-7	TRS-8
Gram stain	+ve	+ve	+ve	+ve	+ve	+ve	+ve
pH optimum (range)	7.0 (6.5–8.0)	7.0 (7.5–8.0)	6.0 (7.0–8.5)	7.0 (5.0–8.0)	7.0 (6.0–8.0)	7.0 (7.0–8.5)	7.0 (7.0–8.5)
Temperature optimum (range) °C	30 (30–40)	30 (28–40)	30 (30–40)	37 (28–45)	37 (28–45)	28 (28–40)	28 (28–40)
Motility	Motile	Motile	Motile	Motile	Motile	Non- motile	Non- motile
Nature	Facultative Anaerobic	Aerobic	Aerobic	Aerobic	Aerobic	Aerobic	Aerobic
Biochemical tests							
Indole test	-	-	-	-	-	-	-
Methyl red test	+	-	-	-	-	-	-
Voges Proskauer test	+	+	+	+	+	+	+
Starch hydrolysis	+	+	-	-	-	+	+
H$_2$S production test	+	-	-	-	-	-	-
Catalase test	+	+	+	+	+	+	+
Glucose utilization	+	+	+	+	+	+	+

Physiological and biochemical characterization of rhizobacterial isolates was carried out under identical conditions. '+', positive; '-', negative result for the test.

Table 2. Characterization of rhizobacterial isolates for plant growth promoting activities.

Isolate	PS	CP	SP	ZS	HP	IP	PP	BF	AD	Antibacterial		Antifungal	
										Xac	*Xoo*	C	F
TRS-1	-	-	+	+	+	+	+	+	-	++	+++	++	-
TRS-2	-	-	++	-	++	+	-	-	+	-	-	-	-
TRS-3	-	-	+	+	+	++	+++	-	-	-	-	-	-
TRS-4	-	-	+++	-	+	++	-	-	+	-	-	-	-
TRS-5	+++	-	++	-	+	++	-	-	-	+	+++	-	-
TRS-7	-	-	+++	-	++	+++	-	+	+	-	-	-	-
TRS-8	-	-	+++	+++	+	+++	+	-	+	-	-	-	-

PS- Phosphate solubilization; CP- Chitinase production; SP- Siderophore production; ZS- Zinc solubilization; HP- HCN production; IP- IAA production; PP- Phytase production; BF- Biofilm formation; AD- ACC deaminase activity. '+', positive; '-', negative result for the test. For phosphate and zinc solubilization, siderophore and chitinase production, antifungal and antibacterial assay: '+', zone of clearance <0.2 mm; '++', zone of clearance 0.2–0.4 mm; '+++', zone of clearance >0.4 mm. For IAA production: '+', absorbance <0.1; '++', absorbance 0.1–0.3; '+++', absorbance >0.3. *Xac- Xanthomonas axonopodis* pv. *citri; Xoo- Xanthomonas oryzae* pv. *oryzae*; C- *Curvularia* sp.; F- *Fusarium* sp.

Table 3. Rhizobacterial isolates and their identity based on 16S rRNA gene sequence similarity.

Isolate	Isolation medium	NCBI strain	Similarity (%)	GenBank Accession No.
TRS-1	M1	*Bacillus licheniformis*	99	KJ572792
TRS-3	M1	*Bacillus subtilis*	100	KJ572793
TRS-5	M1	*Bacillus pumilus*	99	KJ572791
TRS-2	M2	*Bacillus* sp.	99	KJ631602
TRS-4	M2	*Bacillus* sp.	100	KJ631603
TRS-7	M2	*Bacillus* sp.	99	KJ631604
TRS-8	M2	*Bacillus* sp.	99	KJ631605

Homology and phylogenetic identity of the rhizobacterial isolates were obtained by comparing the 16S rRNA gene sequence similarity with that of related strains available at the NCBI database.
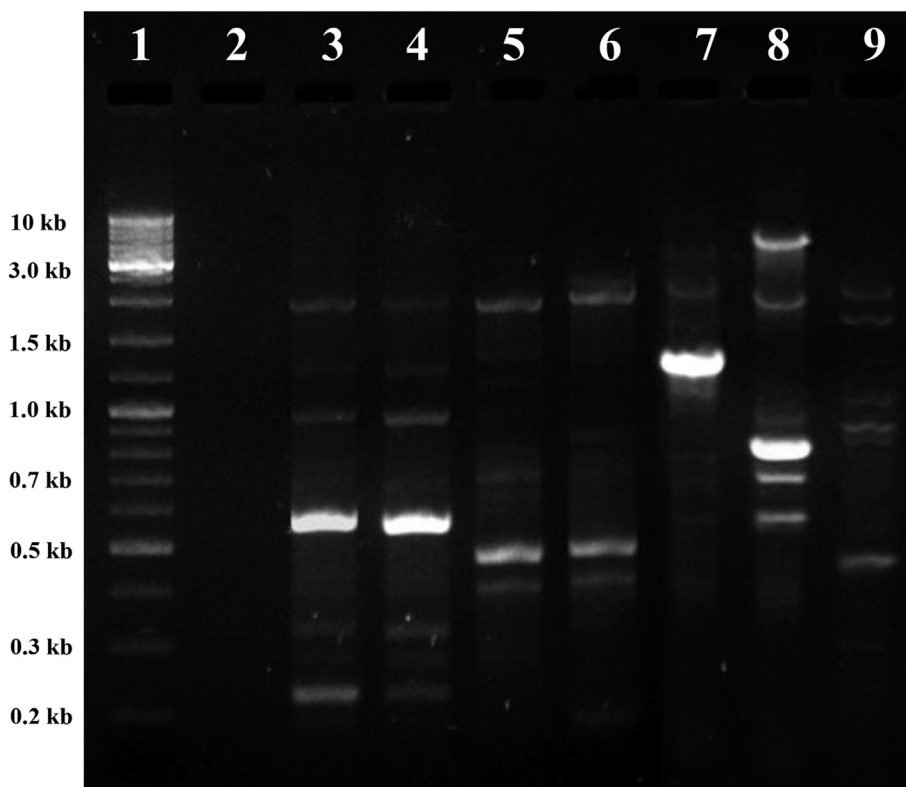

Figure 1. BOX-PCR patterns of the seven TRS isolates of *Bacillus* genus. (L–R) Lane 1, DNA molecular mass standard (Generuler 2-Log DNA Ladder, New England Biolabs, USA; size indicated in the left-hand margin); lane 2, no DNA control (sterile water); lanes 3–9, *Bacillus* spp. TRS-2, TRS-4, TRS-7, TRS-8, TRS-1, TRS-3 and TRS-5. Full, non-adjusted gel images are provided in Supplementary File 1.
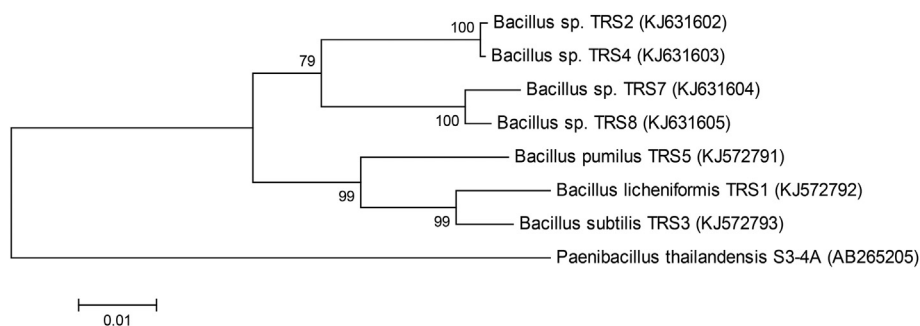
3.6. Plant growth promotion in tissue culture

The overall growth response of tomato seedlings to seed bacterization by the *Bacillus* isolates was assessed in MS medium (Figure 3). Rhizobacteria treated tomato plants exhibited variations in root length (Figure 3A). The response to seed bacterization with TRS-4 and TRS-5 did not vary significantly in comparison to both the controls. The remaining five treatments, along with the commercial isolate (C*Bli*), significantly increased tomato root length in comparison to the CMC control. TRS-8 notably increased the root length more than the other isolates. Shoot length response to bacterial isolates differed considerably (Figure 3B). Isolates TRS-2, TRS-4, TRS-7, and TRS-8 improved shoot length more than the other isolates. Plants arising from TRS-8, followed by TRS-7, TRS-2, and TRS-4 bacterized seeds showed significant improvement in shoot length as compared to control (CMC) and C*Bli*.

An increase in the dry weight due to seed bacterization varied significantly (Figure 3C) on treatment with TRS-2, TRS-7, and TRS-8. Isolate TRS-8, followed by TRS-7, significantly enhanced the plant dry weight in comparison to CMC control. A comparison of root colonization by the test rhizobacterial isolates was also done (Figure 4). All the isolates colonized tomato roots. There was a significant increase in root colonization from 5 days with the isolates TRS-1 and TRS-3. At 10 and 15 days, all the isolates colonized roots extensively except TRS-4. TRS-8 and TRS-7 were better colonizers of the root compared with C*Bli*.

3.7. Plant growth promotion in greenhouse

The greenhouse soil used in this study had the following physicochemical characteristics: red colour, sandy texture, 7.5 pH, 1–1.5% oxidizable organic carbon, 10–15 kg ha^{-1} ammoniacal nitrogen, 10–20 kg ha^{-1} nitrate nitrogen, 56–73 kg ha^{-1} available phosphorus, and 112–280 kg ha^{-1} available potassium. There was a gradual increase in shoot length, root length and dry weight of the bacterized tomato plants at 20-, 40- and 60-days post-inoculation (Figure 5). The isolates TRS-8 and TRS-7 produced significant responses on tomato plant growth parameters after 20, 40, or 60 days with respect to the CMC control.

4. Discussion

The growth and development of plants often depend on the type of plant-microbe interactions functioning in the rhizosphere. Several cultivation-dependent studies revealed the occurrence of multiple species of *Bacillus* in the soil and rhizosphere which were reported as PGPR (Kumar et al., 2012; Singh et al., 2014; Mumtaz et al., 2017; Akinrinlola et al., 2018) as they promoted plant growth and/or suppressed phytopathogens. Based on the 16S rRNA gene sequences, the seven tomato rhizobacterial isolates, in this study, had 99–100% similarity with the genus *Bacillus*. Identification of *Bacillus* species exclusively based on the 16S rRNA gene sequences was considered insufficient (Lima-Bittencourt et al., 2007). BOX-PCR fingerprinting is a well-documented and widely employed phylogenetic informative tool for molecular typing of various bacteria (Marques et al., 2008; Zhu et al., 2014). Köberl et al. (2011) showed BOX-PCR fingerprinting to be an effective tool to explore intraspecies diversity within *Bacillus* populations. In this study, BOX-PCR generated distinctive electrophoretic patterns among different *Bacillus* strains with clear and identifiable bands. The presence of similar or variable bands enabled differentiation of the *Bacillus* isolates.

To improve the screening approaches for selecting effective PGPR strains, identification of traits predicting PGP will be useful. Most of the seven *Bacillus* isolates exhibited multiple PGP characteristics. Phosphate solubilization by rhizobacteria promotes plant growth and yields (Lyngwi et al., 2016). Some species of bacteria, including *Bacillus* spp., possess the ability to mineralize and solubilize organic and inorganic phosphorus in the soil for quick access to the plant (Barea and Richardson, 2015). Microbial phytases, specially produced by *Bacillus* spp., were studied due to their PGP effects and diverse agrobiotechnological applications (Kumar et al., 2013; Sanguin et al., 2016). Besides solubilizing phytate phosphorous, extracellular phytases produced by *Bacillus* spp. release essential mineral nutrients like Ca^{2+}, Zn^{2+}, and Fe^{2+} from chelate-forming phytates (Sansinenea, 2019). Only one isolate (TRS-5) solubilized mineral phosphate effectively, and three of them (TRS-1, TRS-3, and TRS-8) produced phytases.

IAA and ethylene are growth regulators that regulate different stages of plant growth (Etesami et al., 2015). All seven isolates produced IAA. Highest IAA produced by TRS-7 and TRS-8 might play a role in enhancing the growth of tomato plants. The auxin IAA is known to strongly affect root growth and architecture. Exogenous IAA of rhizobacterial origin can increase root length and biomass, and enhance plant growth by regulating the expression of host genes related to auxin response, defense, hormone and cell wall synthesis (Ruzzi and Aroca, 2015; Backer et al., 2018). The isolates TRS-2, TRS-4, TRS-7 and TRS-8 produced ACC deaminase that breaks down the ethylene precursor ACC into ammonia and α-ketobutyrate, alleviating ethylene stress in plants and delaying senescence (Etesami et al., 2015). Bacteria can further metabolize these end products for their growth. *Bacillus* spp. exhibiting ACC deaminase activity are reported to be halotolerant and can promote plant growth under salinity stress conditions (Santoyo et al., 2019; Orozco-Mosqueda et al., 2020).

Bacillus spp. enhance plant growth and yield by solubilizing insoluble zinc compounds and increasing bioavailability of zinc in the soil (Mumtaz et al., 2017). TRS-1, TRS-3, and TRS-8 solubilized zinc with TRS-8 being the best zinc solubilizer. The presence of iron-chelating siderophore producing microorganisms in the rhizosphere makes iron available to the plant, aids in plant growth under iron-deficient conditions while limiting iron availability for phytopathogens (Saha et al., 2016; Sansinenea, 2019). Siderophore and HCN were produced by all the seven isolates. TRS-4, TRS-7, and TRS-8 produced more of siderophore, while TRS-2 and TRS-7 produced more of HCN. The PGPR that produce HCN suppress plant pathogens and reduce the severity of disease and also indirectly increase phosphorous availability through sequestration and metal chelation (Rijavec and Lapanje, 2016; Backer et al., 2018). All the seven isolates were positive for the Voges Proskauer test, indicating the production of acetylmethyl carbinol (acetoin) from glucose fermentation. Volatile organic compounds like acetoin produced by *Bacillus* strains
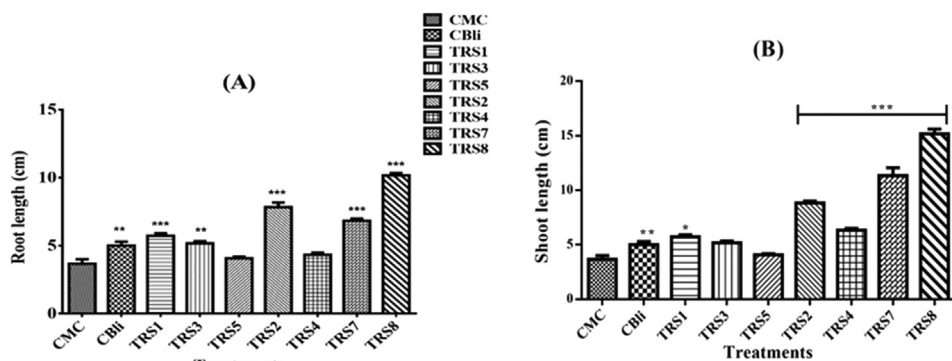
Figure 3. Effect of seven rhizobacterial isolates, a commercial *Bacillus licheniformis* strain (*CBli*) and a CMC control on tomato plants in MS medium after 15 days of treatment: root length (A), shoot length (B), and dry weight (C). Values represent mean (n = 3), and the vertical lines represent ±standard error of the mean. For each of the growth response, i.e., root length, shoot length and dry weight, statistical analysis has been performed using one-way ANOVA followed by Dunnett's post-hoc test for multiple mean comparisons. *** highly significant, ** moderately significant, * less significant compared to CMC control (p < 0.05).
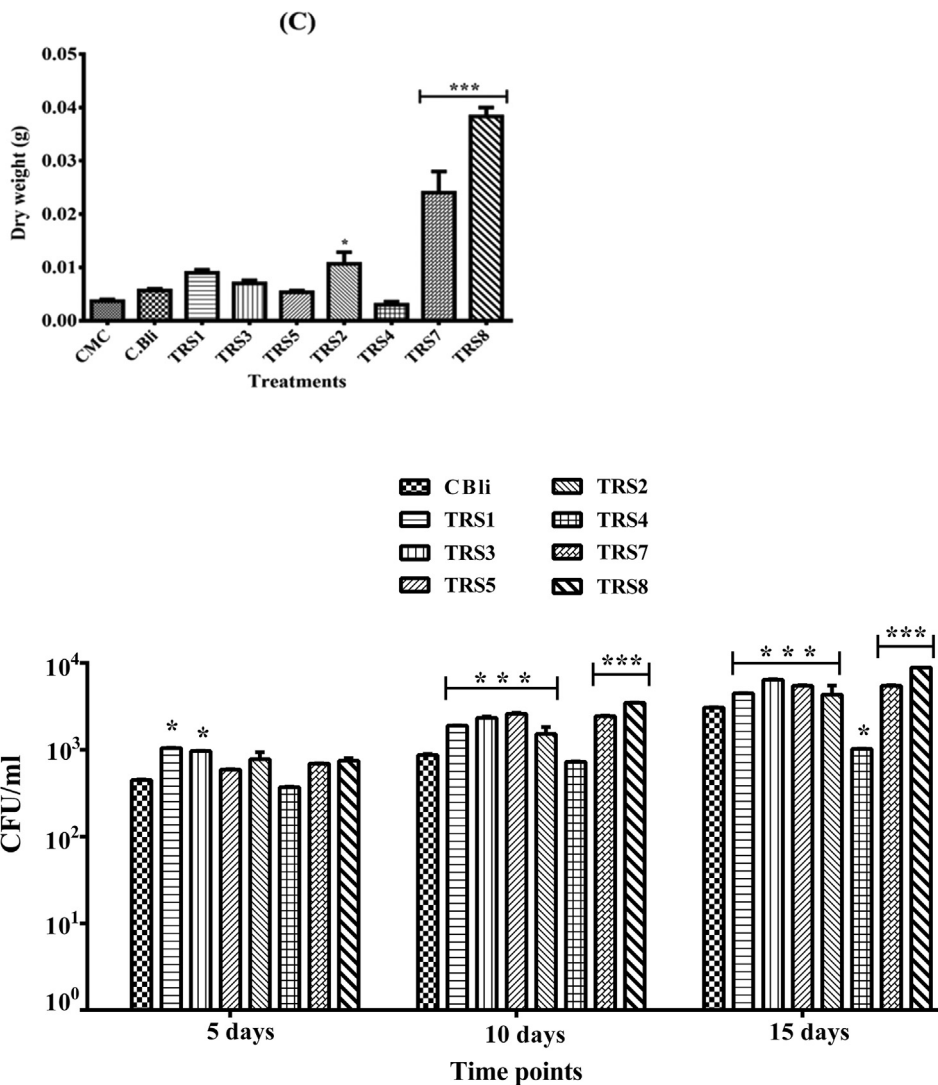
Figure 4. Comparison of root colonization by test rhizobacterial isolates. Values represent mean (n = 3), and the vertical lines represent ±standard error of the mean. Statistical analysis was performed using two-way ANOVA for each of the test bacterial isolate against three different time points, followed by Bonferroni's post-hoc test for multiple mean comparisons. *** highly significant, ** moderately significant, * less significant compared to the *CBli* positive control (p < 0.05).

were reported to increase leaf surface area and induce systemic resistance in *Arabidopsis thaliana* (Ryu et al., 2003).

Bacillus spp. exhibit remarkable antibacterial and/or antifungal activity against different phytopathogens (Kumar et al., 2012) that make them suitable biocontrol agents in agriculture. They are reported to be reservoirs of several biologically active molecules, including those with potential antifungal activity (Sansinenea, 2019). Rhizospheric Bacilli can aid in suppressing several soil-borne phytopathogens (Singh et al., 2014;

Cao et al., 2018). In the present study, antagonistic activity was observed with TRS-1 and TRS-5 against both *Xanthomonas axonopodis* pv. *citri* and *X. oryzae* pv. *oryzae*. TRS-1 exhibited antifungal activity against *Curvularia* sp. The antagonistic effect of the test bacterial isolates could be useful for an effective biocontrol and other plant growth promotion activities.

Extensive root colonization is a prerequisite for the PGPR to establish successfully in the rhizosphere and rhizoplane (Zhou et al., 2016). The
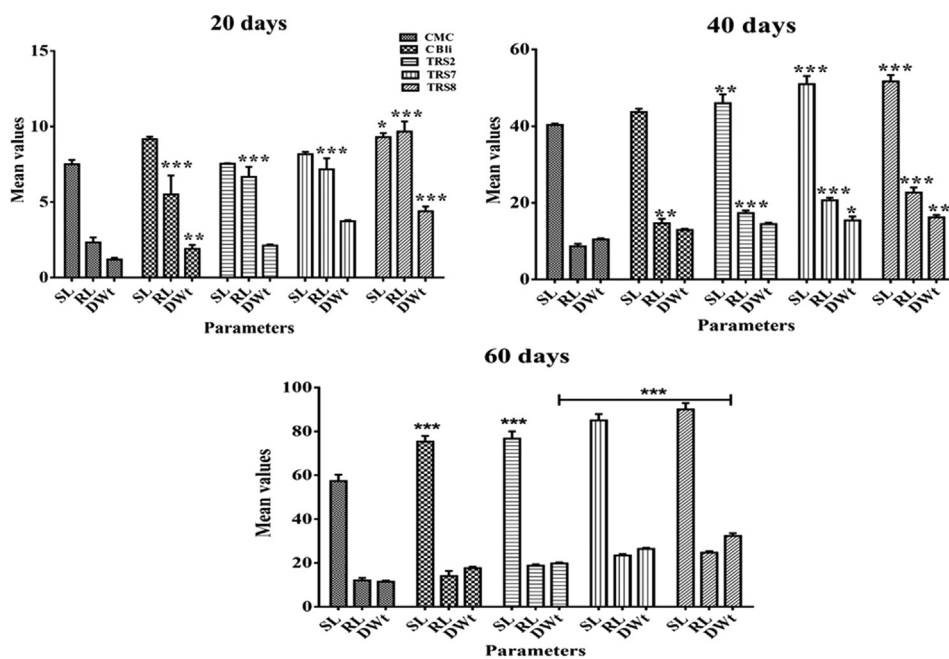
Figure 5. Influence of three rhizobacterial isolates (selected) along with a commercial strain (C*Bli*) on tomato plant responses in greenhouse (20d, 40d and 60d): root length (RL in cm), shoot length (SL in cm), and dry weight (DWt in g). Values represent mean (n = 3), and the vertical lines represent ±standard error of the mean. For each of the growth response, i.e., root length, shoot length and dry weight with or without the isolates, statistical analysis has been performed using two-way ANOVA followed by Bonferroni's post-hoc test for multiple mean comparisons. *** highly significant, ** moderately significant, * less significant compared to CMC control (p < 0.05).

isolates TRS-8 and TRS-7 were better root colonizers in comparison to other test isolates. Root colonization by PGPR is often enhanced by the formation of biofilms on root surfaces that facilitates retention of moisture and protects plant roots from harmful microorganisms (Kalam et al., 2017b). *Paenibacillus polymyxa* and *Bacillus subtilis* colonize *Arabidopsis* roots by forming biofilms and render biocontrol activity (Timmusk et al., 2005; Beauregard et al., 2013; Vlamakis et al., 2013). Two isolates TRS-1 and TRS-7, from tomato rhizosphere formed biofilms. As active colonization of tomato roots by *Bacillus subtilis* PTS-394 (Qiao et al., 2017) promoted growth and suppressed soil pathogens with no durable impact on the tomato rhizosphere microbial community composition, a combination of factors may be essential for PGPR to be successful.

Seed bacterization was employed to monitor the effects of rhizobacterial strains on plant growth (Vaikuntapu et al., 2014; Kalam et al., 2017a). The response of tomato plants to seed bacterization in terms of shoot length, root length and dry weight varied significantly. PGPR enhance plant growth and yield by facilitating the uptake of mineral nutrients, synthesizing several phytohormones, and protecting plants from diverse phytopathogens. The genus *Bacillus* represents one of the most diverse Bacilli genera commonly used as bioinoculants to promote plant growth. Akinrinlola et al. (2018) identified 12 *Bacillus* strains promoting the growth of corn, wheat, and soybean. The strains exhibited multiple PGP traits, including phosphate solubilization, nitrogen fixation and IAA production. Similarly, wheat rhizobacteria were screened for multiple *in vitro* PGP attributes and were evaluated under controlled conditions in pot experiments (Rana et al., 2011).

The use of beneficial, environmentally safe microbial inoculants has been regarded as an alternative to synthetic agrochemicals. *Bacillus* species, present in the immediate vicinity of crop rhizospheres, are most widely used as bioinoculants. The present study selected TRS-8 and TRS-7 as potential PGPR isolates from tomato rhizosphere that can enhance plant growth and fertility. Further characterization of these isolates is required to assess their suitability as effective bioinoculants.

Declarations

Author contribution statement

Sadaf Kalam: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Anirban Basu: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Appa Rao Podile: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Competing interest statement

The authors declare no conflict of interest.

Additional information

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RESEARCH

Evidence that cultural food practices of *Adi* women in Arunachal Pradesh, India, improve social-ecological resilience: insights for Sustainable Development Goals

Ranjay K. Singh[1,2*], Arvind Kumar[2], Anshuman Singh[2] and Poonam Singhal[3]

Abstract

Introduction: Sustainable use of biocultural diversity can help achieve Sustainable Development Goals (SDGs) in many ways. The tribal communities of bio-culturally rich Northeastern India rely heavily on local food resources and knowledge for livelihood security. In this study with *Adi* women of East Siang district, Arunachal Pradesh, India, we aimed to understand (i) access and use patterns for biocultural plants and animal species from diverse ecosystems and (ii) species diversity and conservation strategies deployed by *Adi*s. We employed a novel participatory approach called "recipe contest" to mobilize *Adi* women from 15 randomly selected remote and transitional villages. Data collected through interviews, focus group discussions and transect walks from 75 women, were analyzed using qualitative and quantitative methods.

Results: We found that traditional local food was linked intricately with women's knowledge, access patterns, and socio-cultural institutions. From an end-user perspective, remote *Adi* villages had more widespread use of plant species (14 as food; 34 as food-cum-ethnomedicine and 13 as income-generating species) and animal (17) as food in remote than in transitional villages. Further, there were significant differences ($p < 0.000$; 0244 and 0.000, respectively) across the social groups for use of plant- and animal-derived food and ethnomedicine. Among different land use systems (i.e., *Jhum* lands, home gardens, and *morang* forests), the highest diversity for food plant was found in home gardens while that for food-cum-ethnomedicinal plants and animal species was observed in *morang* forests. *Adi* women apply traditional agronomic, cultural, and harvest strategies, based on local knowledge and institutions and varying with social systems, to conserve their key local resources. Significant correlations ($r = 0.63$ to 0.92) were seen between conservation and use of food and animal species. Traditional knowledge on these aspects was linked intricately to the socio-cultural milieu and is transmitted inter-generationally through various social institutions.

Conclusions: Cultural food practices, crucial to social-ecological resilience and livelihood security of *Adi* and similar indigenous communities confronting global environmental challenges, need to be mainstreamed with relevant policies for achieving some targets of SDGs.

Keywords: *Adi* women, Arunachal Pradesh, Conservation, Diverse ecosystems, SDGs, Traditional food species

* Correspondence: ranjaysingh_jbp@rediffmail.com
[1]College of Horticulture and Forestry, Central Agricultural University, Pasighat, Arunachal Pradesh 791102, India
[2]ICAR-Central Soil Salinity Research Institute, Karnal, Haryana 132001, India
Full list of author information is available at the end of the article

Introduction

Human-environment interactions shape socio-cultural and ecosystem diversity in several ways (Marten 2001). Social-ecological diversity, in turn, helps determine intrinsic relations between food-based cultures and livelihood patterns (Pretty et al. 2009). Learning about local biodiversity-based traditional food systems requires continuous acquisition and integration of knowledge from diverse sources (Turner and Turner 2004; Reyes-García et al. 2009). Such knowledge varies with eco-cultural diversity and local strategies for accessing food-based resources (Singh et al. 2010). In the recent past, erosion of traditional food and related cultural knowledge, and the importance of characterizing, validating, and promoting this knowledge has been stressed, particularly relating to impacts on peoples' nutrition and health (Kuhnlein 2005; Kuhnlein et al. 2013; Turner 2005). Mainstream society can learn from traditional knowledge holders to better understand and strengthen location-specific adaptive practices for sustaining biocultural diversity and reducing land degradation (Pretty et al. 2009).

Women are generally considered more knowledgeable than men regarding the ecology and conservation practices of plants used as food and ethnomedicine (Merétika et al. 2010), since they need this knowledge for ensuring family food security (Ellena and Nongkynrih 2017). In mountainous regions in particular, where subsistence economies prevail, strategies for food production and sustenance are major responsibilities of women (Singh et al. 2017), who possess significant knowledge of harvesting, processing, and use of food and ethnomedicine (Magni 2016). Women in general also play an important role in intergenerational transfer of traditional knowledge (TK), thus ultimately assuring food and livelihood security, as well as careful observation and testing of particular foods (Turner and Clifton 2009; Turner and Spalding 2013). Women's knowledge of processing and sustaining food and nutritional resources varies with socio-cultural systems (Gupta 1996).

Arunachal Pradesh (Ar P), a state in northeastern India, occupies 7.7% of the total geographical area of the country and is one of the biodiversity hot-spots of the world (Myer et al. 2000). It harbors a large proportion of India's flora [(8000 species) (Rao 1994)], with about 4% (2526 plant species) being endemic (Nayar 1996). Community forests—which include forests under clan control, cultivated and non-cultivated floras in *jhum* land (slash and burn agriculture), and home gardens—play a pivotal role in conservation of indigenous biodiversity and securing food and livelihoods for local communities (Ramakrishnan 2007). In Ar P, the women often take the lead in managing TK, and in development, care, and well-being of families and their food systems. However, in most conservation programs for biodiversity of India—including Ar P—the roles of women and their knowledge for strengthening and promoting community survival are often not acknowledged (Mishra et al. 2009). An understanding of women's roles and the ways in which they uphold TK—either in harvesting or adding cultural value to plants and animals used as food and ethnomedicine—and in conservation of related biodiversity is critical to achieving some of the SDGs (Sustainable Development Goals) in India. This study focuses on the *Adi* women of Ar P relating to understand (i) access of culturally valuable local plant and animal species from diverse ecosystems for use as food and ethnomedicine and (ii) assessing species diversity and location-specific traditional conservation strategies.

Conceptual framework

Tribal communities living in remote locations of India, particularly of mountainous ecosystems differ from those of more settled and manipulated landscapes in terms of available resources and socio-cultural and political resource base (Joshi 1989) (Fig. 1). These economically marginal communities, unable to access external resources, have developed ecosystem-based knowledge systems (Gupta et al. 1999; Subramanian and Pisupati 2010), to survive and maintain biocultural diversity (Toledo 1999). Over time, they have also evolved culturally and ecologically responsive strategies for adapting to multiple stressors (Davidson-Hunt et al. 2012). Identifying these strategies can provide meaningful insights for policymakers attempting to develop place-based approaches to land and resource use, and meeting targets of SDGs, especially for biocultural hot-spots (Maffi 2010; UNESCO 2019). Based on insights from Toledo (1999), Maffi (2010), and Davidson-Hunt et al. (2012), we conceptualized *Adi* biocultural resources as "a plant-animal-cultural continuum contributing to resilience and sustainable livelihoods".

Development approaches relating to food and environmental security for those having predominant biocultural resource bases require a different lens for understanding people's worldviews and practices for sustainability (Davidson-Hunt et al. 2012). The Indian government has prepared a stakeholder framework to meet the SDG targets (particularly goal 1, "No Poverty"; goal 2, "Zero Hunger"; and goal 15, "Life on Land") (Aayog 2017). Scrutiny of this framework, however, shows a lack of focus on SDGs on ensuring biocultural resources and inclusion of *bottom-up* perspectives from marginal and tribal communities. Schleicher et al. (2018) stress the importance of biocultural diversity in understanding the constraints of such peoples and their priorities and agendas. We have conceptualized biocultural knowledge as how tribal communities assign cultural values to local plant and animal species used as traditional foods and
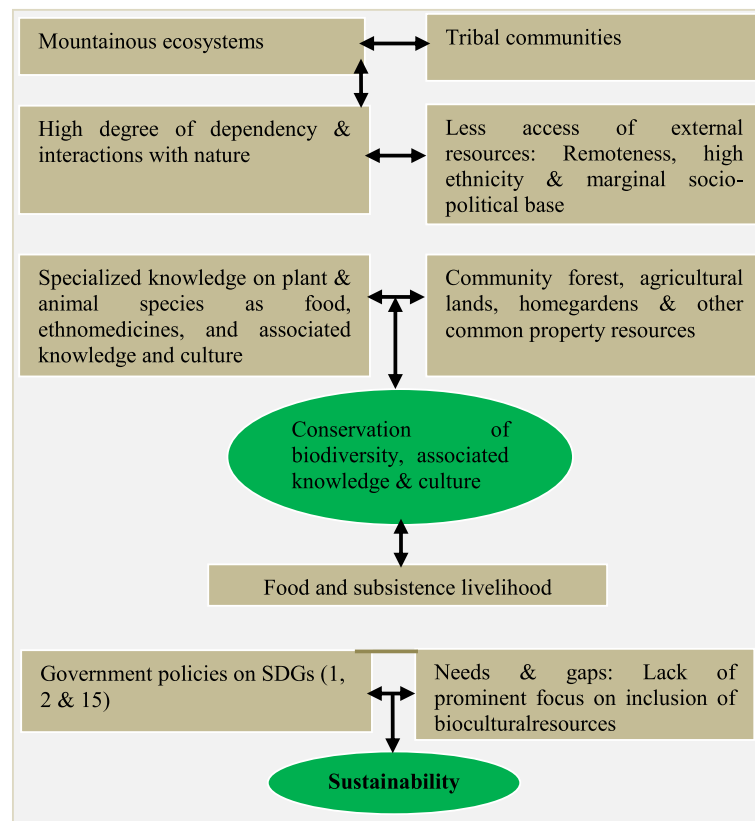
Fig. 1 Conceptual framework on biocultural resources helping to sustain food security and subsidence livelihood, and contributing to the Sustainable Development Goals

how they apply their knowledge to access, use, and conserve these species in diverse ecosystems (Fig. 1). We assumed that inclusion of insights from the biocultural resources conserved by more nature dependent and marginal communities (in our case *Adi*) in making strategies on SDGs can provide multiple-scale outcomes helpful in addressing the challenges of SDGs (Fig. 1).

In this study, we documented *Adi* food species, their origins, use as food and/or ethnomedicine, and importance in local culture including beliefs, festivals, and marriage (Maffi 2010). We also documented *Adi* women's traditional management practices, growing and maintaining local species in particular land use types (Fig. 1). We first present *Adi* women's use and access patterns for local plant and animal species as food, ethnomedicine, and income provisions and how these vary in remote and transitional villages. We then highlight similarities and differences in food species and document how different traditional agronomic and cultural practices can strengthen conservation of these species within different social systems. Finally, we highlight connections between culture, learning (biocultural dimensions), and food species conservation. We discuss probable determinants shaping the key trends reflected

in our results, and suggest how policy and planning agencies can gain insights to strengthen some targets of SDGs relevant to fragile mountainous ecosystems.

Research methodology
Study area
Arunachal Pradesh (Ar P) state of India (26° 28′ and 29° 30′ N, 90° 30′ and 97° 30′ E) covers an area of 83,743 km². The entire state is mountainous, with altitudes ranging from 200 to 6,400 m above sea level. The climate is humid at lower elevations; the valleys are covered by swampy rainforest, particularly in the eastern section. Altogether, the state's forest cover is 81.22%. Forests comprise 10.03% of India's total forest cover (DoEF 2010). Most of "unclassified" forests in Ar P are *morang* (community owned). Forests in and around *jhum* lands are managed by particular clans/tribes (Singh et al. 2018).

Ar P is home to 26 tribes and about 110 ethnic groups. The *Adi* (also known as *Abor*) are a major collective tribe living in West Siang, East Siang, Upper Siang, Upper Subansiri, and Dibang Valley districts. The *Adi* have distinct customs, dialect, and life-ways (Danggen 2003). Their economy is primarily agrarian, revolving predominantly around *jhum* and wet rice cultivation.

Their staple foods include rice, maize, millets, and forest-based traditional food products. Women collect culturally important plants from diverse ecosystems for their food, ethnomedicine, marketing, firewood, and other needs. Men hunt wild game from *morang* community forests, including for feasting on cultural occasions such as festivals and weddings (Singh et al. 2010). A traditional measure of a family's wealth is the possession of "*mithun*," a native ox (*Bos frontalis*). Folk dances are performed as part of important rituals, during festivals and other occasions, generally accompanying serving of traditional foods.

Research design

Exploratory research design is widely applied in assessing human-environment interactions to develop a better understanding of how people perceive and use local resources for their well-being (Cuthil 2002). The purpose of this research was to explore the culturally valuable plant and animal species being used as food and/or ethnomedicine and for income generation, and how they are being conserved by the *Adis*. We used an exploratory research design (Roscoe 1975) involving both conventional social-ecological tools and unique participatory approaches (Wagner and Alexander 2013) to accomplish the study objectives (Fig. 2).

Sampling of study area

We applied a mixed method of sampling to the study area (Onwuegbuzie and Collins 2007). At the outset, we approached each village community, building rapport with key village communicators (customary chief, elders, cultural experts and hunters) to describe our study objectives. The study was carried out within three purposively selected circles (State Government administrative units): Pasighat and Mebo of East Siang, and Maryang of Upper Siang. This purposive selection was based on *Adi*'s ethnicity, high dependency on local bioresources, remoteness of the region, high level of diversity and endemism of plant resources (both terrestrial and aquatic), and communal nature of the local resources. Within each circle, five villages (a total of 15 from three circles) were selected randomly. Seven of these were relatively remote and 8 were transitional, being situated closer to towns.

Recipe contest: participatory methodology for sampling participants

In each sampled village, a participatory recipe contest (traditional food competition) was organized among *Adi* women, being publicized about 15 days prior to the event. Potential women participants were informed about the event with the help of a team, consisting of *Gaon Burha* (customary chief of an *Adi* village), elders
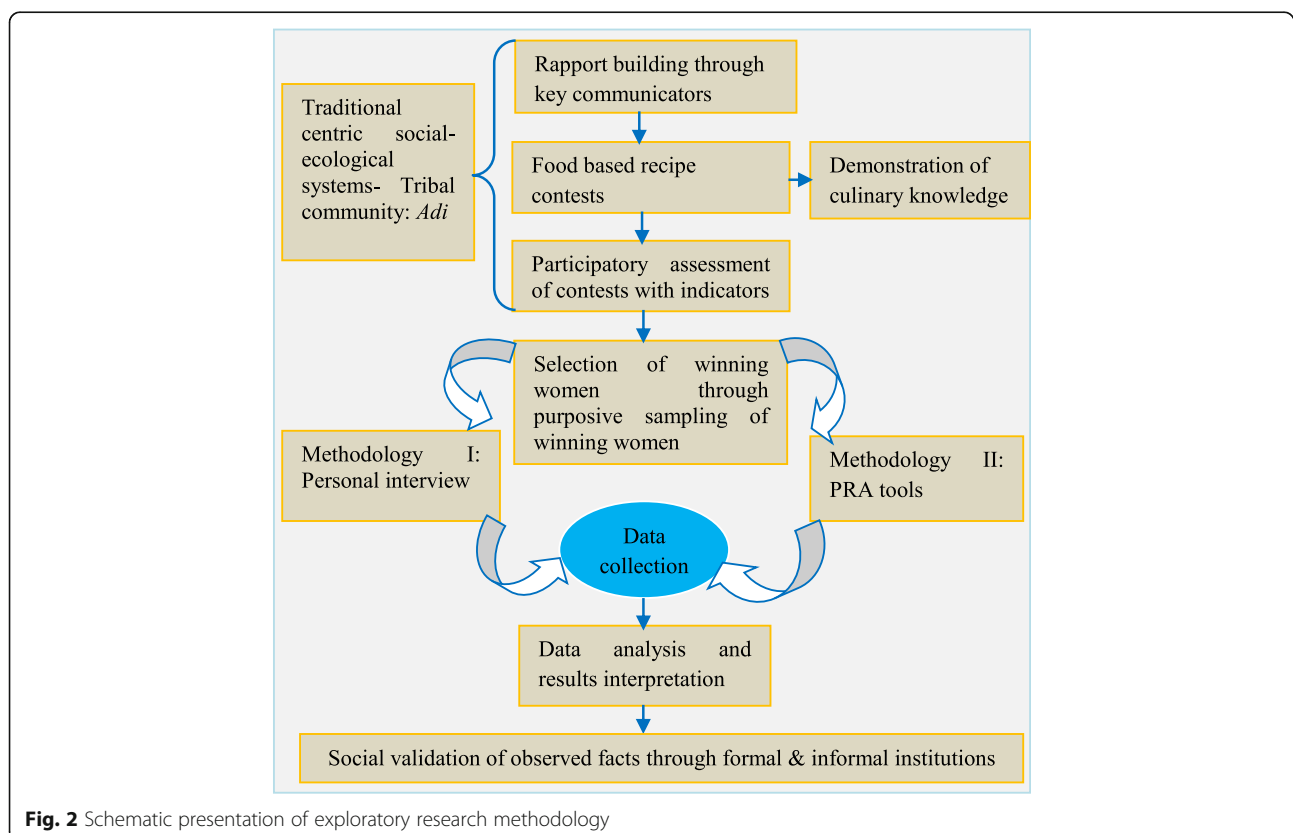

Fig. 2 Schematic presentation of exploratory research methodology

and hunters of selected villages. The contest allowed us to explore, fairly readily, each village's relative diversity of culturally important plant- and animal-based traditional food (Singh and Singh 2013). On a fixed date, interested women presented dishes prepared from local plants and animals in village *mosup* (community hall) (Fig. 3), along with plant samples and dried parts of animals (stored already). Their plant samples were identified by plant taxonomists of the Botanical Survey of India, Ar P and zoological resources were identified by wildlife experts, with the help of hunters and the participating women.

The number of participants in the selected villages varied from 15 to 35. The plant- and animal-based foods were assessed by a team consisting of 4–5 elderly women, *Gaon Burha* (~ 4; 60 from 15 villages) and study team members. Assessment categories included local species used as food, traditional cooking methods applied, learning sources (parents, grandparents, or other members of community), and knowledge about the ecosystems from which species were accessed. Each category was assigned a potential high score of "1"; thus, a species fulfilling all criteria would be assigned a maximum score of "4." Based on the highest mean scores (scores assigned from village food expert and scientists added together) obtained by women for their total foods presented in the "recipe contest," first, second, and third prizes were awarded, with two additional consolation prizes. The winners received their awards at a public gathering in each village, and the proceedings were broadcasted by All India Radio, Pasighat (East Siang district). Based on scores obtained in the contest, a total of 35 women were finally selected randomly (based on the equal probability to each participant of being selection) from remote villages and 40 from transition villages. Thus, we interviewed a total of 75 women (five from each village) for recording study observations (see Additional file 1: Table S1).

Fig. 3 An *Adi* woman displaying diversity of traditional food prepared from local plant and animal species (photo by Ranjay K. Singh)

Data collection through interviews

Our interview schedule with open-ended questions was based on a local literature search and scopic study (Orchard et al. 2016) conducted earlier (Singh and Adi 2010), allowing us to become familiar with the local context. We applied a combination of data collection approaches, both qualitative (use and access patterns of species, agronomic practices and cultural dimensions) and quantitative (diversity of plant species, conservation practices and income). The interview schedule was first applied in a pilot test with 5 *Adi* women in a non-sampled area to assess the reliability of our questionnaire and make any needed improvements in questions and language (see Additional file 1: Table S2). Throughout the study a local guide assisted us in our documentation, and the women participants were interviewed in the *Adi* dialect. On average, interviews were conducted in segments over 1–2 days per woman, with a total of about one week of interviewing for each village.

Data collection through participatory tools

Transect walks were done with elders and sampled respondents to identify land use types. Visits to various food production and conservation areas (*jhum* lands, home gardens, *morang* community forest, and aquatic bodies under common property resources) were made, with the support of village elders (4 per village), who helped in our assessments during the recipe contests, recording and validating the different food species (see Additional file 1: Table S3). Focus group discussions helped to document complex aspects such as changes in food systems over time, cultural dimensions and a deeper sense of ecological aspects of food species. Participant observations with some respondents helped to document agronomic and harvesting practices and connections between festivals and traditional foods. Participatory audio recording helped to cross check data taken through interviews and FGD (see Additional file 1: Table S4).

Measurement of major variables

Learning modes were assessed in terms of number of socio-cultural institutions (e.g., family and indigenous institutions) through which respondents acquired local knowledge of plant and animal species. Land use systems (home gardens, *jhum* land, *morang* community forest and water stream or community river) determined scores assigned to a species: 1 for species conserved in home gardens (less in area), 2 for those in *jhum* land (moderately higher in area), and 3 for species conserved in *morang* forests or water streams (highest in area). The conservation of each species was considered based on their cultivation within a particular land use type or how the *Adi* women allowed them to grow naturally

(prefixing cultural value). The food species included in this study were classified based on the purpose of use and access from different land use types. Use, as a variable, was quantified by assigning a score of "1" if a species was used by less than 50% of respondents, followed by "3" (51–75%) and "5" (76–100%). This score also helped in assessing significance of difference between two social groups about species used for diverse purposes. Similar patterns were followed in species used both in food and ethnomedicines, and those used as animal-based food.

Criteria for selection of species for economic and livelihood aspects

Data were collected for 13 of the most popular ethnobotanicals and their overall economic value was assessed using the following criteria: (i) most frequently used in foods, (ii) easy availability, (iii) conservation in various habitats, (iv) economic value in local market, and (v) adaptive capacity for climatic, ecological, and socio-institutional stresses.

Data analysis and statistics

A thematic technique was applied to determine patterns and trends in particular aspects of the data (Stringer et al. 2017). Diversity indices of species found in different land use systems were calculated drawing on insights from Coddington et al. (1996), Camargo (1997), Nagendra (2002) and Eguia-Aguilara et al. (2005). The indices were computed using PAST software (Hammer et al. 2001). STAR statistics (version 2.0.1) (IRRI 2013) was applied to compute the Spearman correlation between usage and conservation of species. The significance of difference between usage of plant and animal food species and income of women from remote and transitional villages was tested applying t test. Other data, including use percentage, income, and agronomic practices, were analyzed using descriptive statistics in a spreadsheet. Key results obtained were shared back with the study respondents to socially validate our conclusions.

Results

Use and accessibility of food species

Plant food species

Fourteen plant species were identified as key ingredients in the foods prepared by *Adi* women. The percentage of use of plant species as food varied from as low as 50.4% for *dilap* (*Allium hookeri*) to the highest at 97.7%, for *onger* (*Zanthoxylum rhetsa*) in remote villages (Table 1). These two species were used relatively less in transitioning villages (55.8 and 25.0%, respectively). *Marshang* (*Spilanthes acmella*) was the second most used species in remote villages (94.6%), while *ongin* was used highly (65.0%), followed by *marshang*, by the respondents of

Table 1 Major local plants species used in foods

Local name	Botanical name	Parts used[a]	Habitats[b]	Use %[c]	
				Rural	Transitioned village
Lai saag	*Brassica* sp. L.	Leaf	JL and HG	65.9	50.5
Dilap	*Alleum hookeri* L.	Whole plant	JL and HG	50.4	25.0
Epuk-tangum	*Bidens pilosa* L.	Leaf	JL and HG	87.5	40.5
Onger	*Zanthoxylum rhetsa* (Roxb.) DC.	Leaf	JL, HG, and MCF	97.7	55.8
Ongin	*Clerodendrumcole brookianum* Walp.	Leaf	JL, HG, and MCF	93.9	65.0
Marshang	*Spilanthes acmella* L.	Leaf	JL, HG, and MCF	94.6	58.0
Kopi	*Solanum khasianum* C.B. Clarke	Fruits	JL and HG	80.5	47.0
Koppir	*Solanum spirale* Roxb.	Fruits	JL and HG	60.3	41.4
Paput	*Gnaphalium affine* D. Don	Leaf	JL and HG	59.1	20.2
Mamang	*Physalis minima* L.	Leaf	JL and HG	58.4	15.0
Fayong	*Polygonum chinensis* Meissn.	Leaf	JL and HG	68.9	10.5
Nayang	*Erigeron Canadensis* L.	Leaf	JL, HG, and MCF	91.2	35.7
Tangum	*Bidens pilosa* L.	Leaf	JL and HG	68.6	19.0
Gende	*Gynura crepidioides* Benth	Leaf	JL and HG	86.8	38.4
Statistics				*t* value = 5.36, *p* < 0.000	

JL Jhum-land, *HG* home gardens, *MCF* Morang community forest

[a]Plant parts data: 11 species as leaves (78.57%), 2 species each as fruit (14.28%), 1 species as whole plant (7.14%)

[b]Species data: JL and HG = 10 species (71.42%); JL, HG, and MCF 4 species (28.57%)

[c]Multiple percentages

transition villages. Overall, women from remote villages used a greater percentage of local plant species as food than their counterparts of transition villages and these two groups varied significantly ($t = 5.36$, $p < 0.000$). Out of 14, most of the species were used in the form of leaves (78.57%) and fruits (14.28%) across the social systems. The majority (71.42 %) of the food plant species were accessed from *jhum* lands and home gardens, and the remainder from a set of three land use systems: *jhum* lands, home gardens, and *morang* community forests (28.57%).

Food plant species with ethnomedicinal values

We identified 33 plant species used as both food and ethnomedicine for treating certain diseases and disorders (Table 2). *Namdung* (*Perilla ocymoides*) was recorded to be a highly used dual purpose species (for stomach disorders and supplementary food for pregnant women) by *Adi* women of remote villages (97.5%), but somewhat lower among women of transition villages (58.5%). *Gende* (*Gynura crepidioides*) was the second most used species, for both remote and transitional *Adi* women (96.6% and 41.5%, respectively), for curing sickness. Overall, based on a total of 33 species, the dependency of women from remote villages on use of plants as food and ethnomedicine was significantly greater than those of their transitional village counterparts ($t = 5.11$, $p < 0.000$). Leaves (45.45%) and fruits (27.27%) of these species were the most usable parts across both the social

systems. Home gardens were the most accessed land use system (45.45%), followed by *jhum* lands (24.24%) and *morang* community forests (30.30%) for dual purpose species.

In terms of medicinal species, some like *kekir* or ginger (*Zingiber siangensis*) and *oik* (*Pouzolzia hirta*), were used for coughs and colds, and for treating snake bites and improving lactation among mothers, respectively. However, their use varied between remote (85.5%) and transition villages (57.8%). Some local medicinal species were also sometimes prescribed by professional doctors, according to our interviewees. For example, *ongin* (*Clerodendrum colebrookianum*) is recommended for controlling diabetes and high blood pressure (35.8 % in remote and 39.8% in transitioning villages). Similarly, the green leaves of *onger* (*Zanthoxylum rhetsa*), rich in antioxidants (Bhardwaj et al. 2005), are used in controlling dysentery and diarrhea (66.8 % in remote and 45.7% in transitioning villages). *Onger* leaves are also cooked with pork and other meats (as are other leafy vegetables), to improve the taste and reduce the incidence of potential parasitic diseases like tapeworm infestation (Yadav and Tangpu 2009).

Animal-based food species

A total of 17 wild animal, fish and insect species were found to be traditional *Adi* foods (Table 3). *Ngopi* (*Labeo dero*) and *ghongha* (*Lymnaea* sp.)/snails (various indigenous species) were used by almost every woman (100%) in remote villages, while having relatively lower use (60.5 and

Table 2 List of major ethnobotanicals used as food-cum-ethnomedicine by the *Adi* women

Local name	Botanical name	Part used[a]	Habitats[b]	Disease/disorders/ailments	Percentage of use[c]	
					Remote villages	Transitioned villages
Amta	*Hibiscus sabdariffa* L.	Flowers and leaves	JL	Diarrhea and dysentery	95.2	21.0
Bangko	*Solanum spirale* Roxb.	Leaves and fruits	JL	High blood pressure and diabetes	94.6	32.5
Kordoi	*Averrhoa carambola* L.	Fruits	MCF	Jaundice	24.2	8.0
Champa	*Dillenia indica* L.	Peel of fruit with small fishes	MCF	Stomach pain	22.4	6.5
Dipatalo	*Campylandra aurantiaa* Baker	Rhizomes	MCF	Tonic in stomach disorders	93.5	47.6
Doni-homgkang	*Plantago erosa* Wall	Leaves	MCF	Improving digestion	19.8	7.5
Fayong	*Polygonum nepalens* Meissn	Leaves	HG	For sick persons	87.5	36.0
Gende	*Gynura crepidioides* Benth	Leaves	HG	For sick persons	96.6	41.5
Haryo	*Eryngium foetidum* L.	Stem and leaves	HG	Headache and madness	87.2	39.7
Kadokaro	*Drymaria diandra* Bl	Fruits	MCF	Gastric problem	86.2	40.5
Kekir	*Zingiber siangensis*	Rhizome	MCF	Cough and cold, wound healing, and in snake bites	85.5	57.8
Koppi	*Solanum torvum* Swartz	Fruits	HG	Gas and indigestion	80.2	60.0
Koppir	*Solanum khasianum* Clarke	Fruits	HG	Gas and indigestion	79.5	62.0
Kulu	*Musa sapientum* L.	Fruits	MCF	Swollen feet and skin disorders	27.9	9.5
Lalada	*Zingiber* sp. Roscoe	Rhizome	HG	Cough and cold, wound healing	80.4	43.8
Mamang	*Physalis minima* L.	Leaves	HG	For sick person	78.2	47.6
Namdung	*Perilla ocymoides* L.	Seeds	JL	Stomach disorders and supplementary food for pregnant women	97.5	58.5
Nayang	*Erigeron canadensis* L.	Leaves	JL	For sick persons	27.3	9.5
Oik	*Pouzolzia hirta* Hassk	Leaves	HG	Improving lactation	68.4	33.2
Onger	*Zanthoxylum rhetsa* Roxb.	Leaves	HG	Stomach disorders and high blood pressure	66.8	45.7
Ongin	*Clerodendrum colebrookianum* Walp.	Leaves	HG	Controlling high blood pressure and diabetes	65.8	39.8
Paput	*Gnepalium affine* D. Don	Leaves	HG	For getting quick energy	59.2	28.5
Rouri	*Piper sylvaticum* Roxb.	Leaves	HG	Sick person	65.9	30.0
Rukdik	*Thelypteris glanduligera* Kunze	Leaves	MCF	Muscle pain	34.2	14.9
Rumdum	*Blumea fistulosa* Roxb.	Leaves	MCF	Diarrhea	32.5	7.8
Sajna	*Moringa oleifera* Lam.	Pods and leaves	HG	control muscle pain and diarrhea	30.2	12.5
Satabayom	*Solanum indicum* L.	Fruits	HG	Diabetes	77.8	51.4
Sirang	*Castanopsis kurzii* Hance	Fruits	JL	Alleviating scurvy, gas trouble, and toothache	34.6	13.7
Talab	*Allium* sp. L.	Bulbs	HG	Improving digestion	75.4	49.5
Tangmeng	*Mussaenda roxburghii* Hook. f.	Leaves	MCF	Stomach disorders	47.9	30.0
Tangum	*Bidens pilosa* L.	Leaves	JL	Sick persons	39.2	12.5
Teetbaigan	*Solanum kurzii* Brace	Fruits	JL	Improving digestion	38.7	14.5
Toko-patta	*Livistona jenkinsiana* Griff.	Fruits	JL	Gastric problems	37.4	18.0
Statistics				*t* value = 5.11, *p* < 0.000		

JL Jhum lands, *HG* home gardens, *MCF* Morang community forest

[a]Leaf parts data: Leaves = 15 (45.45%); fruits = 9 (27.27%); flower, peel, seeds, stem, bulbs, and pods = 6 (18.18%); rhizomes = 3 (9.09%)

[b]Habitat data: JL = 8 species (24.24%), HG = 15 species (45.45%); MCF = 10 species (30.30%)

[c]Multiple responses

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Table 3 Major animal species used in foods[a]

Local name	Zoological name	Habitats[b]	Cultural dimensions	Use %[c] Remote villages	Use %[c] Transitioned village
Kebungs	Malayan giant squirrel (MGS) *Ratufa bicolour* Layard in Blyth[d]	MCF	Used as dowry and cultural food during *aran* festival	66.6	29.5
Chuha	*Rattus rattus* L./*Niviventer fulvescens* Gray	MCF	Used as food in *solung, aran* festivals, and marriage	65.8	22.4
Lomadi	*Vulpes bengalensis* Shaw	MCF	Used during grand celebrations after hunting	67.4	11.2
Porcupine[e]	*Hystrix hodgsoni* Gray	MCF	Part of summer festivals and ethnomedicines	47.2	19.4
Jangalisuar	*Sus scrofa* L.	MCF	Used during *solung, aran,* and *mopin* festival	43.1	23.4
Bug-nyul	*Viverricula indica* É. Geoffroy Saint-Hilaire	MCF	As above	41.9	29.7
Bhalu-biral[f]	*Arctictis binturong* F. G. Cuvier	MCF	As above	42.5	33.2
Kala bhalu[f]	*Selenarctos thibetanus laniger* Pocock	MCF	Used during marriage	40.8	11.4
Neur	*Herpestes auropunctatus* Hodgson	MCF	Part of dowry	39.1	20.4
Kekdakhane-walamoosa	*Herpestes urva* Hodgson	MCF	Part of dowry	46.4	23.8
Mithun	*Bos frontalis* Lambert	MCF	Part of dowry, indicators of social prestige	48.9	35.8
Gandhi pug	*Aspongopus nepalensis* Westwood	MCF	Used in several *Adi* ceremonies	43.8	14.5
Run-kung	*Oecophylla smaragdina* Fabricius	Water stream and CPR	Used as food and applied in curing malaria	42.3	11.2
Ngopi[g]	*Labeo dero* Hamilton	Water stream and CPR	Used as medicines in skin diseases and kept as fermented food	100	60.5
Ghongha[h]	*Lymnaea* sp. P. Rashad and other species also	Water stream and CPR	Considered to be helpful in improving vitality	100	55.4
Sidhari	*Puntius* sp. Hamilton	Water stream and CPR	Fresh and fermented use in parties	98.5	63.8
Chelhwa	*Chelwa laubuca* Hamilton	Water stream and CPR	As above	96.6	60.5
Statistics			t value = 2.05, p < 0.024		

MCF Morang community forest, *CPR* common property resources

[a]Data pertaining to animals species reported in the table were collected by hosting FGD (focus group discussions) with elder women

[b]Habitat data: MCF = 12 species (70.58%); water stream and CPR = 5 species (29.41%)

[c]Multiple percentages

[d]Red-bellied squirrel (RBS) *Callosciurus erythraeus*, hoary-bellied squirrel (HBS) *C. pygerythrus*, and the Himalayan striped squirrel (HSS) *Tamiops maccellandi* are also found there and used occasionally

[e]Other than using in meat, the gall bladder is used for curing malaria

[f]Other than using in meat, bones are used for some rituals and cultural functions

[g]This indigenous breed of fish is used for curing some skin diseases. It is most commonly fish used in fermentation and giving to seek and old person

[h]Used in food and its shell is used to make edible lime

55.4%) in transition villages. *Sidhari* (*Puntius* sp.) and *chelhwa* (*Chela laubuca*) fish were the second and third most used food species: 98.5 and 96.6% use, respectively, among the women of remote villages, and 63.8 and 60.5% among women of transitional villages. Among insects, the *gandhi-pug* (*Aspongopus nepalensis*) was used more widely as food (43.8%) in remote villages than in transitional villages (14.5%). These animal-based food species were integral parts of various *Adi* socio-cultural dimensions (festivals, marriage, and ethnomedicines). Overall, their use as food was significantly higher in remote than transitional villages (t = 2.05, p < 0. 024).

Most of the terrestrial animal species across the two community types were accessed from *morang* community forest (being hunted by the men and provided to the women) (70.58%), while aquatic species, caught by both men and women, came from freshwater streams and community rivers (common property resources) (29.41% use). Proximity to *morang* forest and other common property resources enables the *Adi* of remote villages to hunt listed species more frequently than in transitioning villages. In addition, women living nearby *jhum* lands, especially in remote communities, were able to use local landraces of rice (local variety *amkel* and *geku*) for making fermented rice and taken with fish, animal, and ethnobotanical products.

Dietary shift from traditional to "convenience" foods
Some of forest-based foods harvested and used by *Adi* women have made the transition from being considered hardship fare to being delicacies fit for social and cultural celebrations. Examples include *ongin*

(*Clerodendrum colebrookianum*), *onger* (*Zanthoxylum rhetsa*), *kopi* (*Solanum torvum*), *koppir* (*Solanum khasianum*), and *bangko* (*Solanum spirale*). Nevertheless, the use of forest-based foods has decreased over time (Fig. 4). The majority of respondents (79.8%) noted a higher abundance of deer, rabbits, boar, and forest rodents in the 1970s and earlier. With ever-growing population, changing land use pattern, acculturation, and an erosion of traditional conservation values, the populations of these wild animals have generally declined. Women reported that the *jhum* lands, following the cutting of trees, have become drier than in the recent past, and described numerous declines over the past few decades in wildlife abundance and in plant-based traditional foods around the settlements, especially in the transitional villages.

Use of some fiber species in food was greater among *Adi* women of remote areas. For example, only 5–7 fiber-rich species were used by *Adi* women of transitional villages, whereas in remote locations use of 20–25 such species were documented. In transitional villages, people were consuming only 20–25% boiled foods, with diets rich in fried and fast foods (30–40%), whereas in more remote locations mostly boiled food without spices and marketed oil were consumed. In remote communities, fat was mainly obtained from hunted animals, and plants like *sayong, kekir, onger*, and *dilap* were used as spices and flavoring the food. Women of transitional villages use purified table salt (chemically sodium chloride without trace minerals), while in remote areas, women used natural salts and ash (produced from burning snail shells); presumably rich in both sodium chloride and other minerals.
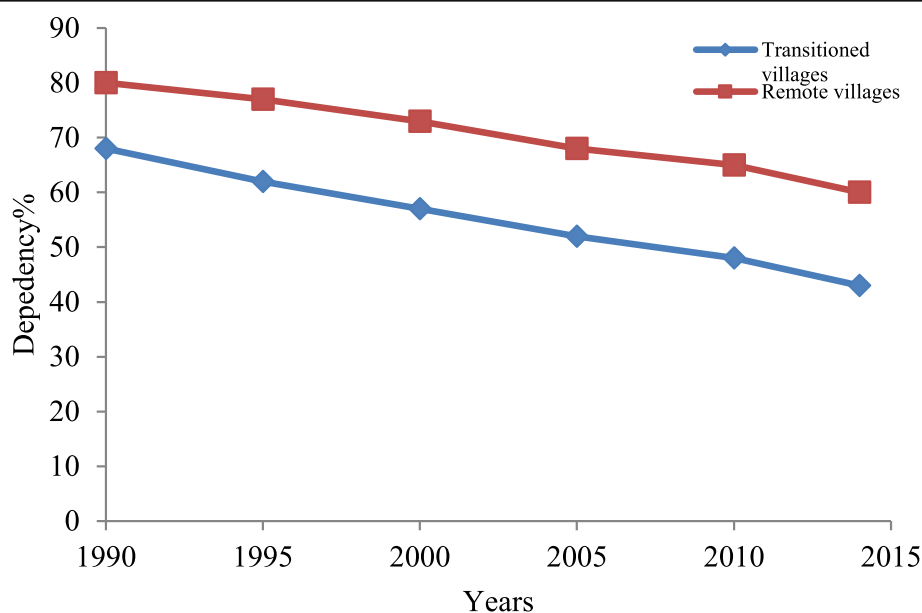

Fig. 4 Dependency percentage of *Adi* on forest-based traditional foods over the year near town and in remote villages

Role of plant species in food and income security

We identified, in all, 13 economically important species harvested by *Adi* women for income, markedly reduce their dependency on external resources (Table 4). These species are adapted to multiple stressors including climatic (flood), ecological (e.g., soil acidity), and institutional (poor accesses of formal institutions and organized market). These species become particularly significant at times when low-lying areas are hit by floods/flash floods, and supplies of marketed foods are curtailed. These species are available in different seasons and vary in economic value, from as low as INR 45/kg to INR 200/kg. However, women of remote areas fetched relatively lower returns from their sale compared to those from transitional village women having access to town markets. For example, the highest difference in market price between remote and transitional villages (109.67%) was recorded for *lalada* (*Zingiber* sp.), while lowest was for *rouri* (*Piper sylvaticum*) (42.86%) (Table 4). There was a significant difference ($t = 2.94$, $p < 0.003$) for the overall market price of 13 species between these two women's groups.

These species were accessed primarily from *jhum* lands (53.84%) and combinations of *jhum* lands, home gardens, and *morang* community forests (23.08%). The *Adi* women of remote villages—often having more family members to collect these local species from different ecosystems—sell them at lower prices to women living near towns. These women augment the diversity of food plants to some extent from their own land use systems, and diversify market demand by assigning different grades (e.g., single species, mixture of two species, mixture of 3–6 species, and mixture of sometimes 7–12 species) to the plant products, according to customer preferences and the price these mixtures bring. The women of transitional villages earned an average of INR 60,000 to 80,000 (US$ 943.1 to 1100) per year by selling such local plants, while women of remote villages earned less (INR 35,000 to 50,000; US$ 487.74 to 696.77).

Species diversity, and agronomic and cultural practices for conservation

Diversity and similarity indices of Adi food species

The Shannon index revealed that with species richness of 8, the diversity (*H*) of food plants was higher in home gardens (2.08) with similarity index (1–*D*) of 0.88, followed by *jhum* lands (*H* = 1.79; 1–*D* = 0.83). Across

Table 4 Economically important ethnobotanicals used by *Adi* women for food and income

Local name	Scientific name	Habitat[a]	Seasonal availability	Economic value in INR[b] per kg Remote villages	Transitioned village	Difference %	Role in adaptations
Koppi	*Solanum torvum*	JL and HG	April to September	70.0	38.0	84.21	A dependable food during flood[c]
Koppir	*Solanum khasianum*	JL and HG	April to September	65.0	31.0	109.67	As above
Onger	*Zanthoxylum rhetsa*	JL, HG, and MCF	Year round	75.0	40.0	87.50	Used year round. Stored after drying for off-season use
Pumpkin	*Cucurbita moschata*	JL	April to August	45.0	20.0	125.00	Easily cultivable
Lalada	*Zingiber* sp.	JL and HG	May to October	120.0	50.0	140.00	Medicinal and used as cash crop
Kekir	*Zingiber siangensis*	JL	May to October	200.0	100.0	100.00	Medicinal and used as cash crop
Poi	*Basella rubra*	JL and HG	April to August	50.0	32.0	56.25	A dependable food during flood
Dhekia saag	*Diplazium esculentum*	JL and MCF	May to September	60.0	32.0	87.50	As above
Marshang	*Spilanthes acmella*	JL, HG, and MCF	April to August	45.0	28.0	60.71	Compatible to grow anywhere
Ongin	*Clerodendrum colebrookianum*	JL, HG, and MCF	May to October	100.0	55.0	81.82	Medicinal and can be used year round. Can be stored after drying for off-season use
Kalmu	A creeper	JL and HG	May to September	65.0	35.0	85.71	Easy in growing and no inputs are required
Rouri	*Piper sylvaticum*	JL and HG	May to September	60.0	42.0	42.86	Easy in growing and no inputs are required
Adi dhania	*Eryngium foetidum*	JL and HG	April to November	100.0	55.0	81.82	Used as substitute for many vegetables. Can be stored after drying for off-season use
Statistics			$t = 2.94$, $p < 0.0035$			Overall 89.69%	

JL, *Jhum*-land; HG, home gardens; MCF, *Morang* community forest
[a]Habitat data: JL = 2 species (15.38%); JL and HG = 7 species (53.84%); JL, HG, and MCF = 3 species (23.08%)
[b]1 USD = 71.76 INR
[c]Since these species are grown at higher altitude in mountainous ecosystems, that is why the flood does not affect these species

these two land use systems, with species richness 14 (Chao-2 = 34.62 ± 26.23), the diversity was 2.64 with a similarity index of 0.93 (Table 5). With regard to plant species used for both food and ethnomedicine, the diversity (2.71) with species richness 15 was again highest in home gardens, with similarity index of 0.93 as compared to *morang* community forest (*H* = 2.40; 1–*D* = 0.78), having 11 species, and *jhum* lands (*H* = 1.95; 1–*D* = 0.86) with 7 species. Across the three land use systems, with species richness of 33 (Chao-2 = 101.5 ± 148), the diversity was 3.50, and the similarity index 0.03. In case of animal-based food species, with species richness 12, the *morang* community forest demonstrated higher diversity index (2.49) with similarity value of 0.92 than those of 5 species being accessed from aquatic bodies. With species richness 17, we noted species diversity 3.85 with Chao-2 value 45.49 ± 38.29. Overall, the species diversity was higher in animal species (3.85), followed by dual-purpose plant species (food and ethnomedicines) (3.50) and species used only for food (2.64). The Berger-Parker index revealed that proportional abundance of species for food and for dual purpose (food and ethnomedicine) was higher in *jhum* lands as compared to other land use systems. In case of animal species used as food, Berger-Parker index (0.20) was greater for species accessed from aquatic body.

Agronomic practices

We found that a great majority (90.7%) of women from remote villages practiced mixed cropping along with relay cropping (62.5%) as traditional agronomic practices. These numbers were fewer among their counterparts in transitional villages (40.6% and 8.5%, respectively). The intended outcomes of these practices were to enhance soil fertility, to strategically use residual soil moisture and to avoid ecological and climatic risks associated with particular species (Fig. 5). Controlled burning (82.4%), applying activator material (62.5%) (elephant and deer excreta) to enrich soil fertility, and active seed dispersal (69.2%) were key agronomic strategies for women of remote rural areas. The activator material also helped break seed dormancy of uncultivated species and enhanced plant productivity. They were not used by women in transitional villages, and the other practices were less frequent (20.4%). Similar trends across community types were seen for vegetative propagation, diversifying harvests, applying special planting techniques, and selective harvesting of plant species to enhance plant populations and species diversity. Creation

Table 5 Shannon diversity and similarity indices of bio-culturally important food species of *Adi*

Major use	Diversity and similarity indices			
	Jhum lands	Home gardens/aquatic bodies[a]	*Morang* community forests	Across the ecosystems
A. Food				
A.1 Plant species				
Species richness	6	8	NA	14
Shannon diversity (*H*)	1.79	2.08	NA	2.64
Simpson dominance (1–*D*)	0.83	0.88	NA	0.93
Berger-Parker index	0.17	0.13	NA	0.08
Chao-2	-	-	-	34.62 ± 26.23
A.2 Animal species				
Species richness	NA	5	12	17
Shannon diversity (*H*)	NA	1.61	2.49	3.85
Simpson dominance (1–*D*)	NA	0.80	0.92	0.98
Berger-Parker index	NA	0.20	0.08	0.02
Chao-2	-	-	-	45.49 ± 38.29
B. Food and ethnomedicine				
Species richness	7	15	11	33
Shannon diversity (*H*)	1.95	2.71	2.40	3.50
Simpson dominance (1–*D*)	0.86	0.93	0.78	0.97
Berger-Parker index	0.14	0.07	0.09	0.03
Chao-2	-	-	-	101.5 ± 148

Note: NA indicates that there were no species recorded in that particular land use
Looking to the assumptions of diversity indices (sample size and number of species for each category), pooled data (across the social systems) were used to draw this table
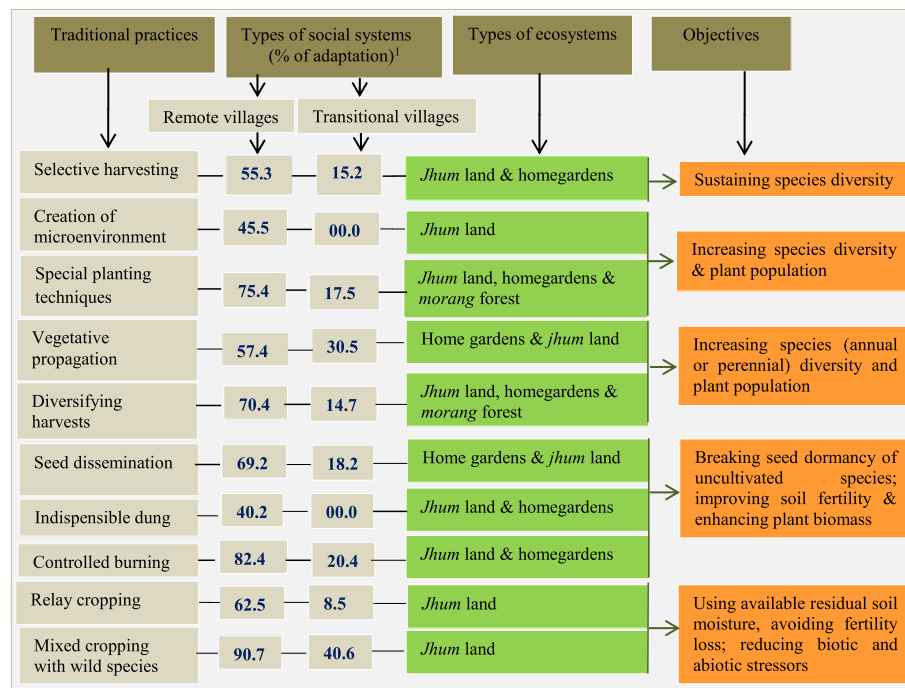[a]Aquatic bodies cover water stream in community forest and river tributaries (under community ownership)

Fig. 5 Traditional agronomic practices adapted by *Adi* women to enrich plant species diversity and enhance ecological sustainability. [1]Figure is based on pooled data of FGDs conducted in each village

of micro-environments (e.g., using exhausted edges of bamboo groves, and digging trenches and lining them with *sisar* tree wood) (45.5%) to increase species diversity and plant populations was an uncommon practice among women of remote villages and not practiced at all among women of transitional villages. All these traditional practices varied across the ecosystems surveyed (*jhum* land, *morang* forest, and home gardens) (Fig. 5).

Food-based knowledge learning

Acquiring knowledge about a particular food and its component species requires incremental experiential learning and practice, which *Adi* women have achieved with the support of various institutions and means (Fig. 6). In remote settlements, family, community, and socio-cultural institutions (*relep*, an informal institution of women, and *Kebang*, headed by *Gaon Burha*) were major venues of food knowledge transfer, whereas in transitional villages food networks were more prominent. Mothers were a valuable source of learning about cultural foods for both the social groups, although the relative importance of these modes of learning varied considerably across both systems.

Sociocultural and institutional practices on animal- and plant-based foods

The women of remote settlements held beliefs that foster conservation of the traditional food species, less so

for their transitional village counterparts (see Additional file 1: Table S5). For example, unlike 70.1% of the remote village participants, only 18.2% of transitional village participants reported that certain fish species are not given to children, due to a belief that this would cause them to urinate in bed, even after they had grown up. Similarly, 89.8% of remote village women, but only 35.0% of transitional village women, maintained that *mithun* (*Bos frontalis*)—a semi-wild animal—is culturally precious and must be carefully treated and never killed without a cultural reason. In remote villages, 56.9% of respondents said that when a wife is pregnant, a male hunter should not hunt monkeys, or they would be haunted by evil spirits, whereas only 23.5% of transitional village respondents held this belief. When they are in the forest at times of their wives' pregnancy, hunters must avoid drinking water from forest water streams, using *Colocasia* leaves for drinking, lest their unborn baby would be attacked by evil spirits. This belief was held by 69.4% of women in remote villages but only 27.0% of transitional villages in transitions. In the event of any of tabooed species being used as food (listed in Additional file 1: Table S5) by mistake, *kekir* (a local variety of ginger) is eaten to drive away the resulting evil spirits. Through practicing these beliefs, the demand for harvesting certain plants and animals is minimized for specific periods, particularly in the remote communities.

Fig. 6 Intergenerational knowledge transfer through various institutions
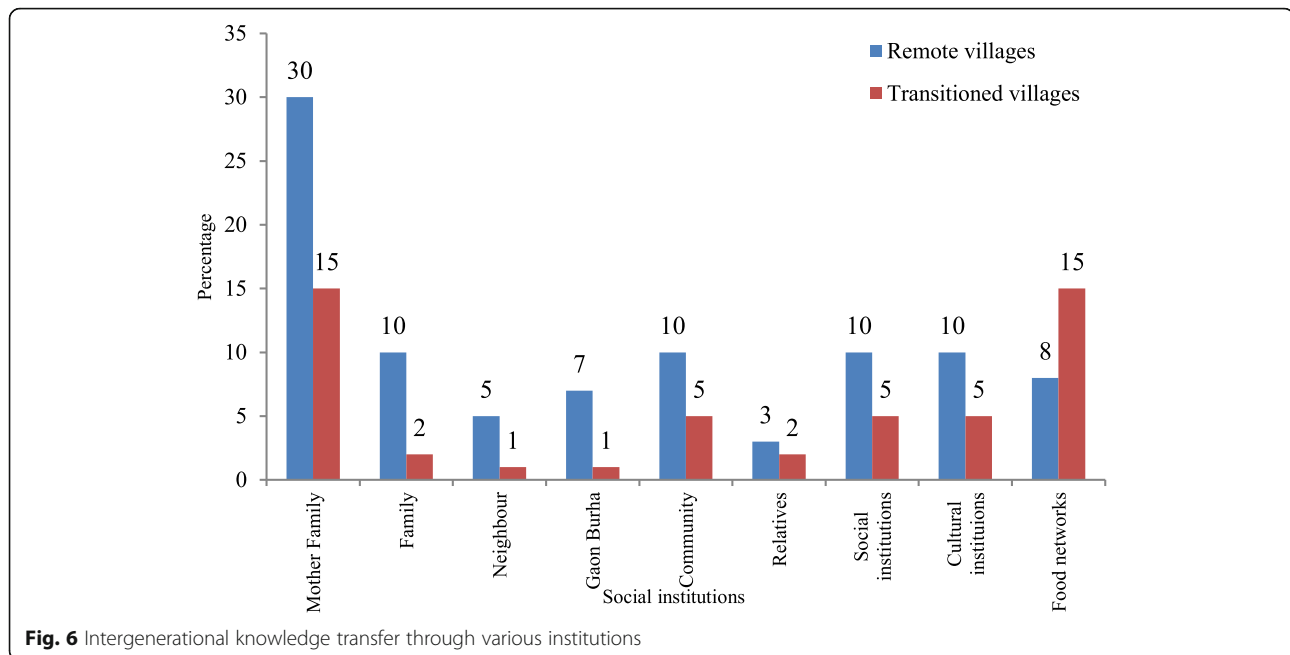
Some of the *Adi* festivals such as *solung*, *aran*, and *mopin* are especially dedicated for hunting; in particular, over-populated animals (e.g. rodents and squirrels [*kebungs*]) are hunted across the social systems. More families in remote (62.5%) than in transitional villages (28.5%) mentioned that they avoid hunting female game animals (e.g., *kebungs*) or catching fish during their breeding periods and that they varied their hunting locations to avoid overhunting. As a result, bioresources formerly used indiscriminately have now received priority for conservation. In addition, sustainability of local biodiversity is maintained by indigenous institutions, including *Kebang* (led by men) and *Reglep* (led by women). These play a significant role in regulating hunting and fishing, and harvesting of forest resources, with violators being fined.

Correlation of use and conservation of species
We found a highly significant correlation between the use and conservation of plant species for both food and ethnomedicine (0.924, *p* < 0.000), and the communities gave greater emphasis on conserving these species in diverse ecosystems (*jhum* land, home gardens, and *morang* forests) than for those used only for food (0.632, *p* < 0.015) (Table 6). The correlation between use and conservation of animals used for food was also appreciably high (0.832, *p* < 0.0005).

Discussion
Two key points arise from this research: first, how culturally rich plant and animal species are used and accessed for food, ethnomedicine, and livelihood security

by *Adi* women and secondly, how the insights of *Adi* women in our study can benefit several targets of Sustainable Development Goals (SDGs) and environmental policies in India.

Biocultural resources: food and livelihood security
Locally harvested plants and animals are important culturally and nutritionally, enhancing health and livelihood security for *Adi* women and their families. Turner and Clifton (2009) and Maffi (2010) reported that local biodiversity is a backbone for cultural identity and overall livelihood security of indigenous peoples across the globe. We found that *Adi* women from different social backgrounds (i.e., from remote versus transitional communities) did not necessarily use and access food species in the same way. Women of remote communities in general used more local plant and animal species than those of transitional villages. This difference might be due to variations in upbringing, different ways of coping with time and labor constraints in food production, and differing degrees of access to marketed food (Power 2008; Mishra et al. 2009). Different local strategies were applied by *Adi* women in accessing food species from different land use types (Turner 2005; Reyes-García et al. 2009). A number of plant species documented were used for both food and ethnomedicine, and this was particularly notable for the remote areas. Our work supports other research showing that local plants and wild game are generally beneficial for the overall nutrition, health, and well-being of indigenous peoples (Kuhnlein et al. 2013; Samson and Pretty 2006). Gregory (2000), Kuhnlein (1980), Kuhnlein (2005), and Kuhnlein et al. (2013) reported that wild plant

Table 6 Association between usage and conservation of species

Biocultural usage of species	Sample size	Spearman rank statistic (r)	Significance (p value)
Plant species used in food	14	0.632	0.015[*]
Plant species used both as food and ethnomedicines	33	0.924	0.000[**]
Animal species used as food	17	0.832	0.0005[**]

"*" and "**" indicate significance at 0.05 and 0.001 probability levels, respectively
Data were analyzed for across the systems (remote and villages under transitions). Categorization in rural and semi-rural was not possible due to statistical assumptions of spearman correlation statistics to avoid misleading conclusions

foods are typically higher in calcium, iron, magnesium, and vitamin C, for example, than cultivated plants. Further, Gregory (2000) suggested that wild fish has less fat, and in particular, less saturated fat, than farm-raised fish. On a worldwide scale, the potential of local species that can be employed as staple foods or in medicinal treatments is very high (Bartlett et al. 2007). While this is especially true for people of remote areas still little affected by planned knowledge, use of local resources also offers many opportunities for enhancing the well-being of town and city dwellers. We found a distinct change in *Adi* peoples' food systems over the last few decades, as is occurring in people's food systems worldwide, which have been transformed at an unprecedented rate due to globalization: a phenomenon termed the "nutrition transition" (Kuhnlein 1980; Bartlett et al. 2007; Power 2008).

We found that women living in transitional social systems closer to urban centers had knew less about traditional ways of cultivating, harvesting, and using local food resources from diverse ecosystems, and used fewer of these foods (cf Turner and Turner 2004; Singh et al. 2010). As a result, their resilience to health and environmental risks is expected to magnify from the erosion of biocultural diversity and the shift away from nutritionally rich local foods to marketed foods, which are often high in unhealthy fats and sugars (Turner and Clifton 2009). Irrigated and mono-cropping systems and implementation of PDS (public distribution system) in India (Kothari 1997), along with emergence of new food stocking and distribution patterns (Behera et al. 2016) with the easy availability of commercial and marketed foods, offer seemingly more efficient alternatives for the people living in or nearby towns, who previously used primarily local and uncultivated species (Kothari 1997). The detrimental effects of new and changed globalized diets are reflected not only in the poorer health of individuals, but also in the farming systems and management of local ecosystems (Parks et al. 2005; Nautiyal et al. 2007).

Biodiversity, conservation, and sustainability
Strategically, the *Adi* access food resources from diverse ecosystems in different seasons and practice a variety of ways that conserve and sustain these resources.

Nonetheless, the variety of foods and the extent of conservation practices vary with social differences, especially in relation to proximity to urban centers. We found a significant correlation between use and conservation of food species. Home gardens (species used for only food), *morang* community forests (species used for both food and ethnomedicines), and common resource areas (aquatic bodies and *morang* community forests) were the major land use types where diversity of species was higher across both remote and transitional *Adi* communities. Conservation strategies were further augmented with traditional agronomic practices, varying from one social system to another. Studies have confirmed that in mountainous ecosystems, local biodiversity being conserved in traditional land use systems contributes significantly to overall biodiversity (Jiang et al. 2003) as compared to the more intensive land use systems where exotic species are grown intensively (Parks et al. 2005).

Cultural practices are governed through beliefs and value systems, and supported by indigenous institutions that shape human behavior in the extraction of plant or animal resources, thus influencing conservation outcomes (Berkes 2017). We found a number of plant species with very high cultural values. Use and conservation of food species governed through cultural practices, socio-ecologically and economically compatible with *Adi* food traditions, might have been enhanced by their location in remote, fragile mountainous ecosystems, and also their need to ensure that local resources from diverse ecosystems are available consistently (cf Charnley et al. 2007). However, recent changes in ecological processes, such as reduction in the *jhum* land cycle due to the increasing human population (Ramakrishnan 2007), conversion of community forests to commercial fields (e.g., fruit orchards), and changes in social norms, are altering the food habits of local community in Ar P (cf Sobrevila 2008). However, the National Biodiversity Strategy Action Plan and Greening India policies, in which women's participation and knowledge are being incorporated in planning and programs on biodiversity conservation (GOI 2019) show promise. The Indian policy of "*Sustaining Himalayan Ecosystems*", which considers land use change and other environmental factors as challenges to biodiversity conservation, may draw

insights from these findings to improve conservation outcomes (GOI 2010).

Insights for Sustainable Development Goals (SDGs)

Despite the fact that women are creative in adding value to plant and animal species by cultivating and using them as nutritionally rich foods and ethnomedicines, their knowledge has been given little attention in the science and policy initiatives of Ar P (GOI 2007). One target of SDGs, "No Poverty", calls on giving equal rights and opportunity to vulnerable people, including women, for management of land and other natural resources to enhance resilience against several environmental stressors (UN 2019). A key result would be to provide opportunities for involving such women, with their different concerns and needs, in the "*bottom-top*" approaches for conservation, development, and overall community well-being (Shimla Declaration 2009). SDG targets two ("Zero Hunger") and fifteen ("Life on Land") call on participation of local stakeholders to strengthen policy and actions required to meet food security, biodiversity conservation, and arresting land degradation (UN ibid; UNESCO 2016a; UNESCO 2019). Engaging women and using their knowledge about access to food resources and their conservation practices thus become crucial inputs to various agencies in order to meet targets for sustainable development (Turner and Clifton 2009; UNESCO 2016b). Our results also hold practical utility as knowledge and practices for plant and animal biodiversity management reported herein can be used for formulating location-specific conservation and genetic improvement programs (e.g., selection for diverse traits) and agronomic manipulations for halting biodiversity erosion and land degradation. Ironically, despite their efforts in managing land and biodiversity resources in ecologically harsh situations, such tribal communities seldom receive policy attention and attendant benefits like equitable benefit sharing for biodiversity conservation.

Empowerment of *Adi* women through location-specific educational endeavors is vital. Culturally responsive education on traditional foods and associated knowledge will be a prerequisite (Blanchet-Cohen and Reilly 2013) to further strengthen the legacy of conserving biocultural resources with emphasis on achieving SDG targets (especially 1, 2, and 15). For this, women's roles within the school education system need to be defined to ensure knowledge transfer and research promotion, in order to provide a scientific and technical base for supporting government policies (Kuhnlein et al. 2013; UNESCO 2016a). Motivating younger women, particularly from transitional systems, to learn from their elders about traditional food-related knowledge, and to form self-help groups (SHG) for value addition and income generation can strengthen the sustainability of biocultural diversity while meeting several SDGs.

Conclusions and policy implications

Our study has demonstrated that *Adi* women, particularly those living in more remote communities, possess a rich knowledge about nutritionally important local food species and their traditional products. They rely heavily on local plant and animal species accessed from diverse land use systems and conserved using proven traditional practices. We found that 14 different plant species were used as food, 33 as both food and ethnomedicine, and 13 species were one of the major means to generating income. Furthermore, we noticed that 17 animal species also play crucial roles in food and sustaining cultural identity of the *Adis*. The knowledge on integrated strategies applied by *Adi* women for accessing and using food resources was found to improve biocultural diversity and social-ecological resilience. Women respondents from traditional and transitional villages differed significantly with each other for use of plant and animal species for food, ethnomedicine, and income generation, with the former using significantly more number of species. Similarly, women from traditional villages had more knowledge on species conservation practices than their transitional counterparts. Home gardens were the most important source of diverse food plants, followed by *jhum* lands. *Morang* community forests and home gardens displayed more species diversity than other land use systems. Unfortunately, changing life styles and land use patterns have accelerated the erosion of biocultural knowledge; particularly in and around transitional villages. Based on key findings, following recommendations, covering socio-economic and ecological dimensions, can be of considerable relevance to future policy initiatives on sustainable development in fragile mountainous and similar ecosystems:

1. Promotion of reciprocal learning between formal and informal knowledge systems can enhance the adaptive capacity of local communities to cope with emerging social-ecological stressors and provide new avenues to science-policy establishments for improving outcomes vis-à-vis SDGs. Increased participation of custodians of biocultural resources, for example, can improve the local acceptability and outcomes of programs like "Organic Farming Policy" (2005) intended to achieve intertwined objectives of social-ecological resilience and well-being.

2. Emphasis also needs to be placed on promoting local/regional dialects as means for rapid diffusion of biocultural knowledge, especially in areas where social-ecological changes are taking a toll natural capital and livelihoods. For example, creation of "Village Knowledge Banks" in collaboration of

science-policy institutions, while retaining local identities and ownership of resources, can provide localized education and learning platforms, particularly in areas rich in biocultural resources but facing threats.

3. There is a need for relevant provisions in government policies and programs [e.g., mid-day meal and public distribution system (PDS)] for promoting nutritional local food. Consequent increase in demand for the locally produced nutritious food can motivate the communities for strengthening species conservation measures as well as increasing livelihood opportunities.

4. Special empowerment drives need to be launched for the capacity building of disadvantaged women groups (e.g., *Adi* women in our case) through Indian policies on promotion of micro and small industries, food processing and value addition, skill development, and eco-tourism such that they are not only better equipped for livelihood security in face of looming threats but may also contribute to achieving some targets of SDGs and biodiversity conservation policies.

5. Coordinated planning between agriculture (including horticulture), forestry, land development corporations, and housing authorities remains critical to reversing and arresting the adverse impacts of land use changes and erosion in biocultural diversity. Aligning local conservation practices with flagship national programs (e.g., Sustaining Himalayan Ecosystems) can specifically help achieve the SDGs targets of "Zero Hunger" and "Life on the Land".

Supplementary information

Supplementary information accompanies this paper at https://doi.org/10.1186/s13717-020-00232-x.

Additional file 1. Characteristics of sampled *Adi* women; major aspects covered in study through pilot testing of interview schedule; visits conducted to various food production/conservation systems (recording and validating species); participatory rural appraisals tools applied in recording data; and beliefs and traditional practices prevalent among *Adi* women relating to wild animals and some plants used in foods.

Abbreviations
Ar P: Arunachal Pradesh; SDGs: Sustainable Development Goals; TK: Traditional knowledge; SHG: Self-help group

Authors' contributions
RKS generated the idea and designed the study, collected and analyzed data, and wrote the article. AK, AS, and PS helped in application of study framework, statistical applications, writing, and reading and revising the manuscript. All the authors read and approved this manuscript.

Authors' information
Ranjay K. Singh is serving as Principal Scientist (Agricultural Extension) at ICAR-Central Soil Salinity Research Institute (CSSRI), Karnal, Haryana, India. His research interest includes human-environment interactions, and he has collaborated with tribal and marginal communities to work out local solutions for community-based natural resource conservation and adaptation to global environmental change. Arvind Kumar is working as a Scientist of crop improvement at ICAR-CSSRI, Karnal, and is specialized in developing crop varieties against abiotic stresses. Anshuman Singh is working as a Scientist at ICAR-CSSRI, Karnal, and has specialization in fruit crops. Poonam Singhal is a Lecturer (Food Science and Technology) at the University of Hyderabad, Hyderabad, India. Her research interests include exploration, characterization and validation of plant-based ethnic foods of India.

Availability of data and materials
Substantial amount of data generated and analyzed is covered in the paper. Further, additional data have been provided in online resources. However, corresponding author may be contacted in case of any clarifications required on data under report.

Ethics approval and consent to participate
Not applicable; however, study participants were informed about the objectives of the study prior to their participation, including the expected outcomes in form of print and digital publications. Accordingly, the consent was obtained in oral form from the studied respondents and Community Chief (*Gaon Burha*) of study villages on knowledge and practices (including photo) on food species to be published. This study was approved by the institute of affiliation.

Consent for publication
Not applicable

Competing interests
The authors hereby declare that they have no competing interests on this article.

Author details
[1]College of Horticulture and Forestry, Central Agricultural University, Pasighat, Arunachal Pradesh 791102, India. [2]ICAR-Central Soil Salinity Research Institute, Karnal, Haryana 132001, India. [3]University of Hyderabad, Hyderabad, Telangana, India.

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Publisher's Note

IMPACT OF VUCA ON CORPORATES

☐ D. Sujatha*

ABSTRACT

Today' business environment is highly dynamic with so much of volatility and uncertainty there is bound to be stress, cut throat competition and Worry. People working in such an environment are focusing on making material gains. Making profits by hook or crook has become the latest mantra. As the world becomes increasingly interconnected through intricate network in technology -laden environments, leadership has become exponentially more complex. This VUCA (Volatile, uncertain, complex and ambiguous) context disrupts long herd leadership constructs. Historically leaders have been able to reflect on past decision making to guide their current and future decisions. As the corporate training provide opportunities for an individual to re-skilling, upskilling and acquire higher order cognitive that are so important to the changing nature of work. These trainings are the next venue of employees to accumulate knowledge and acquire innovative skills after their school and university, but such opportunities are relatively rare to see as every organisation is busy in hoarding the latest technology and they are overlooking the unique aspect they are having in the form of human capital.

Key words** Environment, Cut throat competition**, Leadership, Cognitive, Innovative skills

Introduction

The term, VUCA was 1st coined by North American country St. George Carsey and was wont to train troopers within the North American Country War school. It's Associate in Nursing signifier that has managed to capture our collective imagination by compactly rundown the troubled times we have a tendency to sleep in. VUCA stands for a business world that's a lot of Volatile, a lot of unsure, a lot of complicated and a lot of Ambiguous than ever before. VUCA truly arose from a North American country military term to explain the 'new world order' following the top of the conflict.[1] Over five hundredth of Fortune five hundred firms from the year 2000 don't exist any longer.[2] They didn't introduce, they didn't use information to form selections, and that they didn't survive as a result of they were victimization the past information to predict the long run..VUCA conflates four distinct kinds of challenges that demand four distinct kinds of responses. That creates it troublesome to grasp the way to approach a difficult state of affairs and simple to use VUCA as a crutch, the way to throw off the labour of strategy and planning— after all, you can't steel oneself for a VUCA world, not specializing in whether or not the staff are operating (output), and if they're operating however they're doing i,e the standard of labour (productivity), or maybe why they're Some don't even shrewdness several workers they really have at any given time, particularly

*Lecturer (Department of commerce) College: St. Ann's college for Women, Mehdipatnam, Hyderabad

if you consider temporary employees and contractors. With thirty seventh of mid-sized companies' information left sitting in stand out or similar kinds of databases that's hardly stunning. During a VUCA world there ar such a big amount of threats lying for attention, a number of that have not been seen before. Thus, knowing what keeps leaders awake at midnight may be a valuable insight into what their govt team ought to rate. Once PwC ran their annual leadership survey, they found that the best rated considerations for CEOs were 'Cyber threats as well as lack of knowledge security' (61%) and 'Speed half dozen Technology: The threat and chance technology also are a threat, however additionally as a chance. The speed of technological amendment shows Associate in Nursing underlying worry of obtaining left behind because of identical technological reasons. Each currently then a brand new technology comes into existence and becomes out-of-date among no time nearly as before long as an organization on boards a brand new IT system, Or as before long as you get a brand new product or service onto the market, a younger upstart company comes along side following massive issue to disrupt it.CEOs so progressively need to visualize a watertight affiliation between digital investments and business objectives; eighty six say a transparent vision of digital technologies will facilitate attain competitive market. A lot of complicated Associate in Nursing volatile a trade is, the tougher to predict and thus a lot of unsure it'll be. Of these four represent distinct parts that create the environment - the planet, a market, Associate in Nursing trade - tougher to know, understand and management. The distinct nature of those four parts are often any processed by visualizing them: during this setting we have a tendency to bump into four distinct kinds of challenges visit volatility, uncertainty, quality and ambiguity that demand constructive kinds of responses. Volatility –

visit the character & dynamics of amendment. A lot of volatile the planet is, a lot of & quicker things amendment. A lot of unsure the planet is, the tougher it's to predict. Quality indicates any state of affairs has several interconnected components & variables. A lot of complicated the planet is, the tougher it's to analyse. Ambiguity denotes lack of clarity concerning the way to interpret cause-and-effect. A lot of ambiguous the planet is, the tougher it's to elucidate. These four challenges create an incredible challenge on organization for strategy creating. During this unpredictable setting, organizations cannot believe the normal manner of strategic coming up with, prognostication & analysis. The amendment is thus rapid; to know & harness the amendment, organization has to acquire the proper signals. Actually, this can be a paradigm shift from a digital savvy setting to a brand-new era wherever data is power and innovation is that the driver of growth. The thought is gaining new connectedness to characterize this setting and also the leadership needed to navigate it with success. Lot of corporates within the world of Human Resource, Strategy, coming up with has been speaking concerning the VUCA world within the current decade. To be precise, the signifier has been used a lot of to scare the stakeholders than to guide them. The alphabets, VUCA are brought along to point Volatile, Uncertain, complicated & Ambiguous.

VUCA is an acronym. It stands for Volatile, Uncertain, Complex and Ambiguous.

Volatility -It means a speed of changes in industry, market or world. It is related with fluctuations in demand and turbulence. More the volatile world is the more the changes take place in the market. It means the world is liable to change rapidly. Volatility in business makes it difficult to the businessmen to anticipate or predict. This process of changes in environment makes the decision-making process tough. The

way how JIO transformed data market in INDIA is classic example and how the situations in the world are changed after covid-19 especially related to the companies of Sanitizers and masks. Volatility also means navigate by looking forward but not backward.

Uncertainty - The dictionary meaning: Something which is not sure, which cannot be relied on. Uncertainty in the marketplace leads to absolute lack of predictability

Uncertainty refers to the extent to which we can confidently predict the future. Part of uncertainty is perceived and associated with people's inability to understand what is going on. . The patterns of the past cannot be extrapolated as they do not lead to any conclusive interpretation for the future. Uncertainty, though, is also a more objective characteristic of an environment. Truly uncertain environments are those that don't allow any prediction, also not on a statistical basis. The more uncertain the world is, the harder it is to predict. For example, the largest smartphone maker in the world, naturally Apple or Samsung seems to be the apparent alternates. However, today it's BBK Group (OnePlus) which has bagged this title! Nothing is certain, you see!

Complexity - The dictionary meaning: Consisting of many different and connected elements Complexity refers to the number of factors that we need to take into account, their variety and the relationships between them. Every aspect of business or even social life is liable to get influenced by known (as well as unknown) factors originating from within (or outside) the organisation The more factors, the greater their variety and the more they are interconnected, the more complex an environment is. Under high complexity, it is impossible to fully analyse the environment and come to rational conclusions. The more complex the world is, the harder it is to analyse.

Ambiguity - The dictionary meaning: Open to more than one interpretation. Ambiguity refers to a lack of clarity about how to interpret something. The combination of Volatility, Uncertainty & Complexity leads to an undefined ambiguity, especially for someone who wishes to leap into unknown territory A situation is ambiguous, for example, when information is incomplete, contradicting or too inaccurate to draw clear conclusions. More generally it refers to fuzziness and vagueness in ideas and terminology. The more ambiguous the world is, the harder it is to interpret. There is always a persistent lack of clarity on the path of growth. For example, A student crafting her career path usually lacks clarity on fitment, opportunity and growth possibilities so does an entrepreneur venturing into a new product line.

Due to the influence of medical care and globalisation, the trendy operating world presents tremendous challenges, however square measure the dynamical operating conditions of a world marked by VUCA. In everyday company settings, VUCA means technological and structure shifts square measure happening a lot of and a lot of quickly; as a result, past price systems and best. Also, it's just about not possible to subsume the complexness at hand. Staff nonetheless ought to move selections, assume on the fly, and adapt consequently – all of that ends up in multiplied levels of psychological strain. To succeed and keep healthy, it's so crucial that they be aware of themselves and their desires. This, in turn, makes a robust sense of private responsibility essential. Companies, for his or her half, even have a responsibility to market their employees' health and well-being, together with a positive company culture. Thereto finish, they have to determine associate structure framework which will change them to supply a spread of health services, courses, and connected data to their staff at each level. At a similar time, there square measure robust interdependencies

between a company's culture and therefore the health and well-being of its staff. This conjointly happens to be wherever the key to success lies: within the VUCA world, a healthy company culture helps guarantee semi-permanent business prosperity.

Circle of Training

Training may be a continuous & circular method that helps workers & organization to develop & processes self-empowerment. It helps the new workers to grasp the work and also the existing employees to figure in a very higher approach It provides a chance to live the correlation between coaching & improved growth of a corporation. It is conjointly useful for brand new workers to quickly integrated into the culture of a corporation. How it helps to enhance a personal performance: It helps a personal to realize authorized existence through the invention of their true potential and hidden capabilities. It conjointly provides an opportunity to the workers to use their new concepts for the betterment of the product or ideas It conjointly provides complete self-reflection of a personal. How it encourages individual & organization's Performance: coaching may be a tool to allow AN worker AN insight concerning the abilities, talents & data to remain tune with this and future surroundings. It conjointly conducts numerous competitions to encourage the workers with rewards and awards. It helps in developing the career of a personal and also the prosperous growth of the organization. Its formalized employee's career, it given the chance to be

told new skills or qualification inside their field of labour. Indirectly it aids a corporation in their strategy creating and structural development. Ultimately it lends a hand toward structure growth: coaching narrows down & prepares the delicate workers World Health Organization square measure right for the corporate. By giving coaching to the workers they learn new things in a very shorter time. And their enhanced potency at geographic point can guarantee success that successively can facilitate to realize a singular price position within the competitive market place with their distinctive capabilities. Learning in teams creates interest in workers than individual coaching and conjointly it creates a healthy competition. Unpredictable events happening outside a corporation may be negative or positive, however either gift bigger VUCA, that makes it tougher for leaders to form choices.AN example of positive quality may be a product going infective agent and turning into a web sensation. Move from protective info to group action of knowledge. To empower workers to form choices, create communication resistance. Sure, some may need to remain up late or stand up early for world corporations, however the gain of obtaining the data straight from the horse's mouth is well worthwhile. Accelerate the speed of interaction the maximum amount as doable. Within the VUCA age, speed matters quite perfection. Use easy rules to form fast choices, instead of excellent analyses. Taking any step to start the transition is best than waiting—or worse, being unfit and concealing one's head within the sand.

4's Circle of Training

Learning Objectives

Self-Development

Strategy & Structural Development

Self-refliection

Aim to enhance self-confidence, self-organization, positive & realistic self-concept

Performance Improvement

Aim to develop Accountability & responsibility, support succession planning, update skills, formalize career

Organisation Growth

Help in succesful & sustainable growth, unique value position, strategic continuity, distinctive capabilities

Training Method, Monitoring & Evaluation Plan

Training Need Assessment

System Development

Create Organisation Learning

The executive's area unit remodelling their corporations into learning organizations. Several workers' area unit given numerous forms of coaching facilities and numerous competitions area unit command in conjunction with the costs to encourage them towards operating in an exceedingly higher approach. They are encouraging experimentation with new merchandise, services and procedures. From company R&D "skunk works" to small-scale pilot comes to cross-functional project groups, they're investment in experiments with new ways that of doing business among their markets. They are additionally making conditions wherever the organization will learn from expertise, creating "work" the "curriculum" for improvement and innovation. They are additionally conducting competitions between numerous teams for a healthy several utilized after-action reviews on a routine basis, wherever work teams scrutinise of their collective performance following major events or tasks, viewing what worked, what went wrong and what must be improved. Corporates even have a comprehensive worker educational program that features leadership coaching for managers in any respect levels. Additionally, stretch assignments and cross-functional motion programs develop high-tension leaders for additional senior positions. Companies, WHO perceive that nobody individual will get a lot of accomplished by themselves and believe thus any modification needs a coordinated set of actions through a collective consciousness. it's

an ideal temporal arrangement for corporations Associate in Nursing organizations to be specializing in developing its worldview in an integrative and holistic manner .Due to the attack ofCOVID_19 the total world is facing new challenges therefore is that the sector of company organisations. They have to set up new and long term strategies so as to sustain during this sector and continue for an extended amount of your time.to be ready to create best use of the VUCA opportunities during these dynamic surroundings by ways that of simplification of the context and by desegregation processes, inner drives and outer demands. Economic process has place vital pressure on leadership and entrepreneurship across the corporate levels, requiring fast deciding, necessary protection of the culture and worldview of the organization, talents to integrate diversities of assorted types, and foster cooperative innovation with the speed as necessitated for the given context.

Recommendations for maintaining a healthy corporate culture in the VUCA world

1. Conduct regular employee surveys to assess your company's current culture and identify areas where it can improve
2. Incorporate health and well-being into your company's guiding vision and strategy
3. Make sure that every area of your company understands and embodies this strategy
4. Put together a set of measures designed to promote health and well-being and implement it throughout the company
5. Make mindful leadership part of the soft-skill training your company provides to its managers (they serve as examples and can amplify the message you want to send)
6. Give your company a clear idea of the impact that health management can have on its financial success
7. Review and report on how well these measures are working on a regular basis.

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Green Hrm – Human Resources Management
Case Study on 5 Indian Companies

Mrs. P. SIRISHA[1]
Mrs. D. SUJATHA[2]
Mrs. DHIVYA KUMARI. G[3]

[1]*Faculty of Commerce, St. Ann's College for Women, Mehdipatnam, Hyderabad*
sirisha.ravi8@gmail.com

[2]*Faculty of Commerce, St. Ann's College for Women, Mehdipatnam, Hyderabad*
sujathagadamalla@gmail.com

[3]*Faculty of Commerce, St. Ann's College for Women, Mehdipatnam, Hyderabad*
ayvidg4u@gmail.com

Abstract

Human resources are the valuable resources of all the factors of production. Money without man is unimaginable. Nowadays because of technical advancement in society, businessmen tend to use less manpower toreduce the cost of production. Yet human resource management plays a vital role in the development of business and it Manages all the activities of the organization. Nowadays green resource management is a novel concept to the majority of academics and professionals in HRM. GHRM always aims at encouraging employees of an organization to go green. Green HRM is a human resource principle and policy to encourage sustainable management within the organizations . The prime focus of this research paper is to explore the companies that areapplying Green Human Resource Management and also to study the advantages and limitations of green HRM.This research paper also focuses on how far it is helpful in contributing human and technical sustainability in the organisation to the maximum extent, by taking case study of somecompanies which are using green HRM.Human resources has two keycomponents - a. environmental friendly HRpractices and b. Protection of knowledge capital. The prospect of green human resource management appearsfavorable for all the stakeholders, be it the employees, employers, practitioners and academicians. The purpose of this paper is to motivate the field of HRM and to expand its role asan environmentally sustainable business.

Keywords: Technical advancement, Human Resources, Sustainability, Organizations.

Introduction

Recently, there has been a growing awareness with various organisation and business communities on the significance of going green and implementing various sustainable management techniques. Various organizations and businesses are experiencing a drastic shift from traditional financial structure to modern based economy, which is set to traverse sustainable economic facets of business. Sustainability is the magic word which is used often in every dimension of our personal life and workplace. Our lifestyle both professional and personal level are influencing the environment so harmfully that we cannot let it go unchecked. Undoubtedly, the corporate world is taking initiative about environmental issues and playing an important role by providing solutions to environmental hazard. And one such solution for environmental hazard is Green Human resource management because it helps to begin or 'to green' in the workplace. As we are aware that HRM is an important function of management because it deals with the most valuable asset, which is Human. In an organisation, HR (Human resource) and their system is the base for any business and organisation. HR is the ones who are in chargeof implementing the eco- friendly process and policies which help in creating a green atmosphere.

Green Management is a set of practices, system and policies that helps to create a green behavior in the minds of company's employees. It also helps to create environmentally resource efficient, socially responsible and sensitive workplace in an organisation. GHRM (Green Human resource management) begins even before appointing new recruits in the organisation. Green Human Resource Management includes two keycomponents: a. Environmental friendly HRpolicies/practices and maintaining/protecting knowledge capital. Green initiatives should be part of CSR (corporate social responsibility) and should also involve all the members in the entity for green initiatives. This paper focuses on exploring theGreen Human resource management,benefits, and its implementation procedure and few examples of companies following GHRM.

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II.OBJECTIVE OF THE STUDY:
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1. To explore the significance of Green HRM in the organisation.
2. To comprehend the various benefits of Green Human resource management.
3. To discover the role of Green HRM in the organisation – A case study of 5 companies.
4. To explore the Implementation process of GHRM in companies.

III. RESEARCH METHODOLOGY

The data sources used in this research paper are secondary data due to the complex nature of the subject topic. The sources of secondary data used in this report are:

- **Published printed sources** – books, journals, magazine/newspaper articles
- **Published electronic sources** – general websites, e-journals, weblogs, mobile applications

IV.LITERATURE REVIEW:

The Literature has given importance for implementing environmental practices as a basic objective for organizational functioning by identifying HRM practices.

a. Cherian & Jacob, 2012 and Haden, oyler and Humphrey (2009) in their paper mentioned that implementation of strategies and environmental objectives along with development goals of an organization results in an effective and efficient environment management system.

b. Daily and Juang (2001) said that businesses essentially need to balance the preservation of environment and industrial growth because implementing green principles in an entity may earn more profit than before.

c. Murari&Bhandari (2011) says that the Human resource department in the business plays an important role in promoting sustainability culture.

d. Harmon, Fairfield and Wittenberg (2010) proposed that more the usage of Green human resource principles and policies , the most is the implementation of EMS (environment management system) in different companies.

e. Jackson, Renwick,jabber and Muller Camen (2011) proposed that Green human resource management depends on the identifiable and unique patterns of green behaviour and decisions of HR managers.

f. Dutta (2012) G-HRM includes 2 major elements, one is maintenance of knowledge capital and sustainable friendly human resource practices. GHRM helps in makingrecognition and commitments on the issue of sustainability.

V. GREEN HUMAN RESOURCE MANAGEMENT

V.1. Meaning of GHRM:

GHRM is a managerial concept and also a trending topic all over the world. It has got different meanings to different people. There is no clear definition of GHRM.

The main reason for going green is to reduce the negative impact that energy consumption and pollution can have on the environment.-

Definitions:

Ramachandran defines Green Human Resource Management as the" combination of environmental management into human resource management".

Green Human Resources Management (GHRM) can be defined as" the set of practices, systems and policies that promote a green behavior of a company's employees in order to create an environmentally delicate efficient resource and socially responsible workplace and overall organization."

Green HRM mobilizes every employee to increase their potential. It means using every human resource to help sustainable and environmental practices and increase employee awareness and commitments on the issue of sustainability.

Examples of GHRM: Video recruiting is the use of video and online interviews, electronic filing, car-sharing, job-sharing, teleconferencing, and virtual interviews, recycling, telecommuting, online training, etc.

Evolving a green culture has a positive and sustainable effect on employee behavior and values that build an internal culture. Green behavior is considered to be a tool in the process of green HRM culture and adopting formal environmental plans. According to Mandip, the practice of green Human Resources should be translated into the Human Resource processes, such as training, compensation, recruitment etc.

From the definitions stated above, it can be concluded that Green HRM needs the participation of all the organization's members to create and keep the organization green.

V.2.Benefits/Advantages of Green HRM

The Green Human Resource Management plays a vital role in the industry to promote the environment-related issues.

Organizations must frame HR policies and practices, and train people to increase awareness about the environment. And they must provide information regarding Environmental protection. The Green HRM may also help the employers and employees   in building brand image and reputation & their skills.

Organizations need to pay attention to recycling of the resources, thus changing the organizational culture, waste management, pollution and helping the society and its people.

It will also make employees and society members think innovatively in using un-utilized natural resources and they can bring" best out of waste ".

Few experts identified the following Benefits of Green Human Resource Management:

1. It helps companies to bring down expenses.
2. Organizations which have a scope of huge growth by being green and creating a new friendly environment which helps in high operational savings by decreasing their cost of production.
3. It helps in achieving job satisfaction and commitment which leads to higher productivity and sustainability.
4. GHRM produces a culture of having affected for the health and wellbeing of fellow workers.
5. Development in the sustainability rate of the employees.
6. It helps in recruiting better employees. Reduction in the environmental impact of the company.
7. GHRM helps in overall performance of the organization.
8. Reduction of environmental damage.

The above benefit may help in attaining greener savings and green products from waste elimination. By promoting such values it indirectly increases consumer satisfaction.

V.3.Implementaiton of GHRM:

The following are some of the points need to follow by the companies to implement green HRM.

   a. Select and appoint people who have knowledge regard green hrm.
   b. Evaluate employees' performance based on environmental criteria;
   c. Introduce ways of motivating employees by providing financial incentives for their environmental performance.
   d. Provide training and education programmes on environmental management;
   e. Foster relation between teams to deal with sustainable problems and attempt for continuous development of environmental management activities.

V.4. Case study of 5 Companies:

<u>**Case study –1**</u>

ITC limited

India Tobacco Company is one of India's leading private sector companies with a powerful dedication to the triple bottom line.

- It has been a frontrunner in implementing eco -responsible processes, much ahead of making laws.
- A turnover of over US $ 5 billion and market capitalization of over US $ 22 billion with a varied presence in hotels, cigarettes, paperboards and specialty papers, packaged foods, agribusiness, and a whole range of other services.
- ITC PSPD (Paperboard and Specialty Papers Division) has 4 manufacturing units. All four manufacturing units are OHSAS 18001, ISO 14001 and ISO 9001 are certified.
- In India for the first time ITC has introduced an environment favorable adaptable paper "Paper Kraft Premium Business Paper", for home and office adapting a new technology 'Ozone Treated Elemental Chlorine Free Technology' replacing Elemental Chlorine which was conservatively used in the bleaching process during paper manufacture.

<u>**CASE STUDY- 2**</u>

Wipro Technologies:
- There was a time when the Karnataka State Pollution Control Board indicted the IT solutions division of Wipro Limited for dumping huge volumes of hazardous electronic waste in illegal recycling units in Bangalore.
- **The eco eye**: an inventiveness that had some goals to deplete the organization's carbon footprint, manage its energy and water effectively and efficiently. And expand new benchmarks to minimize the use of hazardous substances, recycling the waste which become cost effectively sustainable, and induce all employees to follow green practices in their professional and personal lives * They comprehensive their green mission to physical infrastructure and substitute CRT(CATHODE RAY TUBE) monitors with LCD monitors .

- The Wipro technologies have a separate green testing lab, it also practices a Carbon Disclosure Project which reduces employee travel.(to report the internal carbon footprint).

- Wipro was the pilot IT company in India to launch eco-friendly notebook anddesktop computers that attach to the restriction of hazardous substances (RHS) regulation which is specified by the European Union (EU)

CASE STUDY - 3 .

Suzlon Energy

- Suzlon Energy The ″green banking″ initiative: State Bank of India tied up with SUZLON to· become the first Indian bank to take lead in harness wind energy.

- The Suzlon One Earth Campus, the corporate headquarter of Suzlon at Hadapsar, Pune.
 India is the most energy efficient building built ever in India with insulated green roof, energy efficient – lighting system, construction time, renewable energy based hot water system, reduced landscape and building water requirement, recycle, reuse and recharge of water, waste management and waste water treatment, health and energy of occupants, carpooling, zero waste management, green education etc. are policies practiced by the companies.

CASE STUDY – 4

HCL Technologies

- HCL Technologies ″Go Green″: a multi-layered corporate program running campaigns to initiate· individual action towards environmental issues.

- It has developed and designed an extensive Green Edge sustainability framework that serves the general needs of manufacturing and production industries.

- HCL technologies are the active members of India Council and the first company in India to launch a Beryllium & Antimony Free laptop. The company extends its

take-back service to consumers for discarding their equipment through Hindustan Computers Limited Green Bag Campaign, and reprocessing collected E-waste in a sustainable friendly manner.

CASE STUDY- 5

Tamil Nadu Newsprint and Papers Limited

- This company has Own power generating facility to make it 100% self-adequate for installation of 61.18 MW PGE (Power generating equipment) at the paper mill site.
- The Excessive Power generated is being exported to the State Grid.
- The Innovative bio-machination project: This project contributes to the sustainable development in terms of generating in-house renewable energy and reducing green – house gases.
- **Other initiatives**: Eco – friendly technologies in process, its proactive role in reducing the use of fossil fuels, increasing the green cover, using energy efficient systems, recycling and reuse of solid and liquid wastes in the process's' argument.

VI. CONCLUSION & SUGGESTIONS

It is evident from the discussion so far made that GHRM promises potential benefits for both organizations and those employed by them.Green pay is also associated with improved financial performance outcomes in the organization. The GHRM practices recognized in this section may have a role to play in improving not only the environmental performance but also the financial performance of the organization. Equally, the GHRM practices analyses here are likely to improve employee well-being in the workplace, not least through humanizing the working environment and satisfying the needs of an increasingly environmentally workforce. To summarize, we believe that Green Human Resource Management has the potential to bestow positively to both improved organizational performance and employee well-beingUnions and employees can help employers to adopt Green HRM policies and practices that help safeguard and augment worker health and well-being.

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Recent Understanding of Soil Acidobacteria and Their Ecological Significance: A Critical Review

Sadaf Kalam[1†], Anirban Basu[2†§], Iqbal Ahmad[3*‡§], R. Z. Sayyed[4‡§], Hesham Ali El-Enshasy[5,6,7‡], Daniel Joe Dailin[5,6‡] and Ni Luh Suriani[8‡]

[1] Department of Biochemistry, St. Ann's College for Women, Hyderabad, India, [2] Department of Plant Sciences, School of Life Sciences, University of Hyderabad, Hyderabad, India, [3] Department of Agricultural Microbiology, Aligarh Muslim University, Aligarh, India, [4] Department of Microbiology, PSGVP Mandal's, Arts, Science and Commerce College, Shahada, India, [5] Institute of Bioproduct Development, Universiti Teknologi Malaysia (UTM), Skudai, Malaysia, [6] School of Chemical and Energy Engineering, Faculty of Engineering, Universiti Teknologi Malaysia (UTM), Skudai, Malaysia, [7] City of Scientific Research and Technological Applications, New Borg El-Arab, Egypt, [8] Biology Department, Faculty of Mathematics and Natural Science, Udayana University, Bali, Indonesia

Acidobacteria represents an underrepresented soil bacterial phylum whose members are pervasive and copiously distributed across nearly all ecosystems. Acidobacterial sequences are abundant in soils and represent a significant fraction of soil microbial community. Being recalcitrant and difficult-to-cultivate under laboratory conditions, holistic, polyphasic approaches are required to study these refractive bacteria extensively. Acidobacteria possesses an inventory of genes involved in diverse metabolic pathways, as evidenced by their pan-genomic profiles. Because of their preponderance and ubiquity in the soil, speculations have been made regarding their dynamic roles in vital ecological processes *viz.*, regulation of biogeochemical cycles, decomposition of biopolymers, exopolysaccharide secretion, and plant growth promotion. These bacteria are expected to have genes that might help in survival and competitive colonization in the rhizosphere, leading to the establishment of beneficial relationships with plants. Exploration of these genetic attributes and more in-depth insights into the belowground mechanics and dynamics would lead to a better understanding of the functions and ecological significance of this enigmatic phylum in the soil-plant environment. This review is an effort to provide a recent update into the diversity of genes in Acidobacteria useful for characterization, understanding ecological roles, and future biotechnological perspectives.

Keywords: acidobacteria, biogeochemical cycles, ecological roles, metagenomics, molecular characterization, plant growth-promoting activities, soil

INTRODUCTION

Prokaryotes, the unseen majority sustaining life on Earth, are involved in a multitude of interactions and biogeochemical processes having global ecological relevance, including decomposition, mineralization, storage, and release of nutrients (Sikorski, 2015). The number of prokaryotes present in a gram of soil can be between 10^6 and 10^9 cells (Bulgarelli et al., 2013). It is apparent from the above data that there is an abundant density of bacteria in the soil, however, the discrepancy is that only less than 1% bacteria from natural environments, including soil, could be cultivated by using conventional culturing techniques (Crits-Christoph et al., 2018; Chaudhary et al., 2019). A large segment of the microbial community gets consistently overlooked during routine

microscopic analysis, indicating them to be novel and hitherto unrecognized with ambiguous physiology and growth requirements (Youssef et al., 2015). A rational investigation stemming from the recognition of this discrepancy is the identification of these refractive bacteria which escape conventional enrichment and isolation procedures. In this direction, the 16S rRNA gene, the gold standard for phylogenetic analysis, proves to be a useful tool for studying the evolution-based taxonomy of microbial communities from various econiches (Rosselli et al., 2016). The rare biosphere comprises of several identified novel bacterial lineages, which appear to be profoundly branching within the bacterial tree and remain unaffiliated with any known bacterial phyla and are termed as candidate phylum (or candidate division) (Solden et al., 2016).

Molecular studies in the soil revealed the presence of unseen, hidden, recalcitrant bacteria referred to as difficult-to-culture, hitherto-unculturable, or yet-to-be cultivated bacteria. In contrast to standard fast-growing bacteria, these underexplored bacterial groups remain elusive to conventional microbiological cultivation techniques, mostly because their optimized growth conditions are still underexplored. The proportion of such bacteria exceeds far more than that of the culturable ones. Among them, Acidobacteria constitutes the most abundant phylum whose members dominate soil bacterial communities (Vartoukian et al., 2010; Chaudhary et al., 2019). Data from 16S rRNA gene inventories signify the vastness and breadth of the phylum with genetic and metabolic diversity (Janssen, 2006). Study of these hitherto-unculturable constituents of soil microbial population through culture-dependent and -independent approaches revealed the essence of their bioactivity and their ecological functions in plant-soil ecosystems (Huber et al., 2016; Lladó et al., 2018; Elmagzob et al., 2019). The underexplored phylum Acidobacteria thus provides an enthralling and seamless source of biological diversity, offering new avenues for exploitation. Henceforth, the most recent advances mining the difficult-to-culture soil acidobacterial diversity for biotechnological advances and comprehensive understanding of the genetic diversity and ecophysiological profiles of Acidobacteria in the plant-soil econiche has been scrupulously presented in this review.

UNDEREXPLORED PHYLUM ACIDOBACTERIA

Acidobacteria represents an enigmatic phylum with its members copiously distributed in different ecosystems (Mushinski et al., 2018). Acidobacteria is ubiquitous in diverse terrestrial environments ranging from tundra soils to desert soils, from peatland soils and sediments to grasslands, forests, and agricultural lands (Janssen, 2006; Eichorst et al., 2018) and constitute about 5–70% of the soil microbial populace (Huber et al., 2016). The members are recalcitrant, rendering this phylum to be feebly understood (Hugenholtz et al., 1998; Huber et al., 2016). The recalcitrance of most Acidobacteria members to grow on conventional growth media can be attributed to

their oligotrophic nature or ecological K-strategy (Ward et al., 2009; Kielak et al., 2016a). Thus, the use of low nutrient media, modified incubation conditions including elevated CO_2, low pH, prolonged incubation periods, and supplementing growth media with amendments like antioxidants, rhizosphere extracts, etc., have led to the isolation of several acidobacterial species (Stevenson et al., 2004; Sait et al., 2006; da Rocha et al., 2009; Tanaka et al., 2017).

Based on the analysis of major 16S rRNA gene sequence clades, the phylum Acidobacteria is phylogenetically classified into 26 subdivisions (Barns et al., 2007). Among these, only seven subdivisions (namely the subdivisions 1, 3, 4, 6, 8, 10, and 23) are represented by taxonomically described members (Dedysh and Yilmaz, 2018; Eichorst et al., 2018). Although more than 12,000 distinct phylotypes and more than 6,500 species-level operational taxonomic units have been reported so far for this predominant soil bacterial group, yet it is described by only 56 cultivable species belonging to 28 genera (Overmann et al., 2017; Vieira et al., 2017; Dedysh and Yilmaz, 2018). All cultured Acidobacteria members are Gram-negative, non-spore formers, and exhibit an oligotrophic mode of nutrition (Fierer et al., 2005; George et al., 2011; Dedysh and Damsté, 2018). Most of the members are acidophilic chemoheterotrophs growing aerobically under mesophilic conditions (Dedysh and Damsté, 2018). To study this phylum, culture-independent approaches have been more successful rather than culture-dependent approaches. Genomic studies provided insights into the genetic make-over of about ten acidobacterial genomes only (Ward et al., 2009; Kielak et al., 2016a). However, owing to the difficulty in cultivating its members, the in-depth ecological purview of this enigmatic phylum has remained evasive. Since information regarding global distribution patterns and apparent ecological roles of Acidobacteria is inadequate, microbial ecologists are deeply engaged to unfurl this obscure phylum.

THE REQUIREMENT FOR HOLISTIC APPROACHES FOR STUDYING PHYLUM ACIDOBACTERIA

Acidobacteria phylogenetic diversity, richness, abundance, ubiquity, especially in soil ecosystems, pin down their roles in various biogeochemical cycles and broad metabolic versatility (Naether et al., 2012). Although their presence and abundance are confirmed through culture-independent studies but their ecological functions, interrelations with environmental parameters and interactions with other soil microbial communities remain obscure. Significant variations have been encountered during isolation of Acidobacteria strains belonging to different lineages and getting cultivated under specific sets of physicochemical conditions or nearly a narrow range of conditions. This suggests the use of various strategies for the successful recovery of ecologically different Acidobacteria groups.

Acidobacteria diversity and dominance is quite pronounced along with high overall ecological and phylogenetic diversity in contrast to its low cultivation success due to radically

different culture laboratory conditions. Although nine different bacterial phyla *viz.*, Proteobacteria, Acidobacteria, Actinobacteria, Verrucomicrobia, Bacteroidetes, Chloroflexi, Planctomycetes, Gemmatimonadetes, and Firmicutes are known to dominate in soil (Janssen, 2006; da Rocha et al., 2009), the phylum Acidobacteria represents the most predominant not-yet-cultured, or difficult-to-culture group of bacteria (Janssen, 2006; Foesel et al., 2014), occupying a significant fraction of the soil microbial community. Henceforth, the determination of their ecophysiological roles becomes quite imperative to understand their functional status in complex bacterial communities. Thus, the ecological roles of Acidobacteria have been studied by analyzing the genetic components obtained by complete and/or draft whole-genome sequencing for culturable species and metagenome sequencing for unculturable species.

16S rRNA ANALYSES AND METAGENOMICS IN STUDYING PHYLUM ACIDOBACTERIA

Meta-microbiomic (16S rRNA gene-based) studies analyze only one specific gene and not the entire genomes of the community members. The 16S rRNA gene sequences, obtained from various environments by employing culture-dependent and culture-independent approaches, provide deeper insights into the species structure and taxonomic diversity of the phylum Acidobacteria. Studies focusing on 16S rRNA genes provide taxonomic status in any bacterial community, while metagenomics can provide both taxonomic and functional profiles of the microbiota. Metagenomic DNA extracted from the environment is often amplified using group or species-specific primers targeting 16S rRNA genes (Kalam et al., 2017a). Metagenomics has facilitated in an apt understanding of the diversity, abundance, genomic make-up, and ecological roles of acidobacterial members in various ecosystems (Tyson et al., 2004; Venter et al., 2004; Parsley et al., 2011). To obtain a complete community structure, 16S rRNA analyses and metagenomics are often used in conjunction (**Figure 1**).

During recent years, the sequencing of collective community genomes employing metagenomics has led to a significant breakthrough in understanding the enigmatic phylum Acidobacteria. Subsequent advent and development of several next-generation sequencing (NGS) platforms further enhanced the metagenomic sequencing efficiencies. Metagenomic approaches, however, at few places fail to retrieve genome scaffolds of sizable length due to soil community complexity, intricacy, and absence of genomes (Tringe et al., 2005; Kowalchuk et al., 2007). Recovering genomic information from the soil
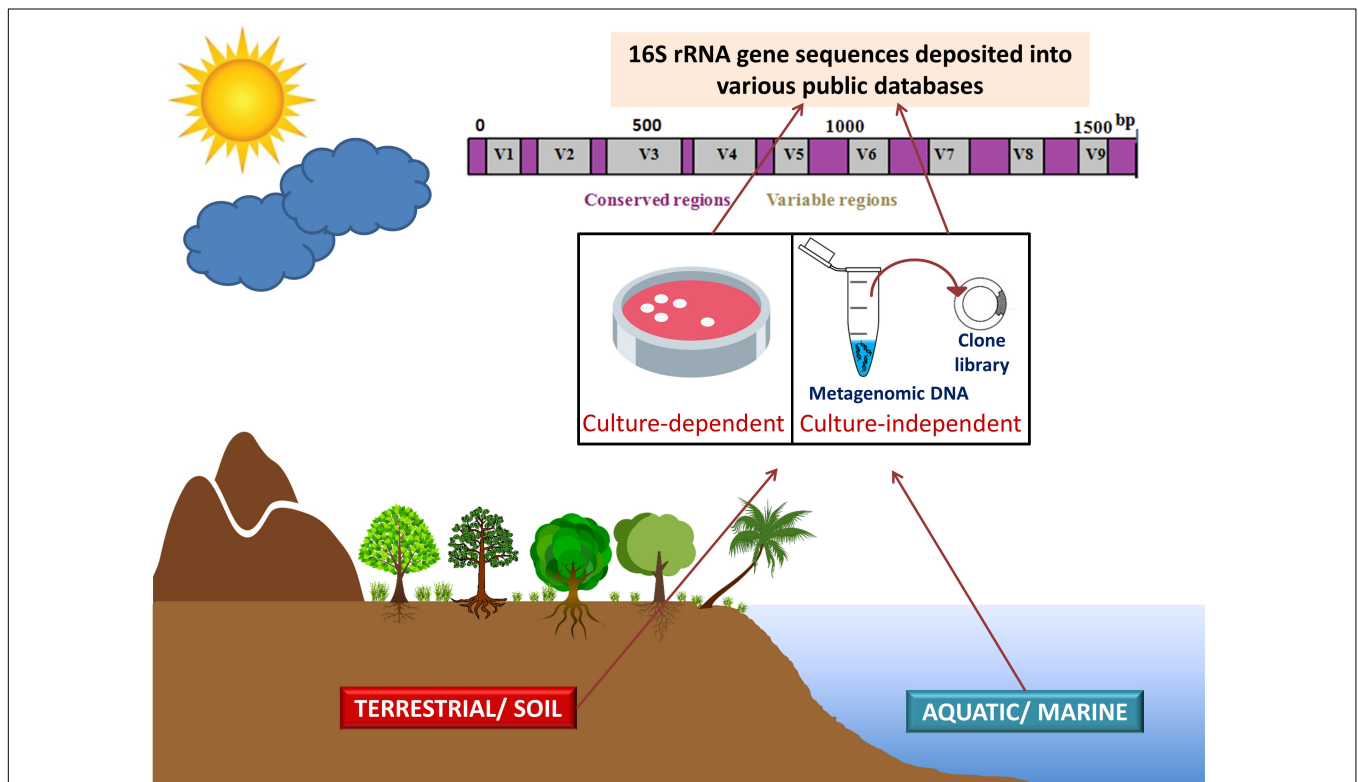
FIGURE 1 | 16S rRNA gene sequencing and metagenomics in studying phylum Acidobacteria. Acidobacterial 16S rRNA gene sequences (from culture-dependent studies) and metagenomic DNA information (from culture-independent studies) obtained from various environments (terrestrial ecosystems including soils and aquatic or marine ecosystems) are deposited in various public databases. The amalgamation of 16S rRNA analyses and metagenomics provides deeper insights into the taxonomic and functional diversity of the phylum Acidobacteria. Figure designed using images from pinclipart.com.

environment requires the use of large insert strategies (Rondon et al., 2000). Metagenomic DNA being high in molecular weights requires meticulous isolation strategies along with novel cloning and screening methods to facilitate the recovery of large DNA fragments from difficult-to-culture bacterial genomes. Large genomic fragments might contain intact metabolic pathways. Acidobacterial genome fragments were successfully recovered from the environment by using a large metagenomic insert (Liles et al., 2003). Henceforth, such approaches provide important platforms for exploring the hidden biotechnological potential of hitherto uncultured bacteria leading to the discovery of novel organo-chemical compounds (Daniel, 2004).

GENETIC INSIGHTS INTO THE PHYLUM ACIDOBACTERIA

Since the recovery of the first Acidobacteria member and subsequent advances in sequencing technologies has provided a platform to study individual Acidobacteria members at the genomic level. It is quite unfortunate that the number of thoroughly studied genomes of this important phylum is very few, despite their numerical abundance in many environments. Genomic studies have unveiled the hidden physiological and metabolic versatility of Acidobacteria members. Comprehensive studies targeting functional characteristics encoded in acidobacterial genomes may provide new vistas into ecological perspectives of phylum Acidobacteria. Ward et al. (2009) conducted detailed genomic studies for the first time, with three acidobacterial strains [two from subdivision 1 *viz.*, *Acidobacterium capsulatum* and *Candidatus* Koribacter versatilis (strain Ellin 345) and one from subdivision 3 *viz.*, *Candidatus* Solibacter usitatus (Ellin 6076)]. Further genomic profiling and comparative genomic studies (Männistö et al., 2012; Rawat et al., 2012a; Lee et al., 2015; Eichorst et al., 2018, 2020) provided more profound insights into the acidobacterial genome and ecophysiology.

Insights into these genomes reveal large genome size (up to 10 Mbp) and a higher percentage of paralogous genes, which might endow the bacterial strains with potential ecological functions (Challacombe et al., 2011). In the same breath, Acidobacteria genomes also possess a comprehensive physiological set of genes that allows them to adapt to various ecological niches. An overview of the general genomic features of Acidobacteria members and their overall genetic and genomic make-up are respectively represented in **Table 1** and **Figure 2**. Data available from published acidobacterial genome sequences reveal the presence of several genes involved in regulating carbon, nitrogen, and sulfur cycles, and those required for degrading different complex polysaccharides. All cultured Acidobacteria species are reported to produce exopolysaccharide (EPS); the presence of *eps* gene clusters in the genomes further supports this data. The acidobacterial genomes also contain a substantial proportion of genes encoding for various transporters, to tide over stress and starvation, and for the biosynthesis of cellulose, N-acyl-homoserine lactones, polyketides, siderophores, hopanoids, and mobile genetic elements (Ward et al., 2009; Kielak et al., 2016a; Crits-Christoph et al., 2018).

Genes for Carbon Metabolism

Each bacterium carries its enzyme machinery catalyzing the breakdown of diverse carbohydrates and nitrogen-containing compounds, which could be used as an identifying characteristic for differentiating varied bacterial species. Acidobacterial genomes possess genes encoding enzymes for the degradation of complex carbohydrate polymers *viz.*, xylan, cellulose, hemicelluloses, pectin, starch, and chitin; amino acids, alcohols, and metabolic intermediates (Ward et al., 2009; Belova et al., 2018). In addition to these, gene modules for diverse carbohydrate breakdown, utilization, and biosynthesis within carbohydrate-active enzymes (CAZy) family are also present (Männistö et al., 2012; Rawat et al., 2012a), spanning across 131 glycoside hydrolase (GH) families (Gilbert, 2010). Putative chitinases belonging to GH18 and GH19 family were also identified in the genomes of a few select Acidobacteria (Rawat et al., 2012a). Gene calling and annotation studies of *Acidobacteria* Group 1 *Acidipila* sp. strain EB88 genome indicated that it was rich in glycolytic enzymes and contained about 85 glycoside hydrolases in 48 families (Domeignoz-Horta et al., 2019).

The acidobacterial genomes are flexible and novel in their carbon metabolizing activity. Few select acidobacterial genomes exhibit anaplerotic CO_2 fixation. Strikingly, homologs of phosphoenolpyruvate carboxylase and isocitrate dehydrogenase have also been detected across several Acidobacteria genomes (Lee et al., 2015; Eichorst et al., 2018). Studies suggest a crucial role of these genes for carbon metabolism in various nutritional pathways as well as a significant role in desiccation resistance, as evidenced by the *Terriglobus saanensis* genome profile (Männistö et al., 2011). A significant contribution is made by acidobacterial enzyme machinery in regulating the carbon biogeochemical cycle (King and Weber, 2007). Since Acidobacteria are endowed with the potential to degrade polymeric carbonaceous complexes, they act as decomposers in soil and actively participate in the cycling of organic matter arising from plants, fungi, and insects (Dedysh and Damsté, 2018).

Genes for Nitrogen Metabolism

Acidobacteria is well equipped with genes catalyzing the metabolism of inorganic and organic sources of nitrogen (Eichorst et al., 2018). They can effectively reduce nitrate, nitrite, and possibly nitric oxide, as could be evidenced by genomic data, supporting their active participation in nitrogen nutrient circuits. Homolog candidate genes for nitrate reductase (*nirA*) have been identified in Ellin 345, *Geobacter fermentans*, and *Terriglobus aquaticum* (Rajeev et al., 2015), while those for nitrate transport (*nrtABCD*) have also been observed in certain acidobacterial strains. The presence of genes encoding dinitrogenase (*nifD* and *nifK*) and dinitrogenase reductase (*nifH*) have also been reported in one acidobacterial genome (Ward et al., 2009). However, experimental evidence regarding nitrogen fixation by Acidobacteria is missing (Kielak et al., 2016a). Insights into the core genomes provide a wealth of data revealing the presence of

TABLE 1 | General genomic features of cultured Acidobacteria members with complete genome sequences.

Acidobacteria species	Subdivision	Genome size (bp)	G + C content (mol%)	No. of coding sequences	Total no. of genes	Total protein coding genes	Total RNA genes	Pseudogenes	References
Acidobacterium capsulatum ATCC 51196	1	4,127,496	60.5	3,502	3,425	3,377	48	0	Ward et al., 2009
Koribacter versatilis Ellin 345	1	5,650,368	58.4	5,239	4,837	4,779	58	2	Ward et al., 2009
Granulicella mallensis MP5ACTX8	1	6,237,577	57.9	NA	4,960	4,907	53	90	Rawat et al., 2012a, 2013
Granulicella tundricola MP5ACTX9	1	5,503,984	60.0	NA	4,757	4,705	52	163	Rawat et al., 2012a, 2014
Terriglobus saanensis SP1PR4	1	5,095,226	57.3	NA	4,333	4,279	54	99	Rawat et al., 2012a,b
Terriglobus albidus ORNL	1	6,405,582	58.5	NA	5,127	5,010	53	64	Podar et al., 2019
Acidobacterium ailaaui PMMR2T	1	3,686,523	57.2	NA	3,184	3,131	53	NA	Myers and King, 2016
Bryocella elongata DSM 22489	1	5,669,524	62.0	NA	4,620	4,567	53	NA	Pinto et al., 2020
Acidiphilium rosea DSM 103428	1	4,213,726	58.8	NA	3,585	3,531	54	NA	Pinto et al., 2020
Occallatibacter sp. AB23	1	6,278,575	59.1	NA	5,429	5,367	62	NA	Pinto et al., 2020
Terracidiphilus gabretensis S55T	1	5,351,935	57.3	NA	4,610	4,562	48	NA	García-Fraile et al., 2016
Solibacter usitatus Ellin 6076	3	9,965,640	61.9	8,568	8,003	7,940	63	114	Ward et al., 2009
Chloracidobacterium thermophilum	4	3,695,372	61.3	3,054	NA	NA	NA	NA	Costas et al., 2012
Chloracidobacterium sp. CP2_5A	4	3,411,091	64.2	NA	3,083	2,969	55	59	Ward et al., 2017
Pyrinomonas methylaliphatogenes K22T	4	3,778,560	59.36	NA	3,244	3,189	55	0	Lee et al., 2015
Luteitalea pratensis HEG_-6_39	6	7,480,314	64.7	NA	NA	6,295	NA	NA	Huang et al., 2016
Holophaga foetida TMBS4T	8	4,127,237	62.95	NA	3,672	3,615	57	76	Anderson et al., 2012
Thermoanaerobaculum aquaticum MP-01T	23	2,660,928	62.7	NA	2,320	2,253	49	18	Losey et al., 2013; Stamps et al., 2014

NA, not available.
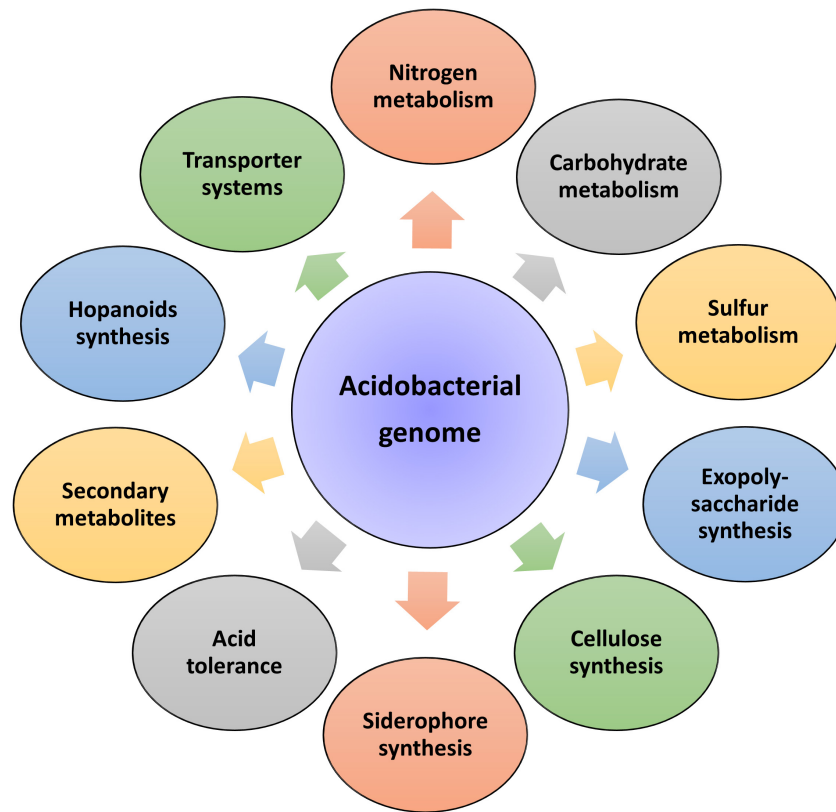
FIGURE 2 | Genetic diversity of the phylum Acidobacteria. Sequencing of acidobacterial genomes and metagenomes revealed a large repertoire of genes responsible for regulating diverse physiological and metabolic functions.

putative homologs encoding for extracellular peptidases, which play significant roles in soils by mobilizing ammonium and other intermediates of N-cycle (Bach et al., 2001; Eichorst et al., 2018). Serine endopeptidases are also found to be widely distributed in most Acidobacteria genomes, indicating their proteolytic activity in soil (Brankatschk et al., 2011). Homologs for various extracellular metalloendopeptidases too span across select Acidobacteria genomes, facilitating them for N-uptake during mineral scarcity. Recent studies on the draft genome of *Acidobacteria* Group 1 *Acidipila* sp. strain EB88, isolated from forest soil, indicated that the genome lacked the genes required for organic acid uptake but was equipped with the genes essential for amino acid, ammonium, and nitrate uptake (Domeignoz-Horta et al., 2019).

Genes for Sulfur Metabolism

Chloroacidobacterium thermophilium, a photoheterotrophic member of Acidobacteria subdivision 4, requires reduced sulfur for its growth (Tank and Bryant, 2015). Draft metagenome-assembled genomes of Acidobacteria subdivisions 1 and 3 from peat soil revealed the presence of putative genes for dissimilatory sulfur metabolism (*dsrAB*, *dsrC*, *dsrD*, *dsrN*, *dsrT*, *dsrMKJOP*, *aprBA*, *qmoABC*, *supP*, *hppA*, *sat*) which function under anoxic conditions (Hausmann et al., 2018). The presence of *dsrAB* genes encoding dissimilatory (bi)sulfite reductase suggests the capacity

of some Acidobacteria members to perform dissimilatory sulfate/sulfite reduction (Huang et al., 2016; Wasmund et al., 2017; Anantharaman et al., 2018). Certain acidobacterial genomes also encode the *dsrL* gene, which is specific to the sulfur oxidation pathway (Hausmann et al., 2018). The presence of *dsrL* is unusual as it is generally found in sulfur oxidizers rather than in sulfate-reducing microorganisms. However, acidobacterial genomes lack any other genes involved in oxidative sulfur metabolism (Hausmann et al., 2018).

Genes Encoding Transporters

Different acidobacterial strains harbor an array of genes encoding different ion channels, high-affinity ABC transporters, several other secretory porters, and transport proteins especially required for tiding on oligotrophic conditions (Paulsen et al., 1998; Ward et al., 2009). Genes encoding iron permease FTR1 and FTR2 family proteins and iron transporter genes *viz*., Mn^{2+} and Fe^{2+} transporters are also present in the Acidobacteria genome. The *feoAB* gene encoding a high-affinity ferrous iron transport protein is invariably present in acidobacterial genomes (Velayudhan et al., 2000). The genome of *Terriglobus saanensis* (isolated from the Tundra region) contains abundant genes involved in carbohydrate metabolism and transport (Männistö et al., 2011). Few Acidobacteria genomes also possess multiple copies of genes for siderophore transport

viz., tonB, exbB, and *exbD* (Postle and Kadner, 2003; Llamas and Bitter, 2006; Ward et al., 2009). In addition to these, *pvuE* (candidate gene for vibrioferrin transporter) and *feoB* (candidate gene for enterobactin transporter) are also found in some Acidobacteria genomes (Ward et al., 2009). Acidobacteria members also have genes for the amino acid-polyamine-organocation (APC) superfamily of transport proteins along with dicarboxylate/amino-acid: cation symporter family of secondary transport proteins (Eichorst et al., 2018).

Genes Regulating Cellulose Synthesis

Acidobacterium capsulatum genome contains an operon with a complete set of genes required for cellulose biosynthesis (Ward et al., 2009). Bacterial cellulose is associated with an array of functions facilitating survival in the soil through biofilm formation, retaining moisture under conditions of stress, thus promoting aeration, contributing to soil aggregate formation (Ude et al., 2006; White et al., 2006). Acidobacteria possesses the remarkable capacity to synthesize cellulose, which would promote biofilms to get adhered easily to ferric iron-containing substrates producing biofilm-ferric iron-reducing "bioshrouds" in acidic environments (Johnson et al., 2008). Cellulose biosynthesis via *bcs* operon has been reported in *Terriglobus saanensis* as evidenced through genome sequencing data (Rawat et al., 2012b; de Castro et al., 2013). The genome of *Acidipila* sp. strain EB88 contained genes for biosynthesis and export of capsule and cellulose (Zhang et al., 2018).

Genes for Oxygen and Hydrogen Utilization

Genomes of particular soil acidobacterial strains possess the potential to respire oxygen at atmospheric and microoxic levels due to the presence of affinity oxidases. Thus, such strains possess an extra selective advantage in the soil microenvironment, where low oxygen concentrations exist (Morris and Schmidt, 2013; Eichorst et al., 2018). The genome of *Acidipila* sp. strain EB88 contains a low-affinity group A heme-copper oxygen oxidase and five high-affinity cbb3 terminal oxidases that enables it to grow under both hypoxic and hyperoxic conditions (Morris and Schmidt, 2013; Domeignoz-Horta et al., 2019). Few Acidobacteria strains possess hydrogen scavenging property due to the presence of nickel-iron [NiFe] hydrogenases (Greening et al., 2015). The occurrence of multiple structural genes (*hhyS, hhyL, hhyE*) and maturation genes (*hypABCDEF*) required for hydrogenase activity further supports acidobacterial hydrogen utilization (Eichorst et al., 2018).

Genes Regulating Secondary Metabolite Biosynthesis

Acidobacteria genomes contain biosynthetic gene clusters that encode a vast repertoire of polyketide and non-ribosomal peptide synthases (Crits-Christoph et al., 2018). Additionally, genes regulating the synthesis of diverse secondary metabolites and other natural compounds like siderophores, antifungals, antibiotics, antivirals, antitumor agents, and antinematodal agents have been reported in Acidobacteria genomes (Challis, 2005; Ward et al., 2009; Parsley et al., 2011; Hadjithomas et al., 2015; Crits-Christoph et al., 2018).

Genes Regulating Stress and Starvation Response

Addiction modules encode various genes, including those required for plasmid maintenance, and has been documented in certain Acidobacteria (Ward et al., 2009). During stress or starvation, the addiction modules operate rapidly and inhibit DNA and protein synthesis (Kroll et al., 2010). This mechanism of stress and starvation tolerance in Acidobacteria enables tiding over environmental oligotrophic nutritional conditions. The prokaryotic transcriptional regulator sigma factor is commonly utilized to control the expression of several gene sets in response to various stresses, including starvation, oxidative stress, heat stress, and exposure to heavy metals that enables the microorganisms to adapt to a stressful environment (Rhodius et al., 2005; Challacombe et al., 2011). The genomes of *Candidatus* Solibacter usitatus [Ellin 6076] and *Candidatus* Koribacter versatilis [Ellin 345] contain a vast repertoire of sigma E (σ^E) homologs (70 and 28 homologs, respectively) that are induced during starvation and other stress conditions (Challacombe et al., 2011).

The presence of hydrogenases enables specific acidobacterial strains to consume atmospheric hydrogen (Greening et al., 2015), which could be a strategy to overcome starvation (Eichorst et al., 2018). Certain Acidobacteria members are equipped with the genes responsible for the dissimilatory reduction of nitrite to ammonia (*nrfHA*), which not only provides energy supply but also aids in the detoxification of nitrosative stress (Rajeev et al., 2015; Eichorst et al., 2018). A very recent study by Pinto et al. (2020) reported that carotenoid production by *Occallatibacter* sp. (belonging to Acidobacteria subdivision 1) can confer tolerance to environmental oxidative stress. Thus, the production of carotenoids and related compounds may offer competitive benefits to soil Acidobacteria.

Genes Regulating Acid Tolerance

Most Acidobacteria members prefer acidic conditions (3.0–6.5 pH) for their growth (Sait et al., 2006; Ward et al., 2009). Also, lower pH levels support a higher abundance of Acidobacteria (Männistö et al., 2007; Lladó et al., 2018). Microorganisms equipped with acid resistance (AR) systems are likely to survive in highly acidic conditions (Sun et al., 2012). Certain moderately acidophilic acidobacterial strains *viz., A. capsulatum*, Ellin 345 (*K. versatilis*), and Ellin 6076 (*S. usitatus*) contain candidate genes in the AR3 (arginine-dependent AR) system indicating the presence of an acid tolerance system (Ward et al., 2009; Kielak et al., 2016a). However, the strains lack the genes involved in other inducible AR systems, *viz.,* AR1 (oxidative AR), AR2 (glutamate-dependent AR), and AR4 (lysine-dependent AR).

Genes for Synthesis of Hopanoids

Hopanoids are pentacyclic triterpenoid bacterial membrane lipids facilitating cell membrane permeability, especially during

extreme environmental conditions (Damsté et al., 2017). Genes related to hopanoid biosynthesis (*shc* gene) have been detected in *Cadidatus* "K. versatilis" belonging to subdivision 1 isolated from soil (Joseph et al., 2003). The presence of C_{30} hopanoids and bacteriohopane polyols was reported in multiple Acidobacteria subdivisions, evident from the occurrence of respective biosynthesis genes (*hpnA*, *hpnG*, *hpnH*) in acidobacterial genomes (Damsté et al., 2017).

Genes for Exopolysaccharide Biosynthesis

Genomic mining studies suggest that Acidobacteria belonging to subdivision 1 can encode EPS biosynthesis genes (Ward et al., 2009). Additionally, several cultured Acidobacteria species are known to secrete EPS (Eichorst et al., 2007; Pankratov and Dedysh, 2010; Whang et al., 2014; Kielak et al., 2017). Gene prediction, along with functional annotation data, identifies a gene cluster encoding capsular polysaccharide in the genomes of three acidobacterial strains isolated from tundra soil. *Granulicella mallensis* genome possessed the *epsH* gene involved in exopolysaccharide synthesis (Rawat et al., 2012a).

Genes for Acyl-Homoserine Lactones (AHL)

Quorum sensing molecules, like acyl-homoserine lactones (AHL), aid in coordinating gene expression in bacterial populations (Parsek et al., 1999). Genomes of Acidobacteria contain a considerable fraction of genes encoding for biosynthesis of N-acyl homoserine lactones (Ward et al., 2009).

Genes for Mobile Genetic Elements

Mobile genetic elements like transposons, bacteriophages, insertion sequence (IS) elements, and integrative and conjugative elements (ICEs) present in several Acidobacteria are known to confer shape and plasticity to the acidobacterial genome. These elements are speculated to mediate horizontal gene transfer, aid in the evolution and ecological success of Acidobacteria (Frost et al., 2005). A recent meta-study by Eichorst et al. (2018) identified 35 putative prophages across 19 acidobacterial genomes. Insertion sequence families encoding their mobility patterns also have been reported to be spanning across Acidobacteria genomes (Challacombe and Kuske, 2012). Multiple genes encoding mobile elements, including transposases and IS elements, have been identified in the genome of *Candidatus* "Solibacter usitatus" Ellin 6076. Additionally, the genome also harbors genes encoding phage integrase family proteins and several other proteins containing a retroviral integrase catalytic region domain, catalyzing site-specific recombinations (Challacombe and Kuske, 2012).

EXCAVATING THE ECOLOGICAL ROLES OF PHYLUM ACIDOBACTERIA IN SOIL

Despite the recent progress in the field of acidobacterial ecology, there is still a paucity of complete information regarding their ecophysiological roles. Significant ecological functions have been reported in forest soil for Acidobacteria members (García-Fraile et al., 2016; Liu et al., 2016). Acidobacteria members in plant-soil ecosystems play pivotal ecological roles, including modulation of biogeochemical cycles and influencing plant growth. The key findings from relevant studies on soil Acidobacteria, highlighting their salient genomic features and ecological roles, are summarized in **Table 2**. An overview of their ecological roles in the plant-soil ecosystems is diagrammatically represented in **Figure 3** and is discussed below.

Acidobacteria as "Keystone Taxa" in Soil Ecosystems

A recent study by Banerjee et al. (2018) has reviewed and re-defined "keystone taxa" in microbial ecology. Microbial keystone taxa have been often ascribed as "ecosystem engineers" as they are the unequivocal drivers of microbial community structure and function in different ecosystems. Acidobacteria was reported to be one of the keystone bacterial taxa in soil associated with the decomposition of soil organic matter (SOM), implying their significance in carbon turnover (Banerjee et al., 2016). Computational inferences obtained from analyses of various terrestrial ecosystems and habitats indicate several subdivisions of phylum Acidobacteria represent the keystone taxa in grasslands (subdivision 4), forest or woodlands (subdivision 4), agricultural soils (subdivision 17), and plant-associated microbiota (subdivisions 1, 3, and 6) (Banerjee et al., 2016, 2018; Jiang et al., 2017; Li et al., 2017). Speculations suggest that

TABLE 2 | Salient genomic features and ecological roles of soil Acidobacteria.

Salient features	References
Involvement in carbon cycle	King and Weber, 2007; Banerjee et al., 2016; García-Fraile et al., 2016; Belova et al., 2018; Dedysh and Damsté, 2018
Involvement in nitrogen cycle	Ward et al., 2009; Rajeev et al., 2015; Eichorst et al., 2018
Involvement in sulfur cycle	Wasmund et al., 2017; Hausmann et al., 2018
Involvement in plant growth promotion	Kielak et al., 2016b; Kalam et al., 2017a
Involvement as "keystone taxa"	Banerjee et al., 2016; Jiang et al., 2017; Li et al., 2017; Banerjee et al., 2018
Involvement in soil matrix formation	Kielak et al., 2016a; Kielak et al., 2017
Establishment of biofilms	Ward et al., 2009; Kielak et al., 2016a; Kielak et al., 2016b
Production of exopolysaccharides	Ward et al., 2009; Rawat et al., 2012a; Kielak et al., 2017
Biosynthesis of secondary metabolites	Ward et al., 2009; Parsley et al., 2011; Hadjithomas et al., 2015; Damsté et al., 2017; Crits-Christoph et al., 2018
Tolerance to stress, starvation, and acidity	Ward et al., 2009; Challacombe et al., 2011; Morris and Schmidt, 2013; Greening et al., 2015; Rajeev et al., 2015; Eichorst et al., 2018; Domeignoz-Horta et al., 2019; Pinto et al., 2020
Presence of mobile genetic elements	Frost et al., 2005; Challacombe and Kuske, 2012; Eichorst et al., 2018

FIGURE 3 | Ecological roles of Acidobacteria members in the soil. Acidobacteria members in the plant-soil ecosystem facilitate modulation of critical biogeochemical cycles *viz*., carbon, nitrogen, and sulfur cycles. Exopolysaccharides (produced by almost all cultured members) aid in soil matrix formation and contribute to plant growth promotion by facilitating water and nutrient uptake by plants. The representatives belonging to *Granulicella* and *Acidocapsa* genera exhibit *in vitro* plant growth promotion traits in the model plant *Arabidopsis thaliana*. These acidobacterial strains effectively produced the phytohormone indole-3-acetic acid (IAA) and siderophores, which significantly enhanced the plant growth parameters (shoot and root biomass). Figure designed using images from pinclipart.com.

these microbial residents exert beneficial effects by selectively modulating the ecological processes of host ecosystems. These bacteria offer two crucial ecological services *viz*., SOM decomposition, and denitrification, thereby enhancing carbon stability (Banerjee et al., 2018). Successful manipulation of these soil dwellers might augment the key ecological process of soil carbon sequestration. Since Acidobacteria members have been identified as structural and functional keystones in plant-soil microbiomes and agroecosystems, they can be exploited to enhance crop performance and productivity.

Modulation of Biogeochemical Cycles

The gamut of living organisms invariably depends on the supply of essential elements such as oxygen, hydrogen, carbon, nitrogen, phosphorus, and sulfur for growth and survival. In any ecosystem, albeit biogeochemical cycles or nutrient cycles are regulated by the biotic and abiotic components of that system, but microorganisms play a key role in modulating them (Falkowski et al., 2008). The critical metabolic processes *viz*., nitrogen metabolism,

carbon fixation, and methane metabolism, sulfur metabolism operating in microbes effectively control global biogeochemical cycling.

Genomic studies highlight the carbohydrate utilization potential exhibited by Acidobacteria members (Ward et al., 2009; Kielak et al., 2016a). The presence of genes encoding enzymes for the degradation of complex carbohydrate polymers like cellulose, hemicellulose, chitin, xylan, and lignin derivatives signifies their active participation in the carbon circuit as decomposers in soil (Ward et al., 2009; Wegner and Liesack, 2017; Banerjee et al., 2018; Belova et al., 2018) and cycling of organic matter derived from plants, fungi, and insects (King and Weber, 2007; García-Fraile et al., 2016; Dedysh and Damsté, 2018).

Soil bacteria convert the inorganic nitrogen to organic compounds (Canfield et al., 2010), which are, in turn, utilized by the plants and other microbes. In plant-soil ecosystems, Acidobacteria communities represent an important microbial guild, central in nitrogen cycling. Genomic evidence coerces the crucial role of Acidobacteria in N-cycling in soils (Ward et al.,

2009). Specialized microorganisms metabolize diverse sulfur compounds via redox reactions and contribute to driving the global sulfur cycle (Wasmund et al., 2017). Acidobacteria genome harbors genes regulating sulfur metabolism that were found to be expressed in native peat soils and upregulated in diverse anoxic conditions, indicating their active role in modulation of the environmental sulfur cycle (Hausmann et al., 2018). Hausmann et al. (2016) reported that consortia of diverse sulfate-reducing microorganisms, including Acidobacteria members, drive the sulfur reduction in peatlands and Acidobacteria members (*Holophaga*) responded positively to sulfate stimulation in peat soil microcosms.

Exopolysaccharide Production

Exopolysaccharides are bacterial polysaccharides synthesized extracellularly (Nwodo et al., 2012), which play a critical role in the soil matrix and aggregate formation (Bogino et al., 2013). EPS plays a significant role in the development of mature biofilm by acting as bridges between cell surfaces (Bura et al., 1998). In the rhizosphere zone, EPS production by bacterial populations contributes toward nutrient and water uptake by plant roots through modification of the physicochemical properties of rhizosphere soil (Degens et al., 1994; Kalam et al., 2017b) and aids in establishing interactions with the root appendages for successful plant-microbe interactions (Bianciotto et al., 2001, 2009).

Several cultured Acidobacteria species are known to secrete EPS (Eichorst et al., 2007; Kielak et al., 2017). Genomic analyses indicate that Acidobacteria belonging to subdivision 1 can encode EPS biosynthesis genes (Ward et al., 2009). These EPSs might protect Acidobacteria, endowing them the ability to survive for prolonged periods in soil (Kielak et al., 2017). Recently, the characterization of EPSs derived from two subdivisions 1 acidobacterial strain (*Granulicella* sp.) has indicated them to be possessing potential environmental, bioremediation, and biotechnological applications (Kielak et al., 2017). These EPSs could be used as natural eco-friendly binders and formulants in the biofertilizer industry.

Acidobacterial genome profiling validates the presence of cellulose synthesis genes along with related accessory proteins. Studies postulate several Acidobacteria members to possess the capability to establish biofilms, exhibit resistance toward desiccation, and aid in the formation of soil aggregates (Kielak et al., 2016a). However, physiological data confirming actual EPS production by Acidobacteria and validating their ecophysiological roles remains obscure.

Acidobacteria as Plant Growth Promoting Rhizobacteria (PGPR)

Plant root microbiome harbors beneficial microbes, which offer an eco-friendly alternative to improve plant growth and protect against phytopathogens (Bulgarelli et al., 2013; Philippot et al., 2013). Plant growth promoting rhizobacteria (PGPR) have been defined as free-living soil bacteria dwelling in the rhizosphere and endowed with the potential to stimulate plant

growth and crop yield (Dutta and Podile, 2010; Sayyed et al., 2019; Zope et al., 2019). A plethora of reviews exhaustively documents almost all aspects of PGPR (Ahmad et al., 2008; Lugtenberg and Kamilova, 2009; Etesami et al., 2015; Parray et al., 2016; Backer et al., 2018). PGPR, through their direct and indirect effects, can bring about substantial plant growth promotion (PGP). They act directly by facilitating nitrogen absorption and assimilation, mineral solubilization, production of phytohormones (Ahmad et al., 2008; Parray et al., 2016; Shaikh et al., 2018; Kalam et al., 2020). Indirectly, PGPR, through their biocontrol mechanisms, produce siderophores (Wani et al., 2016; Sayyed et al., 2019), lytic enzymes (Jadhav et al., 2020a,b), antibiotics (Vinay et al., 2016; Reshma et al., 2018; Kenawy et al., 2019), 1-aminocyclopropane-1-carboxylic acid (ACC) (Glick, 2014; Goswami et al., 2016; Sagar et al., 2020), guarding host plants against pathogens (Shaikh et al., 2018).

Several studies reported Acidobacteria to be avid rhizosphere colonizers (Lee et al., 2008; da Rocha et al., 2010, 2013). The first evidence for PGP by Acidobacteria subdivision 1 members was provided by Kielak et al. (2016b). Their experiments confirmed interactions between three acidobacterial strains belonging to the genera *Granulicella* and *Acidicapsa* and the host plant *Arabidopsis thaliana*. Determination of *in vitro* PGP traits provided evidence that all the test strains were active producers of phytohormones IAA and siderophore. There was a significant increase in root growth parameters, although shoot biomass variations were non-significant in comparison to the controls. All three strains were able to adhere to roots, form a biofilm, and grow along the root surface. The pioneering study provided the first time with a direct confirmation regarding the Acidobacteria-plant interactions and PGP by Acidobacteria members. Acidobacteria subdivision 1 member dominates among other subdivisions, followed by subdivisions 4, 3, 8, and 23. Their preponderance in the soil environment in comparison with other subdivisions and their genetic profiles surmise their active roles in plant-soil ecosystems. Also, analysis of biosynthetic gene pathways from few publicly available genomes of soil-inhabiting acidobacterial isolates suggests the presence of genes linked to the production of secondary metabolites involved in plant growth (Parsley et al., 2011; Hadjithomas et al., 2015).

The diversity and composition analysis of broomcorn millet rhizosphere using 16S rDNA sequencing indicated Acidobacteria to be a core bacterial component among rhizobacterial assemblages, comprising 10.7% of the total operational taxonomic units (OTUs) obtained (Na et al., 2019). In another interesting study, Kalam et al. (2017a) explored the effects of three PGPR strains *viz.*, *Sphingobacterium* sp.*, Variovorax* sp., and *Roseomonas* sp. on crop rhizospheric population densities of acidobacterial members. They reported that the equivalent cell numbers of Acidobacteria members gradually increased over time with a simultaneous increase in plant growth promotion by PGPR inocula. The study speculated the existence of beneficial interactions between the triad of difficult-to-culture bacteria, PGPR, and host plants. Additional experiments in plant-soil ecosystems are

still required for unveiling the exact ecological roles of rhizospheric Acidobacteria members.

CONCLUSION

Soil represents a luxurious pool of microorganisms, including the shadow biosphere dwellers, the difficult-to-culture bacteria which might be strategic sources for novel and successful biotechnological products. Acidobacteria is an important and among the most abundant difficult-to-culture phylum harboring the soil ecosystem. Acidobacteria plays significant ecological roles, as evidenced through their active participation in key carbon, nitrogen, and sulfur biogeochemical circuits. At the same time, the production of EPS further strengthens their ecophysiological functions in establishing plant-soil beneficial interactions. Since this divergent phylum predominates rhizosphere microbial communities, these bacteria might be a significant component of the crop's natural environment. Initiatives for manipulating crop rhizosphere with acidobacterial populations to increase plant growth in laboratory and greenhouse studies could be considered to be future challenges and thrust areas for research. The correlation and information presented in this review might prove to be relevant for future modeling of experiments to expose the potential biotechnological roles of Acidobacteria members and exploit them as prospective alternatives to agrochemicals, facilitating sustainable agriculture.

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ENHANCING LANGUAGE COMPETENCE AMONG SLOW LEARNERS IN AUTONOMOUS LEARNING USING MOBILEAPPLICATIONS

T. Sreevani[1], Dr.Mutyala Suresh[2]

[1]Research scholar in ELT, Dept. of English, Koneru Lakshmaih Education Foundation (Deemed to be University), Vaddeswaram, Guntur District, A.P., India&

Lecturer, Dept. of English, St.Ann'sCollege For Women, Hyderabad, TS, India.

[2]Asso. Professor, Dept of English, Koneru Lakshmaih Education Foundation(Deemed to be University), Vaddeswaram, Guntur District, A.P., India.

sreevani.vips@gmail.com; msphd@kluniversity.in

Abstract   English language, being the International official language for communication in today's world, plays an important role in student's life. Teaching-learning process in English classrooms among the undergraduates face many challenges due to different levels of proficiency of the learners. The classroom is always a combination of slow learners and advance learners. Teaching only the syllabus in framed methods will not bring desired outcome. Teacher should motivate autonomous learning among slow learners to take responsibility of learning independently. Learning through mobiles became lifestyle for the new generation learners. Mobile learning is more spontaneous and less formal. It has the advantage of self-learning and learning anywhere, everywhere and at any time. Language learning through mobile was first time invented by Stanford learning lab (Beown-2001). Hence, the teachers can fill the gap between slow learners and advance learners. This study focuses on investigating the self-learning model using mobile applications in autonomous learning to enhance English Language Competences among slow learners in first year undergraduate students. For this purpose a questionnaire about autonomous leaning using mobile applications for the slow learners was administered to 150 under- graduate students. A pre-test was conducted and students underwent intervention programme for the period of 20 days, at the end post-test results were show than the students enhanced in language

competences.

Introduction

English is popularly considered as the International language and also as the lingua Franca in the world. According to British Council (2013), "English is the dominant international language of the 21st century. It is spoken at useful level by some 1.75 billon people- a quarter of the world population". English language learners are increasing rapidly worldwide. In India English language is practiced as a second language in the classrooms, due to which there are many challenges confronted by learners and teachers. Researchers have designed many remedies in the area of ESL (English as second Language). One of the challenges faced in teaching English is with the slow learners in the classroom. Slow learners have difficulty of grasping the teachings in the classroom, where as other learners can grasp the content easily. Teaching in a class with different levels of learners is a great challenge to a teacher in given time and curriculum frame.

Dr. Praveen Sharma, Associate professor from Hindu college of Education, Haryana in her lectures for CEC(Consortium for Educational Communication) spoke about Slow learners and gifted children and mentioned that "Intellectually, slow learners score most often between 75 and 90 IQ between the borderline and low average classifications of intelligence (knoff 1987)". Actually, parental disharmony, poor health, economy and social background etc. are the affecting factors on the slow learners even though they have good IQ. To address this problem of slow learning in the English language classroom, teacher should motivate slow learners to adopt autonomous learning. According to Guo(2011), as mentioned byYiwen Hu(2016) was of the opinion that "learner autonomy refers to a learner taking charge of his or her own learning".

Autonomy learning in language gives better opportunities to slow learners outside the classroom and increases their proficiency in language skills. Language teacher needs to make slow learners self- motivated, self-reliant providing self-learning modules. Over 3 decades vigorous research on language acquisition through autonomous learning happened around the world. Researchers explored to bring forth new strategies, modern teaching techniques and approaches to help autonomous learners. Using technology in Autonomous learning is one of the current trends in the autonomous learning. Learners of all ages are learning this global language through this new mode. Rapid growth of enrolment in online English courses forms the basis for this opinion. The accessibility of online courses on mobile devices and availability of language learning Apps on smartphones made the path brighter for the learners in this new trend.

Mobile –Assisted Language Learning research is increasing rapidly from 2008. (Duman, Orahon, & Gedik,2015).Mobile –Assisted Language Learning also referred as MALL, also known as M- learning , is absorbed by language learners more than CALL(Computer –Assisted Language Learning) due to the number of advantages .M- learning creates various

learning opportunities, because Mobile technology industry and language experts invented number of mobile Applications to learn language. At present there are many Apps available for English Language learning, to name few: BBC learning English, British council, knudge, me, let's talk, Two min English, Game to learn English, Real English and etc.

Aim of the research

The study aims at investigating self-learning modules using mobile applications in Autonomous learning to enhance English language competences among slow learners of first year undergraduate students at St. Ann's College for Women, Mehdipatnam, Hyderabad, Telangana.

Statement of the problem

There are slow learners and advance learners in every classroom. Slow learners have to take responsibility to learn independently to get desirable outcome of the class. Teaching alone doesn't help slow learners to get their proficiency in the classroom

Hypotheses

1. No proper self-learning module is provided to make the slow learners self-sufficient.
2. Autonomous learning is given least priority by the slow learners.
3. Slow learners do not know how to use Mobile applications for learning Language Competences.
4.

Objective of research

1. To enable slow learners in developing their English language competences.
2. To make use of Autonomous leaning to enhance Language Competences among slow learners.
3. To equip slow learners with self-learning module using mobile applications..

Research Design

The study focuses on investigating self-learning module using mobile applications in Autonomous learning to enhance Language Competences among slow learners in first year undergraduate students, so that they become proficient in English language. For this purpose, a preliminary study was conducted by the researchers in various first year under-graduate class rooms in the college. It was found that not much focus was given to slow learners to enhance English language outside the classroom. After few brain storming sessions, motivating on autonomous leaning using mobile applications, a structured questionnaire was administered to 150 randomly selected slow learners. After collecting the data from the slow learners, their perception was analyzed, tabulated and interpreted. For each question, five options such as not Agree strongly, Agree, Not a Strong Opinion, Disagree, Disagree totally.

Score for each option is given as:

Table: 1: Evaluation scale for the research

Not Agree strongly	1
Agree,	2
Not a Strong Opinion	3
Disagree	4
Disagree totally	5

Responses for the questionnaire:

The slow learners were asked various questions, to find out how they use mobile applications for autonomous learning. The sample learners were asked to respond to every question in the column by marking tick.

- Use of autonomous learning outside classroom is an elaborate process and time consuming.
- I enjoy learning English language through the use of various Mobile Applications.
- I love listening to podcast on my mobile for enhancing my vocabulary.
- I thoroughly enjoy YouTube videos to enhance English.
- Reading stories and news on mobile helps me in improvising my grammar and comprehension.
- With the use of educational mobile Applications, I strive to improve language.

Methodology

Likert scale was used for analyzing and calculating the data. The score for the questionnaire is as follows

Table: 2 Analyzed data through Likert Scale

Autonomous learning using Mobile Applications	3.8

A pre-test was conducted based on the scores students divided in to two groups. Each group consisting of 75 members. The first group is the experimental group and the other group is the control group. The experimental group underwent intervention programme forthe period of 20 days.

the researcher designed an intensive training program and provide training to the experimental group to develop English language competences in autonomous learning with help of mobile applications.

many language activities were incorporated in the training programme. On the other hand, The control group continued with the same classroom teaching.

The training was conducted for 20 sessions. Each session was 60 minutes and two days per week. Researcher has given few mobile applications for the learners, such as BBC Leaning English, knudge.me (excel English), Let's talk(learn anywhere. Anytime), British Council English score, ESSAY, English Conversations, Google News and Google classroom for them to practice. The slow learner worked on the following activities given by the researcher to improve their language competences and concepts in autonomous leaning using mobile applications.

I. Activities on Listening Competences:

Listening competences is one of the most significant skills among LSRW skills. The benefits of practicing listening skills help the students understand spoken language, acquire pronunciation, word stress, intonation, vocabulary and etc

Activity 1:

The researcher gave outline of the story to create interest among students and instructed them to focus only on understanding the message comfortably in their first attempt. While listening for the second time, students can make a note of new words or phrases. After giving needed inputs, researcher asked the students to listen to "Alice in wonderland: part1 to 5"From BBC learning English App in 1st session, each part comprising of 5 to 6 minutes. The students were very excited and enjoyed listening to the audio-stories. Students improved listening skills and recollected the already known words such as curious, daisies, marmalade, telescope, creep, curious, shrinking, creature, chop off, hookah pipe and etc. At the end of the session, the students got motivated to listen to the remaining part of the story without the intervention of the researcher and picked up new vocabulary

Activity 2:

After listening to the remaining part of Alice adventures in wonderland in the next session, students were asked to observe tone and intonation from part 6 to 10. When they completed their listening of all the audio files, researcher asked them to imitate any one of the intonations related. In the beginning students showed less interest, however after sometime few students made an attempt to make dialogues and very few students came forward to narrate entire episode. Here are some dialogues students made at the end of the session:

I wonder who could live in such a little house
The Duchess!!
No room here! I'm sorry, no room!
Ooh, good. Riddles! I love riddles!

I'm Alice, Your Majesty.
Silence! Call the first Witness!

II. Activities on Speaking Competences:

Listening and Speaking are interdependent skills in language learning process. Developing effective listening skills in language learning gives ability to speak effectively. Speaking skills can be improved by putting them into practice. Language teacher should educate on strategies and provide opportunities to students in advancing their speaking skills

Activity 3:

The Researcher gave tips on 'Story Telling' and gave choice to pick a story or prepare their own story. After that students were asked to record a story on Recorder APP, and then listen to correct their own mistakes in grammar, punctuation, intonation, accent etc. and record again. Initially, students were afraid of making mistakes, stammering and stuttering. But the researcher encouraged them to overcome fear and do persistently, which will lead them to perfection. Finally, Students recorded their stories, after several corrections made they recorded again and they identified that they have ability to get all the corrections themselves. This method of autonomous learning aid Students to speak in English confidently

Activity 4:

Language learners need to practice conversational English to empower the communication competence in the society. Researcher introduced YouTube video on "Learn English conversation-English comedy-English Speaking Part 1" in the class to enhance Conversational English. While watching this videos, students couldn't control the laugh and the same time they learnt new phrases to using in the conversations. Students were given time to watch remaining episodes and practice conversational phrases to present a small talk in the next session.
   • Introducing yourself
   • Asking information
   • Giving information
   • Talking about favorite things
   • Describing people
   • Situation : at the travel office
   • Asking directions
   • Telephone conversations
Students presented topics in the class with great zeal and interest. Some of them are mentioned above.

III. Activities on Reading Competences:

Listening and Reading skills are called receptive skills. Reading skills help the reader to recognize the meaning of the word and its relationship

with sentence and also relationship between and among the sentence. There are many types, some are oral and silent reading, creative reading, skimming , scanning, extensive reading and intensive reading. Language Learners should practice all types of reading skills to acquire efficient reading comprehension ability.

Activity 5:

Reading News is a very good activity to develop reading comprehension skills. In this digital age 'Google News app' gives access to read all Newspapers on the smartphone. Researcher asked students to read News on Google News App to develop reading comprehension. Students browse the App and find interest to read News on their interesting topics. Every session one or two students were allotted to read News in the class for five minutes.

IV. Activities on Writing Competences

Speaking and writing skills are called productive skills. Becoming proficient in Writing is a great challenge for English language learner. Some of the challenges are limited vocabulary, inaccurate verb tenses, vast grammar rules and little experience in writing. Constant practice in listening, speaking and reading skills enable learner better his or her writing skills. Letter writing, essay writing, descriptive writing, narrative writing, discursive writing, argumentative writing, informative writing, report writing and etc. are types of writing can be mastered with vigorous practice

Activity 6:

Researcher assigned to download TED Talk App to watch any video and asked to paraphrase the content of their choice. Students struggled to attempt in the beginning, whoever they rectified errors and submitted their written assignment.

Activity 7:

Researcher oriented on "How to improve Writing Skills", in addition to that asked students to watch YouTube video from Learn English Lab channel on "POWER Writing". After watching the video, students learnt to Prepare, Organize, Write, Evaluate and Review which are the five stages and tips to improve Writing skills.

Students were asked to write small journal or dairy on their mobile notepad with at least one paragraph every day. They have to notice frequently used adjectives or word and use every time different synonyms. Students identified some of the words repeated very often and realized to improve vocabulary and grammar to excel in Writing Skills.

V. Activities on Grammar and Vocabulary:

Activity 8:

Knudge.me App helps to fine-tune vocabulary. Different types of games on vocabulary are deigned in this App which motivated students to play to learn new words.
  • Game 1: words checker which will help in expand vocabulary
  • Game2: Fly High helps get fluent with new words
  • Game 3: vocabulary builder- Easy
  • Game 4: all goals there are 144 new words with pronunciation, meaning, usage, synonyms, antonyms and after leaning recall test will be conducted on each new word
  • Game 5: take quiz

There are many other exercises, like Substitutes , word store, collections, little- known Facts are very attractive to the students to learn new vocabulary.

Researcher cheers up students to play games and empower their vocabulary. Students got a charge of vocabulary after playing games.

Activity 9*:*

Let's talk App and BBC 6 minute grammar is introduced by researcher to the class to learn English Grammar lessons. Since grammar is vast subject, students were asked to work on for 5 sessions. Articles, Noun, pronoun, verb, adjectives, adverbs, prepositions, tenses, degrees of comparisons and etc. are on the list in Autonomous learning.

Researcher suggested to watch video on "Main Part of a Sentence" from Let's talk App. Subsequently, watching videos sharpened students to identify subject and predicate in different types of sentences

Findings:

  - A great number of students 'Agreed Strongly' that they enjoy learning vocabulary using games on mobile Application.
  - It was learned that most of the students were reluctant to learn grammar. But after few brainstorming sessions, they developed interest in learning grammar using mobile applications.
  - It was proved that sample slow learners can enhance Language Competences in Autonomous learning using Mobile Applications.
  -    Language Proficiency of sample Slow learners is increased at the end of the training programme.

Conclusion:

The study on self-learning module using mobile applications in Autonomous learning to enhance English language Competences among slow learners was more effective than only framed classroom teaching on curriculum. The aim of the research is to create a self-learning module to meet the requirements of the slow learners to develop proficiency in English to get desired outcome of the class .The research and training which was conducted to enhance Language Competences among slow learners in Autonomous learning using Mobile applications was very impressive.

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Increasing Demand In Usage Of Smartphones Among Undergraduatesto Enhance The English Language Skills: An Analysis

Dr.Mutyala Suresh[1] , T. Sreevani[2]

[1]Asso. Professor, Dept of English, Koneru Lakshmaih Education Foundation(Deemed to be University), Vaddeswaram, Guntur District,
A.P., India. Email: msphd@kluniversity.in
[2]Research scholar in ELT, Dept. of English, Koneru Lakshmaih Education Foundation(Deemed to be University), Vaddeswaram, Guntur District, A.P., India &Lecturer, Dept. of English, St.Ann's College For Women, Hyderabad, TS, India.
Email: sreevani.vips@gmail.com

Abstract:
The invention of smartphones influenced every area of people's life. According to the needs of the people, companies are producing the latest versions of Smartphones with modern technologies. Demand in the market shows with much evidence that the usage of smartphones is increasing day by day in the world. Smartphones are not only used for communication purposes but also other purposes like web surfing, entertainment, education, camera, and so on. Educational institutions have introduced Smart-education using Smartphones in all the fields of education. Coming to language learning, Mobile language learning was first started in the year 1980 by Twarog and Pereszlenyi Pinter to take feedback and assistance for distant language learners and gradually increased its usage due to the advantages it offers. This study focuses on analyzing the increasing demand for smartphone users to learn the English language among Undergraduate students and identify the language skills that are enhanced after using a smartphone. As a part of the research, a questionnaire is administered among the undergraduates of urban and rural areas of Telangana state in India. After collecting and analyzing the data, it is considered that the usage of smartphones enhanced English language skills, such as listening, reading, writing, grammar, vocabulary, pronunciation, communication, etc. And for the past 5 years, the usage of smartphones among undergraduates has helped in improving their English language skills tremendously and its influence would be more in the coming years.

Keywords: English language skills, Language Learning,   Usage of the smartphone, Undergraduates.

1. INTRODUCTION

Today, the supremacy of smartphones is very much evident in every walks of human life, it includes communication, social life, business, education, and so on. According to the needs of the people, companies are producing the latest versions of Smartphones with modern technologies. Recent Statics was done by the "Backmycell" organization have proven that in the last 5 years 40% of the smartphone users increased globally and by 2025 the number of users may go up to 75% (Turner, 2020). Demand in the market shows with much evidence that the usage of smartphones is increasing day by day in the world. Educational institutions have introduced Smart-education using Smartphones in all the fields of education. In the present

scenario, It is significantly proven that the development of the Smart-education impacting globally in the entire field of education (Zane L. Berge, Lin Y. Muilenburg, 2014)

Smartphones are used to enhance language learning for many decades. Mobile language learning was first started in the year 1980 by Twarog and Pereszlenyi Pinter to take feedback and assistance for distant language learners and gradually increased its usage due to the advantages it offers (Green, Collier, & Evans, 2001). The impact of smartphones in English language learning has been proven in several research studies due to its abundant advantages. Thus, "Mobile-assisted Language Learning (MALL) was introduced in the English language teaching-learning process" (Hashim, Yunus, Embi, & Ozir, 2017). Anil (2018) mentioned that "using technology in the classroom may create a real world that will develop learners' real world skills".History has proven that from the past few decades learning English through smartphones is accelerating time to time.

Smartphones enable learners to practice languages anytime and everywhere. Accessibility to the internet is adding features to the learners in equipping language skills. Severely studies have approved that smartphones are a powerful tool to drill vocabulary and spellings. Pegrum M. (2014) study found that "This gives rise to repetitive drilling of vocabulary, spelling, grammar, and pronunciation, aiming at consolidation of foundational knowledge through flashcard exercises, quizzes, or simple games (p.89)". According to Aziz et al. (2018, p. 238) Students are not bored with learning new vocabulary. Smartphones facilitate personalized learning, students can listen to their preferable topics to improve listening skills and read online to develop reading comprehension. According to new research learners get motivated to study in formal settings as well as informal settings using Smartphones(Kacetl & Klímová, 2019, p. 179), This feches to learn languages more proficiently.

Research questions

Why is the demand for smartphone usage increasing day by day to learning the English language among Undergraduate students?

What are the language skills that are enhanced after using smartphones?

2. OBJECTIVE OF THE RESEARCH

To understand the increasing demand in the usage of Smartphones to Enhance the English Language Skills among undergraduates.

To study the enhancement of undergraduates in English Language Skills after using Smartphones.

Hypothesis

Increasing Demand in Usage of Smartphones among Undergraduates
to Enhance the English Language Skills

Aim of research

The research aims to study the increasing demand in the usage of Smartphones to Enhance the English Language Skills among Undergraduates of urban and rural areas of Telangana.

3. METHODOLOGY

As part of the research, 149 undergraduate students from urban and rural areas of Telangana state in India were taken as the sample for the research. 118 urban students and 31 rural students. In order to administer questionnaires among students, A questionnaire was prepared using Likert scale related to the usages of the Smartphone to enhance English language skills. Responses were gathered and analyzed systematically.

Findings

Based on the findings of this research it is clear that the usage of smartphones to enhance English language skills is growing at an extremely rapid rate. From Table-1,The finding of the questionnaire shows that the number of students using smartphones increased every year from

2016 to 2020. In the year 2020, 61 participants out of 149 gave the highest rate of using smartphones to improve the English language. Whereas only 12 participants in the year 2016, 13 participants in the year 2017,12 participants in the year 2018, and 21 participants in the year 2019 rated highest.

Table-1

How likely are you to rate your usage of Smartphone to learn English language in the fallowing years?(0- lowest; 5 -highest)
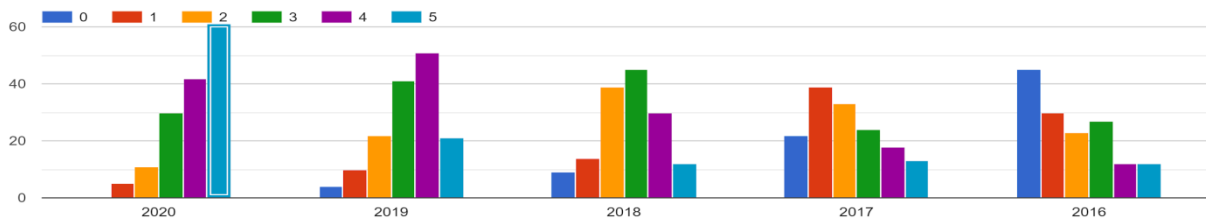
Findings of the questionnaire in Table-2 advocate that the more numbers of students use smartphones to develop English language skills in the coming 5 years. 66 participants out of 149 gave the highest rate, Which is calculated as 44% agreed that they use Smartphones to learn English language skills in the coming 5 years.

Table-2

How likely are you to rate your usage of Smartphone to learn English language in coming 5 years?(0- lowest ; 5- highest)

149 responses
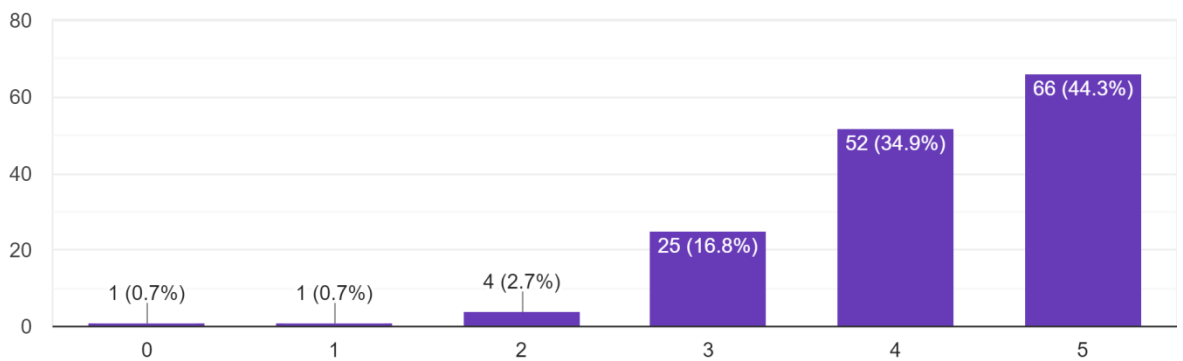
Results of the research in Table-3 propose that Smartphone use is more adequate in equipping English language skills, such as vocabulary, reading, communication skills, writing skills, listening skills, pronunciation, and grammar. 64% of the students greatly agreed that they enhance their vocabulary by using smartphones, 68% of the students greatly agreed that they enhance their reading skills by using smartphones, 62% of the students greatly agreed that they enhance their communication skills by using smartphones, 53% of the students greatly agreed that they enhance their writing skills by using smartphones, 69% of the students greatly agreed that they enhance their listening skills by using smartphones, 61% of the students greatly agreed that they enhance their pronunciation by using smartphones, 62% of the students greatly agreed that they enhance their grammar by using smartphones. Look at table 3, for a detailed description.

Table-3

Rank the following language skills that are enhanced after using smartphone?
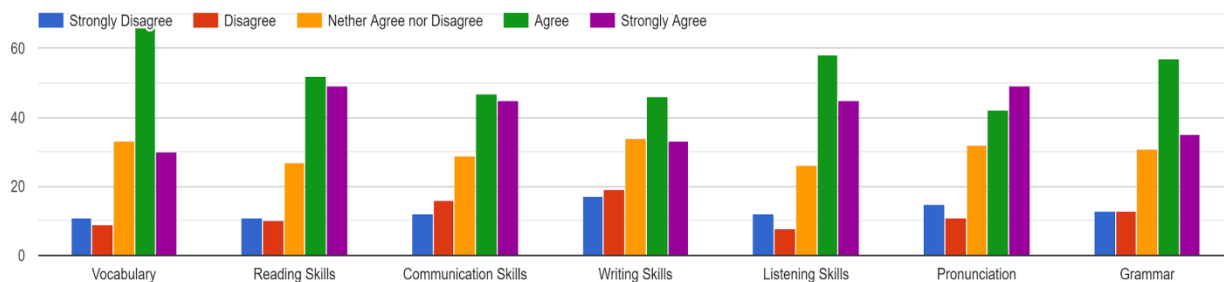
Discussions

Based on the analysis of the data it is proven that the demand for the usage of smartphones to enhance English language skills is increasing every year among undergraduate students. The study revealed that 61% in the year 2020 and 66% in the years 2021to 2025 students are likely to use Smartphones to learn English language skills. Godwin-Jones, R. (2017) research findings also says that technological innovations and new features in the latest smartphones foresee to use these Smartphones as more powerful devices in the teaching-learning process.

In addition to that, analysis of the data showed that 64 percent of all participants use smartphones for vocabulary, 68 percent for Reading skills, 62 percent for communication skills, 53 percent for writing skills, 69 percent for listening skills, 61 percent for pronunciation and 62 percent for grammar. Thus, Analysis of the data attested that the majority of undergraduates are using smartphones to improve their vocabulary, reading skills, communication skills, writing skills, listening skills, pronunciation, and grammar. Research findings of Chiu et al. (2015) also supported that the students learn vocabulary, pronunciation, listening skills, grammar, reading skills, and spelling and improved tremendously by using smartphones.

Recommendations

Findings of the research in increasing demand in the usage of smartphones among undergraduates recommend the following:

1. Use Smartphones for language learning purpose
2. Smartphones usage helps to Improve vocabulary, reading skills, communication skills, writing skills, listening skills, pronunciation, grammar, etc.
3. Smartphone helps to accelerate personalized language learning

Limitations

The limitations of this research follows as:

1. only a very few numbers of students participated in the research
2. The study conducted only from at learner point of view, not from experts from language teaching-learning fields
3. Research used only the quantitative method and the qualitative approach might give a better understanding of the usage of Smartphones to acquire English language skills.

4. Conclusions

As the findings of the research suggest that the usage of smartphones enhanced English language skills due to many advantages. Students can avail themselves of many learning opportunities everywhere and anytime because of their portability and internet connectivity. Finally, it is considered that the usage of smartphones enhances English language skills, such as listening, reading, writing, grammar, vocabulary, pronunciation, communication, etc. And for the past 5 years, the usage of smartphones among undergraduates has helped in improving their English language skills tremendously and its influence would be more in the coming years.

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Effect of magnetic field, heat generation and absorption on nanofluid flow over a nonlinear stretching sheet

Santoshi Misra[*1] and Govardhan Kamatam[2]

Abstract

The study of magnetohydrodynamic flow of a nanoparticle suspension under the influence of varied dimensionless parameters has been the focus of research in contemporary times. This work models the effect of magnetic field, heat generation and absorption parameter in a steady, laminar, two-dimensional boundary layer flow of a nanofluid over a permeable stretching sheet at a given surface temperature and partial slip. The highly nonlinear governing equations are solved numerically using similarity transformations with suitable boundary conditions and converted to ordinary differential equations. A computational model is setup using FORTRAN, where a relevant Adam's predictor–corrector method is employed to solve the equations. The impact of the dimensionless parameters, including the Brownian motion, thermophoresis, magnetic field, heat generation and absorption parameters, on the velocity, temperature and nanoparticle concentration of fluid flow are analysed systematically.

Introduction

The study of magnetohydrodynamic problems, such as nanofluid flow over a permeable stretching sheet, has recently become relevant due to potential applications in various fields of science, such as metallurgy and chemical engineering processes with industrial applications which include glass fibre, paper production, hot rolling, metal spinning, wire drawing, etc. Research involving two dimensional boundary layer laminar flow was initiated by Sakiadis [1], who later on extended these studies in order to include continuous surfaces and the boundary layer behaviour [2]. This research was continued by Crane

[3], who considered the Navier–Stokes equations involving the conservation of mass, momentum, energy and concentration for the boundary layer flow. Other groups, such as Chamka et al. [4], studied the fluid flow using a semi-infinite flat surface with the heat generation and absorption coefficient. Anderson [5] conducted experiments on fluid flow using the finite difference method which seemed amenable to provide accurate results. The problem involving laminar flow due to stretching of the sheet in nanofluids was investigated by Khan and Pop [6] which gained enormous popularity among researchers.

The study of nanofluids (i.e., a fluid containing particles smaller than 100 nm in at least one dimension) is important mainly due to the fact that they can be used to enhance the thermal conductivity and convective heat transfer performance of base fluids such as water, ethylene, glycol, etc. This takes place due to the intense and rigorous distribution of nanoparticle Brownian motion within the base fluid, thus enhancing the uniformity, conductance and properties which have paved the way for substantial research in this field.

Sheet stretching involves analysis using both linear and nonlinear equations (e.g., the polymer extrusion process) as reflected in the numerical study by Rana and Bhargava [7]. This study was extended by Das [8] and Hayat et al. [9], who used partial slip conditions for the boundary layer flow to investigate the velocity, temperature and concentration changes with regard to various dimensionless parameters in the fluid flow under the influence of a magnetic field. Besthapu and Bandari [10] have analysed the heat and mass transfer rates using Casson nanofluids with nonlinear equations. Applications in nuclear waste storage were initiated by Gaffar et al. [11], who focussed on viscoelastic Jeffrey fluid in a porous medium considering the effect of various dimensionless parameters that influence the heat and mass transfer flow. Dogonchi and Ganji [12] examined the velocity and temperature of nanofluids under the thermal radiation effect using Brownian motion. Viscous fluid flow melting following plate thickness variation was systematically investigated by Farooq et al. [13]. Qayyum et al. [14] considered external factors acting upon the fluid focussing on the influence of heat generation/absorption phenomena in the flow problem. Sreekala et al. [15], Rashid et al. [16] and Ahmad et al. [17] included external forces acting on the fluid flow, emphasizing their tremendous effect on the velocity, temperature and concentration profiles with fluid heat and mass transfer. Seth et al. [18] and Soomro et al. [19,20] have extended nanofluid research by considering the effects of various dimensionless parameters on the nanoparticle flow when suspended in different nanofluids. Farooq et al. [21], Irfan et al. [22] and Pal et al. [23] have supplemented the investigation by extending it to the three dimensional flow of different nanofluids which were used to obtain the dimensionless velocity, temperature and concentration profiles under the influence of external fields. In order to study nanofluid heat and mass transfer flow, Shah et al. [24] and Yousif et al. [25] developed mathematical models to obtain the numerical solution of basic single-variable governing equations.

Keeping in mind the prior investigations involving steady boundary layer flow and heat transfer of a nanofluid through a permeable stretching surface in the presence of partial slip, the present research incorporates the magnetic field effect and heat generation/absorption coefficient into the velocity, temperature and concentration profiles. Given that the end product is proportional to the heat transfer rate, the most frequent topic of boundary layer flow is the heat exchange phenomenon which has applicability to real life problems. Here, the elucidation of extrinsic forces acting on the flow with regard to partial differential equations has been reduced to ordinary differential equations with distinct boundary conditions, and the results were interpreted graphically and are corroborated by the literature.

Methods
Problem motivation, governing equations and problem solution
A two-dimensional, laminar, nonlinear steady-state boundary layer flow of a nanofluid across a permeable stretching surface was considered for study, as shown in Figure 1.
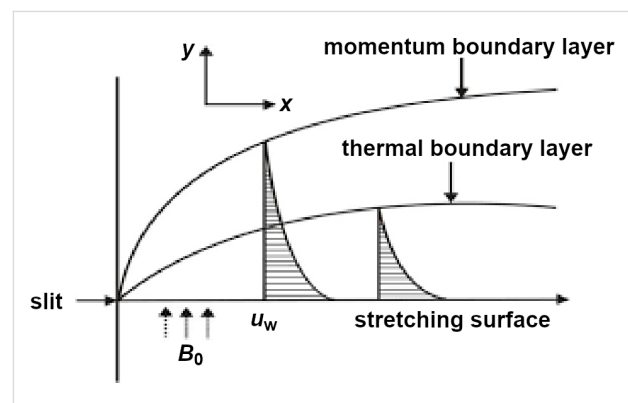
Figure 1: Two-dimensional coordinate system and fluid flow mechanism of a nanoparticle suspension.

Figure 1 describes the stretching surface of a sheet taken along the x and y axes, normal to the surface. Fluid flow occurs when $y \geq 0$, which is triggered when the sheet is stretched out of the slit occurring at the origin (when $x = y = 0$). The flow speed at any arbitrary point on the plate is assumed to be proportional to the power of its distance from the slit, considering boundary layer flow approximations at a distance of x units from the farthest edge. Considering a fixed origin, the sheet velocity is represented by $u_{\mathrm{w}}(x) = ax^n$, where n is the stretching parameter, a is the constant and x is the coordinate along the stretching surface.

The basic nanofluid governing equations in Cartesian form are given in the following where T_{w} and C_{w} represent the sheet temperature and the sheet nanoparticle fraction, respectively. The pressure and external forces are neglected and the nanoparticle fraction is assumed to be constant across the stretching surface. T_∞ and C_∞ denote ambient temperature and the associated

nanoparticle fraction where $T_w > T_\infty$ and $C_w > C_\infty$. This physical model has significant applications in modern nanotechnology and thermal manufacturing in various industries.

Conservation of mass equation

$$\frac{\mathrm{d}u}{\mathrm{d}x} + \frac{\mathrm{d}v}{\mathrm{d}y} = 0 \tag{1}$$

Conservation of momentum equation (velocity)

$$u\frac{\mathrm{d}u}{\mathrm{d}x} + v\frac{\mathrm{d}v}{\mathrm{d}y} = v\frac{\mathrm{d}^2u}{\mathrm{d}y^2} - \frac{\sigma B_0^2}{\rho_f}u \tag{2}$$

Conservation of thermal energy equation (temperature)

$$u\frac{\partial T}{\partial x} + v\frac{\partial T}{\partial y} = \alpha_m\nabla^2 T + \tau\left[D_B\frac{\partial C}{\partial y}\frac{\partial T}{\partial y} + \left(\frac{D_T}{T_\infty}\right)\left(\frac{\partial T}{\partial y}\right)^2\right] + \frac{1}{\rho c_p}Q_0(T - T_\infty) \tag{3}$$

Conservation of nanoparticle equation (concentration)

$$u\frac{\partial C}{\partial x} + v\frac{\partial C}{\partial y} = D_B\frac{\partial^2 C}{\partial y^2} + \left(\frac{D_T}{T_\infty}\right)\frac{\partial^2 T}{\partial y^2} \tag{4}$$

which is subjected to the following boundary conditions

$$\text{At } y = 0: \ u = u_w + u_s, \ v = \pm v_w, \ T = T_w, \ C = C_w \tag{5}$$

$$y \to \infty: \ u \to 0, \ T \to T_\infty, \ C \to C_\infty \tag{6}$$

and

$$\alpha_m = \frac{K_m}{(\rho c)_f}, \ \tau = \frac{(\rho c)_p}{(\rho c)_f} \tag{7}$$

α_m denotes the thermal diffusivity, and τ denotes the ratio between effective heat capacities of the nanoparticle material and the base fluid. In Equations 1–4, u, and v denote the velocity components along x and y axes, respectively, ρ_f is the base fluid density, a is a positive constant, D_B is the Brownian diffusion coefficient, D_T denotes the thermodiffusion coefficient, c is the

volumetric expansion coefficient, ρ_p is the particle density, σ denotes the nanofluid electrical conductivity, B_0 denotes the magnetic induction, v_w denotes the suction/injection velocity and Q_0 (Q) denotes the heat generation (absorption) coefficient.

u_s in Equation 8 represents the slip velocity, given as

$$u_s = l\frac{\partial u}{\partial y} \ \text{at } y = 0 \tag{8}$$

which is proportional to the local sheet stress, and l is the slip length constant.

The similarity transformations

The similarity transformations to solve the governing equations are as follows:

$$\eta = y\sqrt{\frac{a(n+1)}{2v}}x^{\frac{n-1}{2}}, \ u = ax^n f'(\eta),$$

$$v = -\sqrt{\frac{av(n+1)}{2}}x^{\frac{n-1}{2}}\left[f(\eta) + \left(\frac{n-1}{n+1}\right)\eta f'(\eta)\right], \tag{9}$$

$$\theta(\eta) = \frac{T - T_\infty}{T_w - T_\infty}, \ \phi(\eta) = \frac{C - C_\infty}{C_w - C_\infty}$$

By substituting the similarity transformations in Equation 9 into the governing boundary layer Equations 1–4 they reduce to ordinary differential equations:

$$f''' + ff'' - \left[\frac{2n}{n+1}\right]f'^2 - Mf' = 0 \tag{10}$$

$$\frac{1}{\Pr}\theta'' + f\theta' + Nb\theta'\phi' + Nt(\theta')^2 + Q\theta = 0 \tag{11}$$

$$\phi'' + Lef\phi' + \frac{Nt}{Nb}\theta'' = 0 \tag{12}$$

The transformed boundary conditions of Equation 5 and Equation 6, with regard to the similarity transformations in Equation 9, are:

$$\text{At } \eta = 0: \ f = F_w, \ f' = 1 + \xi_p f'', \ \theta = 1, \ f = 1 \tag{13}$$

$$\eta \to \infty: \ f' \to 0, \ \theta \to 0, \ \phi \to 0 \tag{14}$$

The working rules for the boundary conditions are:

At $y = 0$: $v = \pm v_w$, where

$$v = = -\sqrt{\frac{av(n+1)}{2}}x^{\frac{n-1}{2}}\left[f(\eta) + \left(\frac{n-1}{n+1}\right)\eta f'(\eta)\right]$$

$$= \pm v_w - \left[f(\eta) + \left(\frac{n-1}{n+1}\right)\eta f'(\eta)\right] = \frac{\pm v_w}{\sqrt{\frac{av(n+1)}{2}}x^{\frac{n-1}{2}}}$$

at $\eta = 0$:

$$-f(\eta) + 0 = \frac{v_w}{\sqrt{\frac{av(n+1)}{2}}x^{\frac{n-1}{2}}}$$

$$f(\eta) = \frac{-v_w}{\sqrt{\frac{av(n+1)}{2}}x^{\frac{n-1}{2}}}$$

Therefore, $f(\eta) = F_w$ where

$$F_w = \frac{-v_w}{\sqrt{\frac{av(n+1)}{2}}x^{\frac{n-1}{2}}}$$

at $y = 0$: $u = u_w + u_s$ where $u_w = ax^n$, $u_s = l\frac{\partial u}{\partial y}$, $u = ax^n f'(\eta)$ where

$$\eta = y\sqrt{\frac{a(n+1)}{2v}}x^{\frac{n-1}{2}}, \eta' = \frac{\partial \eta}{\partial y} = \sqrt{\frac{a(n+1)}{2v}}x^{\frac{n-1}{2}}$$

substituting the above values into $u = u_w + u_s$ we get:

$$ax^n f'(\eta) = ax^n + l\frac{\partial u}{\partial y} \text{ where } \frac{\partial u}{\partial y} = ax^n f''(\eta)\eta'$$

$$ax^n f'(\eta) = ax^n + l\left[ax^n f''(\eta)\sqrt{\frac{a(n+1)}{2v}}x^{\frac{n-1}{2}}\right]$$

$$f'(\eta) = 1 + l\sqrt{\frac{a(n+1)}{2v}}x^{\frac{n-1}{2}}f''(\eta)$$

$$f'(\eta) = 1 + \xi_p f''(\eta) \text{ where } \xi_p = l\sqrt{\frac{a(n+1)}{2v}}x^{\frac{n-1}{2}}$$

at $y = 0$: when $T = T_w$, $\theta(\eta) = \frac{T_w - T_\infty}{T_w - T_\infty} = 1$

and when $C = C_w$, $\phi(\eta) = \frac{C_w - C_\infty}{C_w - C_\infty} = 1$.

Therefore, at $\eta = 0$: $f = F_w, f' = 1 + \xi_p f''$, $\theta = 1$, $\phi = 1$ when $y \to \infty$: $u \to 0$, $T \to T_\infty$, $C \to C_\infty$ henceforth as $u \to 0$, $f'(\eta) \to 0$, as $T \to T_\infty$, $\theta(\eta) \to 0$, as $C \to C_\infty$, $\phi(\eta) \to 0$

Therefore, at $\eta \to \infty$: $f' \to 0, \theta \to 0, \phi \to 0$

The prime (´) denotes differentiation with respect to η, and the physical parameters involved in the equations are defined as follows:

$$\Pr = \frac{v}{\alpha_m} \text{ Prandtl number, } \text{Le} = \frac{v}{D_B} \text{ Lewis number,}$$

$$\text{Nb} = \frac{(\rho c)_p D_B (C_w - C_\infty)}{(\rho c)_f v} \text{ Brownian motion parameter,}$$

$$\text{Nt} = \frac{(\rho c)_p D_T (T_w - T_\infty)}{(\rho c)_f v T_\infty} \text{ Thermophoresis parameter,}$$

$$F_w = \frac{-v_w}{\sqrt{\frac{av(n+1)}{2}}x^{\frac{n-1}{2}}} \text{ Suction/injection parameter,} \qquad (15)$$

$$\xi_p = l\sqrt{\frac{a(n+1)}{2}}x^{\frac{n-1}{2}} \text{ Slip parameter for liquids,}$$

$$M = \frac{\sigma B_0^2}{\rho_f}\frac{2}{a(n+1)x^{n-1}} \text{ Magnetic parameter,}$$

$$Q = \frac{2Q_0}{\rho c_p a(n+1)x^{n-1}} \text{ Heat generation/absorption coefficient}$$

The abbreviations used for the calculations are summarized in Table 1.

Solving a system of first-order differential equations

The nonlinear and coupled partial differential equations, represented by Equations 10–12, are solved by using the Adam's predictor–corrector method which is the most efficient technique in numerical analysis used to solve distinctive problems related to heat transfer, fluid mechanics, and electrical systems. The first step involves reducing the nonlinear differential equations of third order in f and second order in θ and ϕ to a system of first-order differential equations, in order to simplify the problem as follows.

$$f''' = -ff'' + \frac{2n}{n+1}f'^2 + Mf' \qquad (16)$$

$$\theta'' = -\Pr\left[f\theta' + \text{Nb}\theta'\phi' + \text{Nt}(\theta)^2 + Q\theta\right] \qquad (17)$$

$$\phi'' = -\text{Le}f\phi' - \frac{\text{Nt}}{\text{Nb}}\theta'' \qquad (18)$$

Table 1: Nomenclature for the basic nanofluid governing equations.

Roman		Greek symbols	
a	constant	α_m	thermal diffusivity
n	nonlinear stretching parameter	ξ_p	slip parameter for liquids
C	nanoparticle volume fraction	υ	kinematic viscosity of the fluid
C_w	nanoparticle volume fraction at the sheet	τ	torsion parameter defined by $\frac{\rho c_p}{\rho c_f}$
C_∞	ambient nanoparticle volume fraction	ρc_p	effective heat capacity of nanoparticles
Nt	thermophoresis parameter	ρc_f	heat capacity of the fluid
Nb	Brownian motion parameter	η	similarity V
(x,y)	Cartesian coordinates	$\phi(\eta)$	rescaled nanoparticle volume fraction
T_w	temperature at the sheet	$\theta(\eta)$	dimensionless temperature
T_∞	ambient temperature	ρ_f	base fluid density
T	temperature	ρ_p	nanoparticle mass density
u_s	slip velocity		
v_w	suction/injection		
D_B	Brownian diffusion coefficient		
D_T	thermodiffusion coefficient		
u_w	velocity of the stretching sheet		
M	magnetic field parameter		
Q_0/Q	heat generation/absorption coefficient		
Le	Lewis number		
F_w	suction/injection parameter		
u, v	velocity components along x, y axes		
σ	nanofluid electrical conductivity		
B_0	magnetic induction		
l	slip length constant		
Pr	Prandtl number		

These equations were solved independently using a FORTRAN program which converts each one of them using different variables to first order as follows.

Equation 16 is represented as a system of equations for f as:

$$f = y_1,\ f' = y_2,\ f'' = y_3$$

$$y_1' = y_2,\ y_2' = y_3,\ y_3' = -y_1 y_3 + \frac{2n}{n+1} y_2^2 + M y_2$$

The boundary conditions with respect to η for f are:

$$\text{at } \eta = 0:\ y_1(0) = F_w,\ y_2(0) = 1 + \xi_p y_3(0)$$

$$\text{at } \eta \to \infty:\ y_2(\infty) \to 0$$

We assume an initial guess value as α for y_3, i.e., $y_3(0) = \alpha$. Hence, $y_1(0) = F_w$, $y_2(0) = 1 + \xi_p \alpha$, $y_3(0) = \alpha$ where α needs to be obtained by using Newton's method to satisfy $y_2(\eta_\infty) \to 0$.

Equation 17 and Equation 18 are represented as follows by defining the system of equations for θ and ϕ:

$$y_1 = \theta,\ y_2 = \theta',\ y_2' = -\Pr\left[f y_2 + \text{Nb} y_2 y_4 + \text{Nt} y_2^2 + Q y_1 \right]$$

$$y_3 = \phi,\ y_4 = \phi',\ y_4' = -\text{Le}\, y_4 - \frac{\text{Nt}}{\text{Nb}} y_2'$$

The boundary conditions with respect to η for θ and ϕ are as follows:

$$\text{at } \eta = 0:\ y_1(0) = 1,\ y_3(0) = 1$$

$$\text{at } \eta \to \infty:\ y_1(\infty) \to 0,\ y_3(\infty) \to 0$$

The values of α and β, which are the unknowns with respect to θ and ϕ, are obtained by starting with an initial guess and then correcting the values using Newton's method to satisfy the end conditions as follows:

$$\begin{pmatrix} \alpha \\ \beta \end{pmatrix}^{\text{New}} = \begin{pmatrix} \alpha \\ \beta \end{pmatrix}^{\text{Old}} - \begin{bmatrix} \dfrac{\partial y_1}{\partial \alpha} & \dfrac{\partial y_1}{\partial \beta} \\[2mm] \dfrac{\partial y_3}{\partial \alpha} & \dfrac{\partial y_3}{\partial \beta} \end{bmatrix}^{-1}_{\eta = \infty} \begin{pmatrix} y_{2,N4} \\ y_{4,N4} \end{pmatrix}$$

The boundary value problem is converted to an initial value problem by reducing the equations, as shown above. The initial value problem is solved by using Adam's predictor–corrector method and assigning an approximate initial value with step size $h = 0.01$ and $\eta = 4$ at the maximum value. The solution is obtained for different parameters used and convergence is achieved with a change in a single parameter. The criteria for convergence are the approximation up to three significant digits. The results derived through computation are plotted as graphs for a clear picture of the numerical problem (Table 2, Table 3).

Table 2: Computation of the streamwise velocity, temperature and concentration profiles for different values of the magnetic field parameter, *M*.

η	*M* = 0.5			*M* = 1.0			*M* = 2.0			*M* = 3.0		
	$f'(\eta)$	$\theta(\eta)$	$\phi(\eta)$	$f'(\eta)$	$\theta(\eta)$	$\phi(\eta)$	$f'(\eta)$	$\theta(\eta)$	$\phi(\eta)$	$f'(\eta)$	$\theta(\eta)$	$\phi(\eta)$
0	0.468	1	1	0.428	1	1	0.375	1	1	0.339	1	1
0.2	0.373	0.935	0.714	0.328	0.94	0.726	0.268	0.946	0.74	0.23	0.949	0.749
0.4	0.298	0.855	0.51	0.252	0.866	0.528	0.193	0.881	0.549	0.156	0.889	0.563
0.6	0.238	0.766	0.374	0.193	0.784	0.392	0.138	0.808	0.415	0.106	0.823	0.429
0.8	0.19	0.672	0.286	0.149	0.699	0.302	0.099	0.732	0.323	0.072	0.753	0.335
1.0	0.152	0.581	0.229	0.114	0.614	0.242	0.071	0.656	0.259	0.049	0.681	0.269
1.2	0.122	0.494	0.189	0.088	0.532	0.2	0.051	0.581	0.214	0.033	0.611	0.221
1.4	0.098	0.414	0.159	0.068	0.456	0.17	0.037	0.51	0.18	0.023	0.543	0.186
1.6	0.078	0.342	0.136	0.052	0.386	0.145	0.027	0.443	0.155	0.015	0.478	0.159
1.8	0.062	0.279	0.115	0.04	0.323	0.125	0.019	0.38	0.134	0.01	0.417	0.137
2.0	0.049	0.225	0.097	0.031	0.267	0.107	0.014	0.323	0.115	0.007	0.359	0.119
2.2	0.039	0.179	0.08	0.023	0.218	0.09	0.01	0.272	0.099	0.005	0.306	0.102
2.4	0.031	0.14	0.066	0.018	0.175	0.075	0.007	0.225	0.084	0.003	0.257	0.087
2.6	0.024	0.107	0.053	0.014	0.138	0.062	0.005	0.183	0.071	0.002	0.213	0.074
2.8	0.018	0.081	0.041	0.01	0.107	0.049	0.004	0.146	0.058	0.001	0.172	0.061
3.0	0.014	0.059	0.031	0.007	0.08	0.038	0.003	0.113	0.046	0.001	0.135	0.049
3.2	0.01	0.042	0.022	0.005	0.058	0.028	0.002	0.084	0.035	0.001	0.102	0.038
3.4	0.007	0.027	0.015	0.004	0.039	0.02	0.001	0.058	0.025	0	0.072	0.028
3.6	0.004	0.016	0.009	0.002	0.024	0.012	0.001	0.036	0.016	0	0.045	0.018
3.8	0.002	0.007	0.004	0.001	0.011	0.006	0	0.017	0.008	0	0.021	0.009
4.0	0	0	0	0	0	0	0	0	0	0	0	0

Table 3: Computation of streamwise velocity, temperature and concentration profiles for different values of the heat generation/absorption coefficient, *Q*.

η	*Q* = 0.0			*Q* = 0.2			*Q* = 0.3		
	$f'(\eta)$	$\theta(\eta)$	$\phi(\eta)$	$f'(\eta)$	$\theta(\eta)$	$\phi(\eta)$	$f'(\eta)$	$\theta(\eta)$	$\phi(\eta)$
0	0.524	1	1	0.524	1	1	0.524	1	1
0.2	0.437	0.928	0.697	0.437	0.95	0.682	0.437	1.028	0.627
0.4	0.365	0.839	0.485	0.365	0.88	0.462	0.365	1.035	0.367
0.6	0.306	0.738	0.349	0.306	0.794	0.322	0.306	1.018	0.208
0.8	0.256	0.634	0.263	0.256	0.701	0.238	0.256	0.977	0.124
1.0	0.214	0.533	0.209	0.214	0.605	0.189	0.214	0.918	0.088
1.2	0.179	0.439	0.172	0.179	0.513	0.158	0.179	0.845	0.076
1.4	0.149	0.355	0.143	0.149	0.427	0.135	0.149	0.764	0.076
1.6	0.124	0.283	0.119	0.124	0.35	0.117	0.124	0.678	0.079
1.8	0.103	0.221	0.099	0.103	0.281	0.1	0.103	0.591	0.082
2.0	0.085	0.17	0.08	0.085	0.223	0.085	0.085	0.506	0.082
2.2	0.069	0.129	0.064	0.069	0.174	0.07	0.069	0.424	0.079

Table 3: Computation of streamwise velocity, temperature and concentration profiles for different values of the heat generation/absorption coefficient, *Q*. (continued)

2.4	0.056	0.096	0.05	0.056	0.133	0.057	0.056	0.348	0.074
2.6	0.045	0.071	0.038	0.045	0.1	0.045	0.045	0.278	0.067
2.8	0.035	0.051	0.028	0.035	0.073	0.035	0.035	0.216	0.058
3.0	0.027	0.036	0.021	0.027	0.052	0.026	0.027	0.162	0.048
3.2	0.02	0.024	0.014	0.02	0.036	0.018	0.02	0.115	0.037
3.4	0.014	0.015	0.009	0.014	0.023	0.012	0.014	0.076	0.027
3.6	0.008	0.009	0.005	0.008	0.013	0.007	0.008	0.045	0.017
3.8	0.004	0.004	0.002	0.004	0.006	0.003	0.004	0.019	0.008
4.0	0	0	0	0	0	0	0	0	0

Results and Discussion

To provide physical insight into the flow problem, numerical computations involving various parameters and their influence on the dimensionless velocity, temperature and nanoparticle concentration of fluid flow have been represented graphically as follows.

Impact of ξ on $f'(\eta)$

Figure 2 illustrates the influence of the slip parameter, ξ, on the velocity gradient which decreases with an increase in the value of ξ, converging to zero at the end of the boundary layer, thus causing a reduction in its thickness for nanofluids. This is due to the fact that an increase in the slip parameter causes a reduction in the skin friction at the surface acting between the stretching sheet and the fluid flow, thus drastically decreasing the velocity gradient.

Impact of ξ on $\theta(\eta)$

The temperature variation component, $\theta(\eta)$, increases with an increase in the slip parameter, ξ, which further leads to an increase in the fluid temperature, thus intensifying the thermal boundary layer thickness (Figure 3). An increase in the slip parameter causes friction at the surface which, in turn, generates a frictional force allowing more fluid to flow passed the stretching sheet, causing an increase in the temperature gradient and reducing the velocity of the fluid.

Impact of ξ on $\phi(\eta)$

The nanoparticle concentration distribution, $\phi(\eta)$, increases with an increase in the slip parameter, ξ, at a given constant surface temperature. An increase in the slip parameter causes friction at the surface which, in turn, generates a frictional force allowing more fluid to flow passed the stretching sheet. This causes an increase in concentration distribution of the fluid as shown in Figure 4, which ultimately reduces the fluid velocity.

Impact of *n* on $f'(\eta)$

The nonlinear stretching parameter, *n*, has a significant influence on the fluid flow velocity component, $f'(\eta)$. Figure 5 shows that with an increase in *n*, the velocity gradient
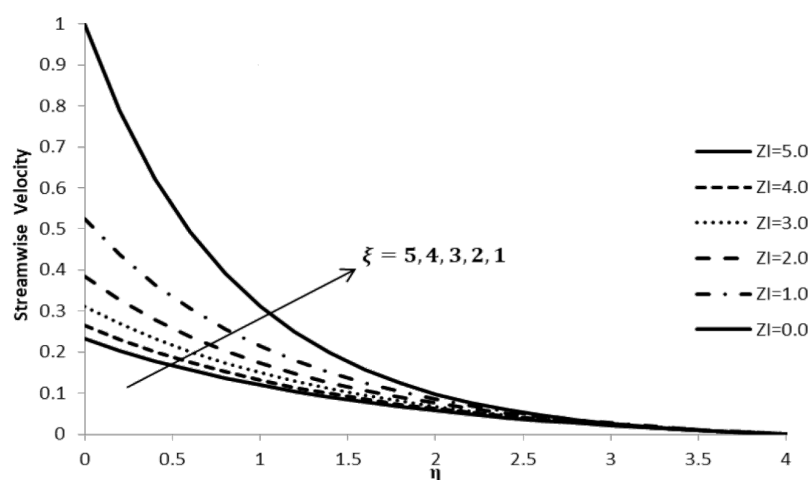
Figure 2: Influence of the slip parameter for liquids ξ on the streamwise velocity component $f'(\eta)$ when *n* = 2.0, *M* = 0.0, *Q* = 0.0, Pr = 2.0, Nb = Nt = 0.5, Le = 5.0, and F_w = 0.2.
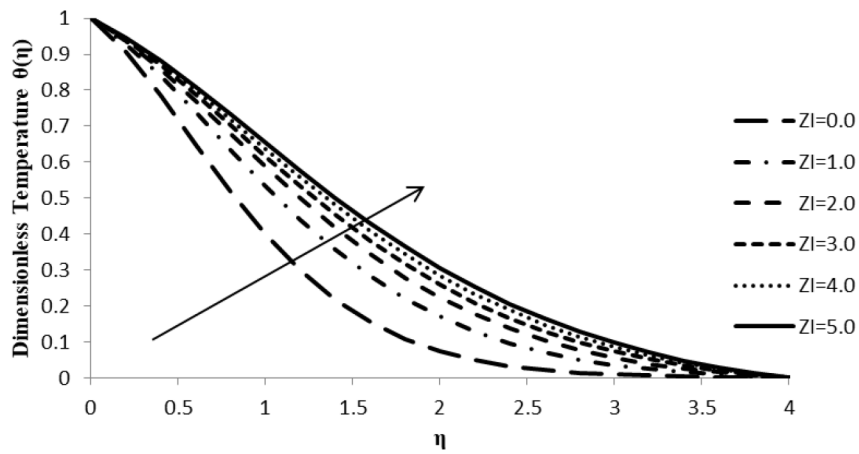
Figure 3: Influence of the slip parameter for liquids ξ on the temperature distribution θ(η) when $n = 2.0$, $M = 0.0$, $Q = 0.0$, $Pr = 2.0$, $Nb = Nt = 0.5$, $Le = 5.0$, and $F_w = 0.2$.
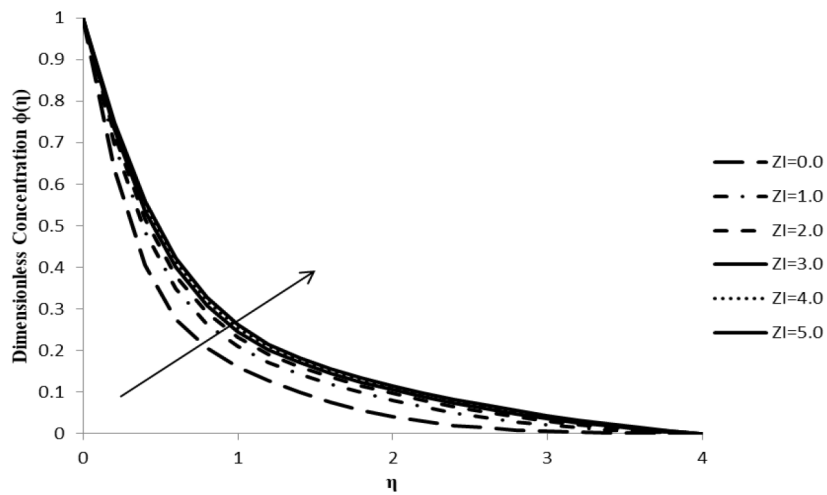
**Figure 4:** Influence of the slip parameter for liquids ξ on the concentration distribution φ(η) when $n = 2.0$, $M = 0.0$, $Q = 0.0$, $Pr = 2.0$, $Nb = Nt = 0.5$, $Le = 5.0$, and $F_w = 0.2$.
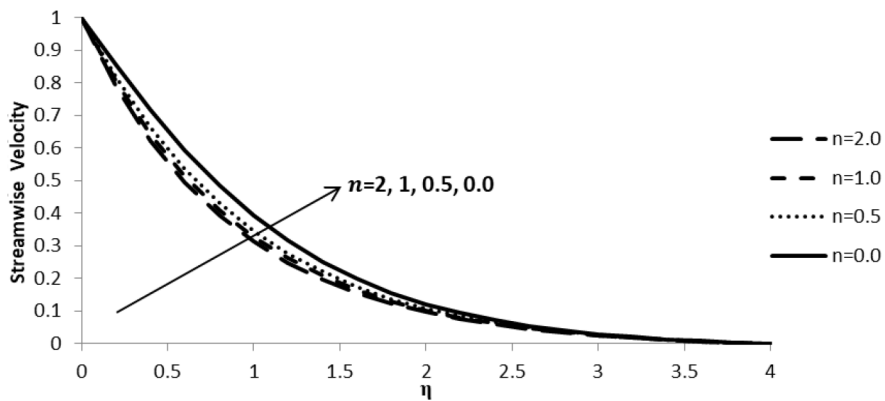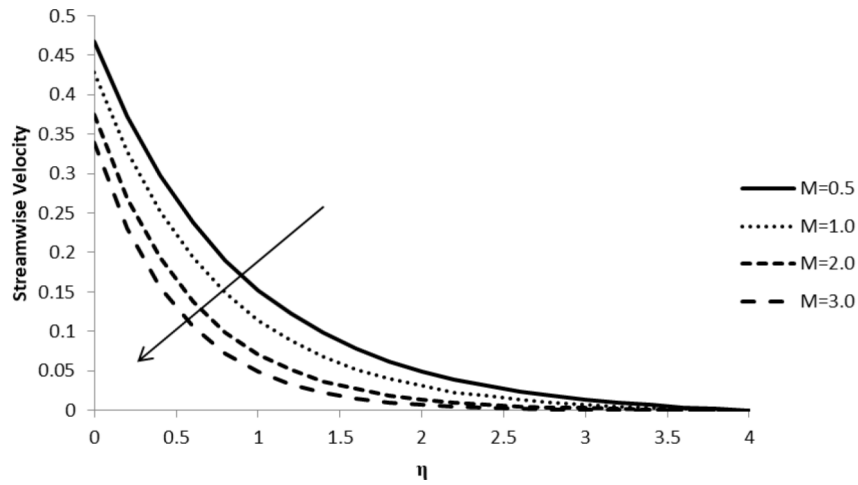

Figure 5: Influence of the stretching parameter n on the streamwise velocity component $f'(η)$ when $ξ = M = Q = 0.0$, $Pr = 2.0$, $Nb = Nt = 0.5$, $Le = 5.0$, and $F_w = 0.2$.

decreases, thus depleting the thickness of the momentum boundary layer. This is due to the fact that, with the sheet being stretched, there is a retarding force in the fluid in which the fluid particles have severe impact on the velocity component leading to its reduction as we move away from the boundary layer.

Impact of *n* on θ(η)

With an increase in the stretching parameter, *n*, the temperature gradient, θ(η), increases leading to an increase in the thermal boundary layer thickness. As the value of the stretching parameter increases, the convection process in the particles of the fluid flow intensifies, thus leading to a rapid increase in the temperature gradient which, in turn, is responsible for an increase in the thermal boundary layer thickness (Figure 6).

Impact of *n* on ϕ(η)

With an increase in the stretching parameter, *n*, the concentration profile of the fluid, ϕ(η), increases slightly but the effect observed is not significant, which is noticed at higher values of *n*. At lower values of the stretching parameter, there is a negligible change in the fluid concentration of the fluid, which is independent of stretch. On the other hand, there is a significant increase in the stretching parameter at extremely high values that does not seem to affect the fluid particle concentration (Figure 7).

Impact of Nb on θ(η)

The Brownian motion parameter, Nb, has a remarkable effect on the temperature gradient, θ(η). When Nb increases, it leads to an increase in θ(η) which causes an increase in the thermal

Figure 6: Influence of the stretching parameter *n* on the temperature distribution θ(η) when ξ = 1.0, *M* = *Q* = 0.0, Pr = 2.0, Nb = Nt = 0.5, Le = 5.0, and F_W = 0.2 (due to the boundary condition, the value of ξ is changed).

Figure 7: Influence of the stretching parameter *n* on the concentration distribution ϕ(η) when ξ = 1.0, *M* = *Q* = 0.0, Pr = 2.0, Nb = Nt = 0.5, Le = 5.0, and F_W = 0.2 (due to the boundary condition, the value of ξ is changed).
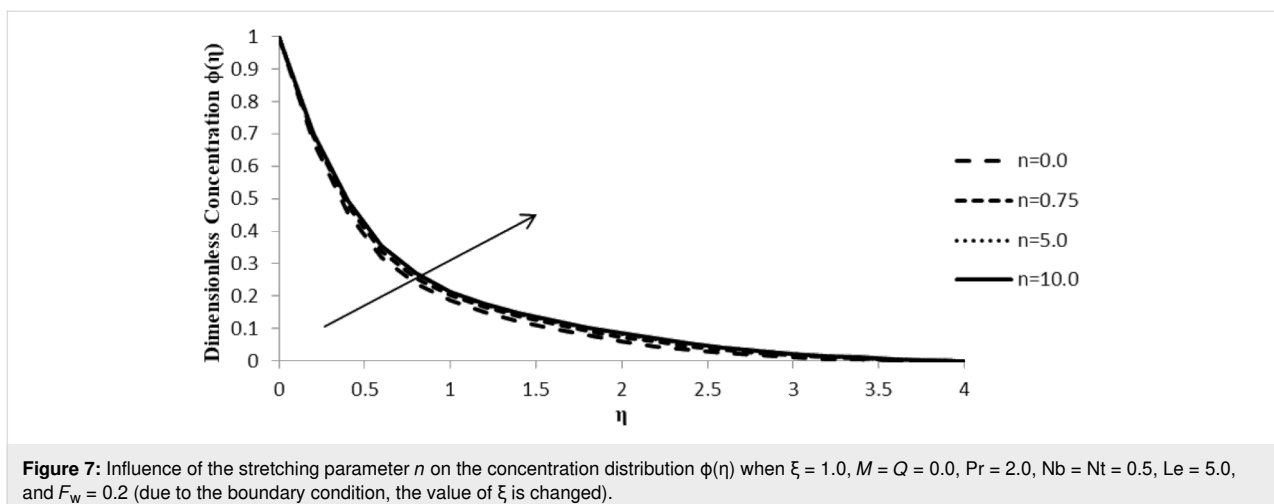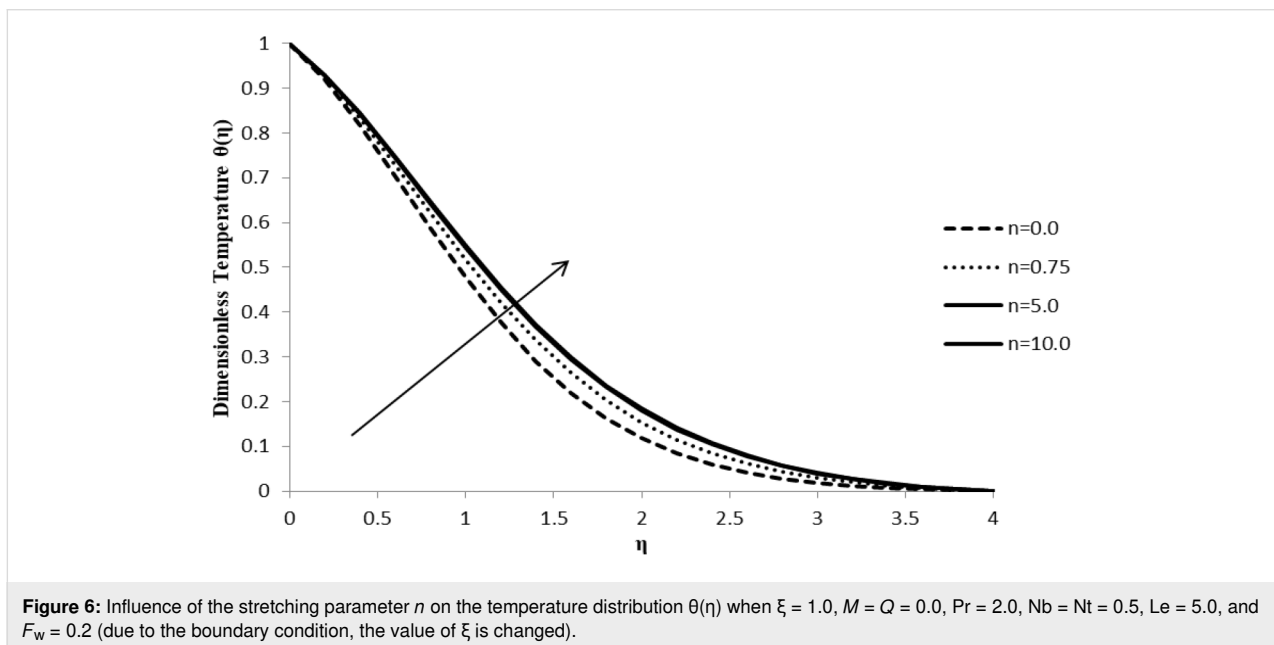
boundary layer thickness of the fluid flow. Higher Brownian motion is responsible for fast movement of fluid particles which, in turn, induces either an increase in acceleration or random acceleration levels resulting in additional energy among particles and, consequently, an increase in the temperature gradient and thermal boundary layer thickness (Figure 8).

Impact of Nb on φ(η)

An increase in the Brownian motion parameter, Nb, results in a decrease in the concentration gradient of the fluid, φ(η), due to the fact that the particles move from a high to a low concentration region. As the movement of the fluid particles intensifies

with an increase in the Brownian motion, the particles start moving rapidly from regions of higher to regions of lower concentration since the random acceleration decreases the concentration gradient of the fluid flow (Figure 9).

Impact of Nt on θ(η)

With an increase in the thermophoresis parameter, Nt, the temperature gradient of the fluid, θ(η), also increases leading to an enhancement in the thermal boundary layer thickness. Due to the presence of a temperature gradient, different particles of the fluid exhibit different responses to the change in the thermophoresis parameter. As Nt increases, the particles start
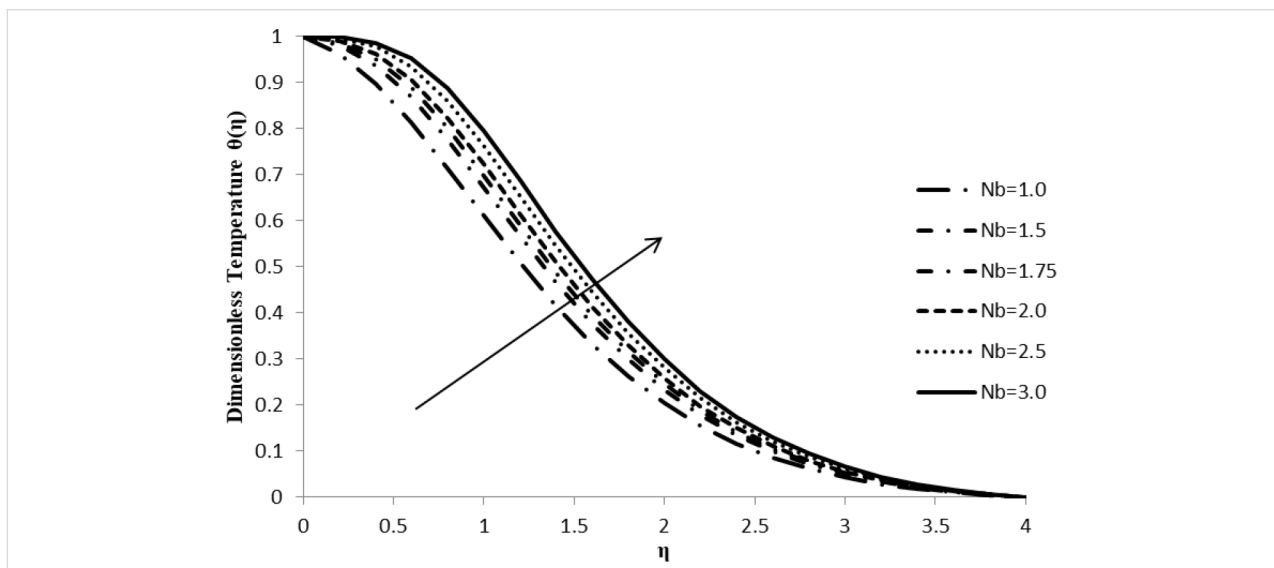
Figure 8: Influence of the Brownian motion parameter Nb on the temperature distribution, θ(η) when n = 2.0, ξ = 1.0, M = Q = 0.0, Pr = 2.0, Nt = 0.5, Le = 5.0, and F_w = 0.2.
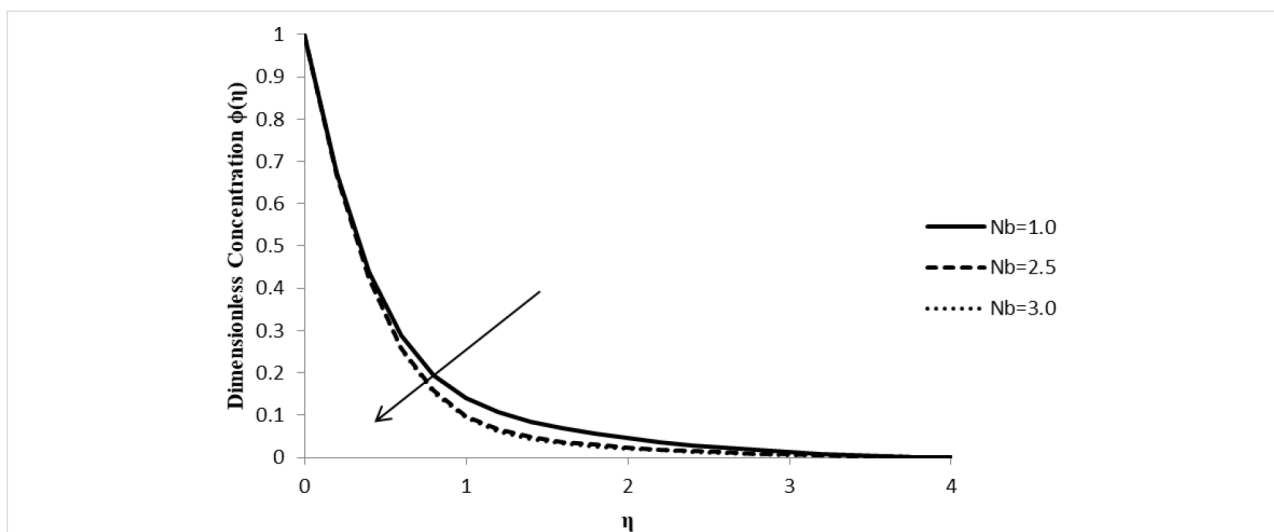
Figure 9: Influence of the Brownian motion parameter Nb on the concentration distribution φ(η) when n = 2.0, ξ = 1.0, M = Q = 0.0, Pr = 2.0, Nt = 0.5, Le = 5.0, and F_w = 0.2.

moving rapidly which causes an elevation in the kinetic energy of the system, resulting in an increase in the temperature distribution and in the boundary layer thickness (Figure 10).

Impact of Nt on ϕ(η)

A small increase in thermophoresis parameter, Nt, causes a massive increase in the concentration distribution of the fluid flow, ϕ(η), which converges to zero at the boundary layer. A small change in the thermophoresis parameter leads to rapid motion in the fluid particles creating excess heat energy and leading to a massive increase in the concentration distribution. Therefore, Figure 11 shows a significant increase in the concentration distribution with a very slight increase in the value of Nt.

Impact of *M* on *f*′(η)

An increase in the magnetic parameter, *M*, leads to a decrease in the streamwise velocity component, *f*′(η), which, in turn, reduces the velocity boundary layer thickness. The external magnetic field has a massive effect on the velocity profile of an electrically conducting fluid, which causes a considerable amount of resistance to its motion and a driving force, called the Lorentz force, which reduces the fluid velocity (Figure 12).

Impact of M on θ(η)

With an increase in the magnetic parameter, *M*, and, consequently, of the fluid resistance, the fluid temperature profile, θ(η), increases and so does the thickness of the thermal bound-
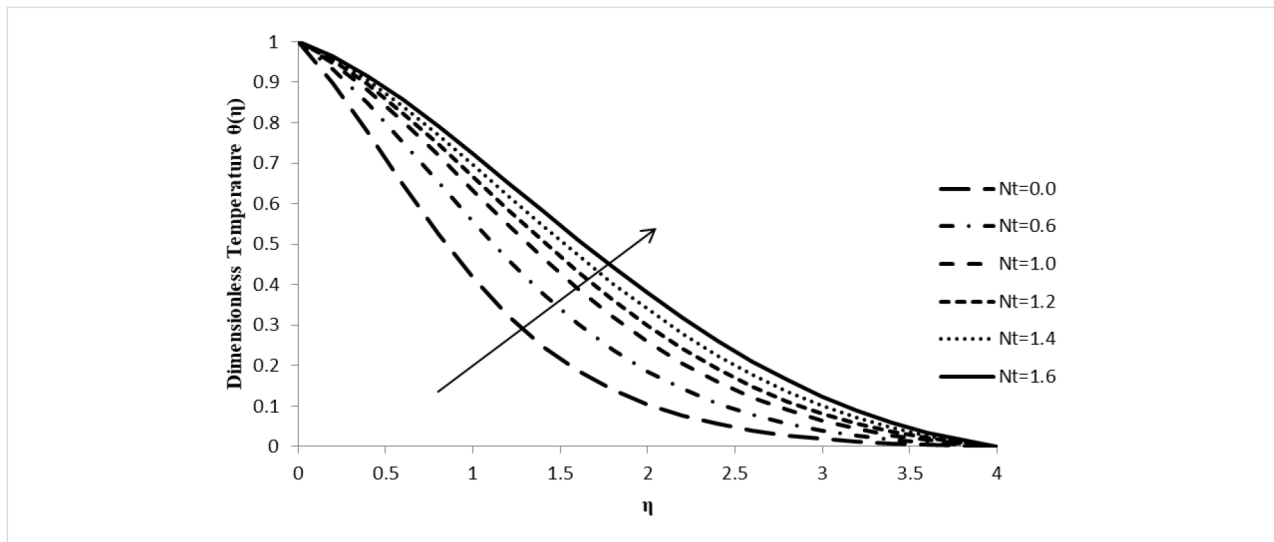
Figure 10: Influence of the thermophoresis parameter Nt on the temperature distribution θ(η) when *n* = 2.0, ξ = 1.0, *M* = *Q* = 0.0, Pr = 2.0, Nb = 0.5, Le = 5.0, and F_w = 0.2.
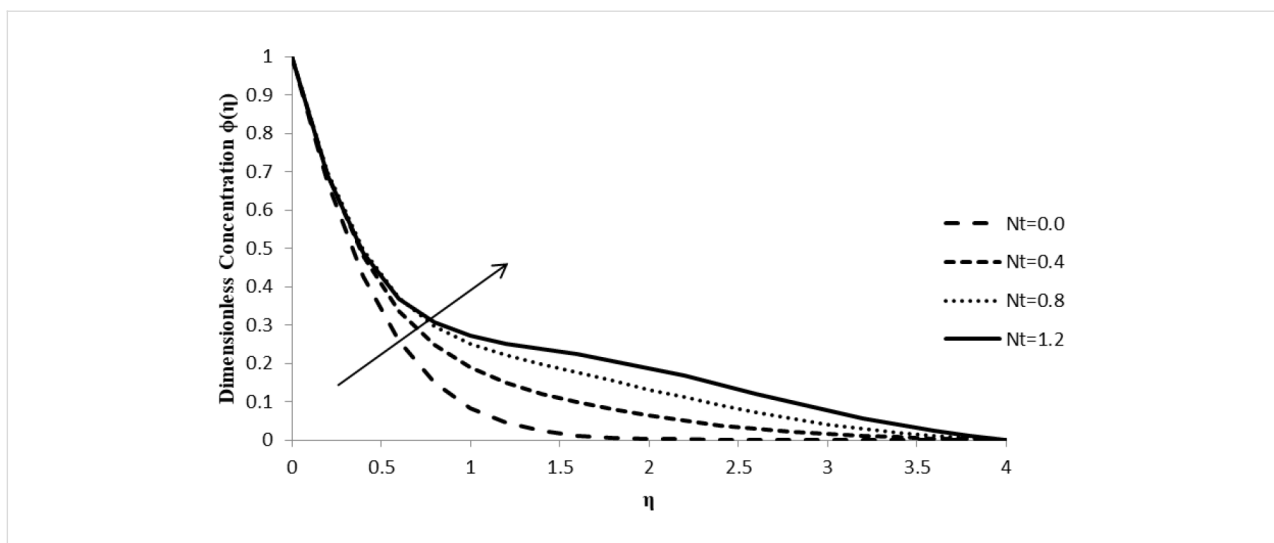
Figure 11: Influence of the thermophoresis parameter Nt on the concentration distribution ϕ(η) when *n* = 2.0, ξ = 1.0, *M* = *Q* = 0.0, Pr = 2.0, Nb = 0.5, Le = 5.0, and F_w = 0.2.

Figure 12: Influence of the magnetic parameter *M* on the streamwise velocity component $f'(\eta)$ when $n = 2.0$, Pr = 2.0, Nb = Nt = 0.5, $Q = 0.0$, Le = 5.0, $\xi = 1.0$, and $F_w = 0.2$.

ary layer. This is due to the fact that with an external magnetic field being employed, the temperature of the fluid increases, resulting in the rapid movement of the fluid particles which increases both the thermal energy and the boundary layer thickness and reduces the heat transfer from the sheet (Figure 13).

Impact of *M* on ϕ(η)

An increase in the magnetic parameter, *M*, does not have much influence on the concentration distribution, $\phi(\eta)$, which is depicted in Figure 14. Even though there is a minimal increasing trend observed, the flow seems invariable. Since the concentration gradient of the fluid flow is not significantly modified by the external magnetic field applied, there is not much of a change in particle motion upon an increase in *M* values (Figure 14).

Impact of *Q* on *f*′(η)

The effect of the heat generation/absorption coefficient, *Q*, on the streamwise velocity component, $f'(\eta)$, is negligible and, therefore, it does not affect the fluid flow velocity. The value of the heat generation/absorption coefficient being positive demonstrates that the heat generated does not have much impact on the velocity gradient of the fluid as the fluid particles move at the same velocity (Figure 15).

Impact of *Q* on θ(η)

The heat generation/absorption coefficient, *Q*, has a considerable effect on the dimensionless temperature profile of the fluid, $\theta(\eta)$, which increases with an increase in the value of *Q* as shown in Figure 16. An increase in the fluid temperature increases the thermal boundary layer thickness. The presence of
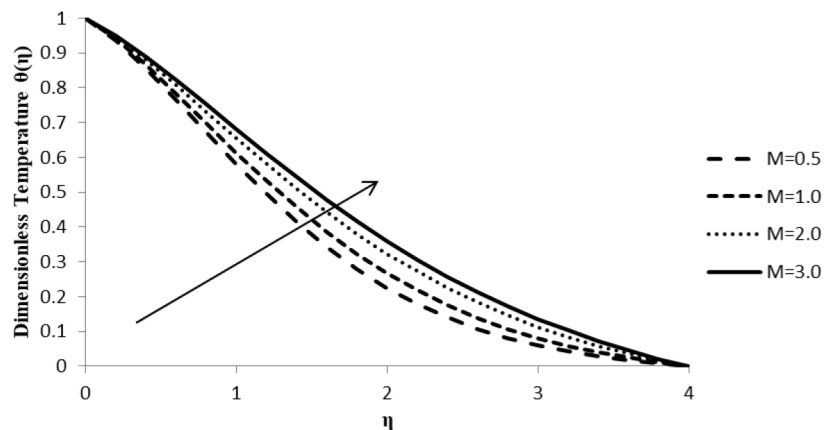
Figure 13: Influence of the magnetic parameter *M* on the temperature distribution θ(η) when $n = 2.0$, Pr = 2.0, Nb = Nt = 0.5, $Q = 0.0$, Le = 5.0, $\xi = 1.0$, and $F_w = 0.2$.
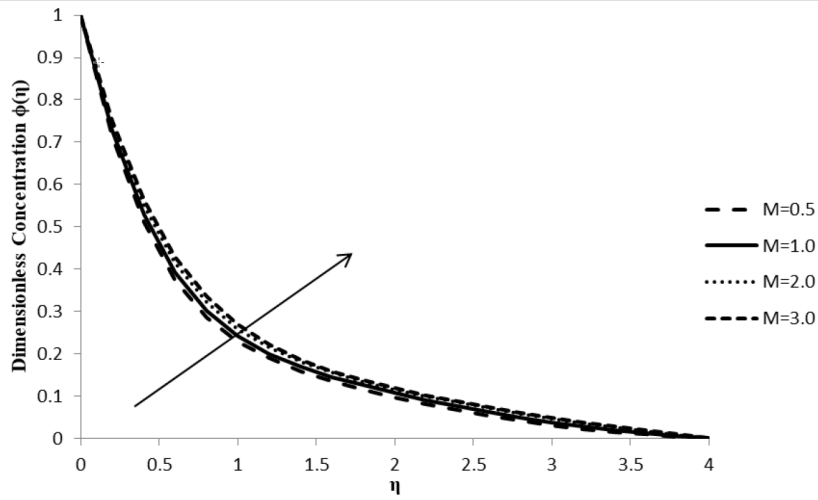
Figure 14: Influence of magnetic parameter *M* on the concentration distribution $\phi(\eta)$ when *n* = 2.0, Pr = 2.0, Nb = Nt = 0.5, *Q* = 0.0, Le = 5.0, ξ = 1.0, and F_w = 0.2.
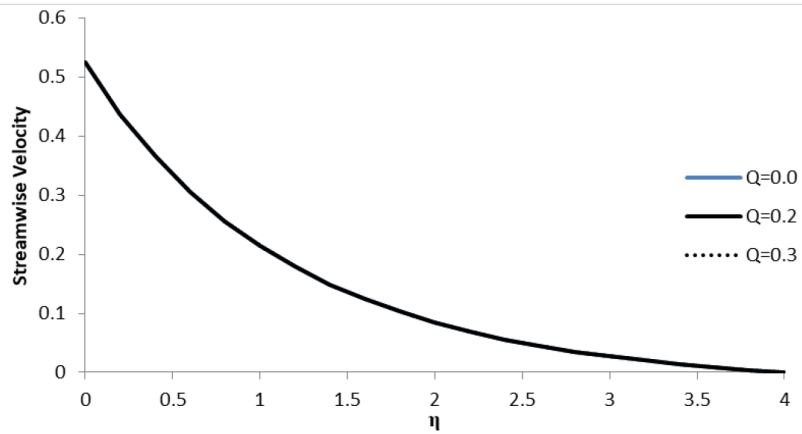
Figure 15: Influence of the heat generation/absorption coefficient *Q* on the streamwise velocity component *f*′(η) when *n* = 2.0, Pr = 2.0, Nb = Nt = 0.5, *M* = 0.0, Le = 5.0, F_w = 0.2, and ξ = 1.0.
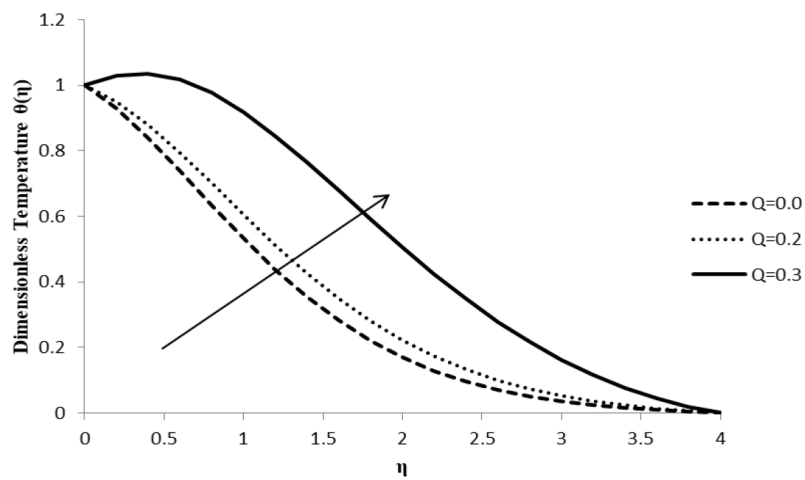
Figure 16: Influence of the heat generation/absorption coefficient *Q* on the temperature distribution θ(η) when *n* = 2.0, Pr = 2.0, Nb = Nt = 0.5, *M* = 0.0, Le = 5.0, F_w = 0.2, and ξ = 1.0.

an external heat source has a significant impact on the temperature gradient of the fluid, resulting in an increase in both the temperature distribution and thermal state of the fluid. With a massive amount of heat energy generated among fluid particles, the thermal boundary layer thickness increases to a larger extent (Figure 16).

Impact of Q on $\phi(\eta)$

The heat generation/absorption coefficient, Q, does not have much of an effect on the concentration distribution, $\phi(\eta)$, as the concentration of the fluid remains unaltered with the change of heat in the fluid. The heat generation coefficient is responsible for changing the heat gradient of the fluid flow, which has a negligible influence on the concentration levels of the fluid particles (Figure 17).

Conclusion

In this work, it was found that an increase in the slip parameter, ξ, significantly reduces the fluid streamwise velocity gradient which, in turn, reduces the boundary layer thickness. On the other hand, an increase in ξ causes an increase in both the temperature $\theta(\eta)$ and concentration gradients $\phi(\eta)$. It was also found that an increase in the stretching parameter n reduces the velocity gradient, thus, depleting the boundary layer; however, the temperature and nanoparticle concentration of the flow were found to increase. An increase in the Brownian motion parameter, Nb, was found to increase the temperature gradient but reduce the concentration of the fluid, whereas the increase in the thermophoresis parameter, Nt, was found to increase both $\theta(\eta)$ and $\phi(\eta)$. The external magnetic field was found to have an enormous influence on the velocity gradient of the fluid which decreases with an increase in M due to a drag-like force, or resistance, that is developed by the fluid. Another observation

was that the temperature gradient considerably increases with an increase in M, thus enhancing the thermal boundary layer thickness of the fluid as the external magnetic field does not have much of an effect on the concentration gradient. It was also shown that an increase in the heat generation/absorption coefficient, Q, results in a significant increase in the temperature profile without changing the velocity or the concentration of the fluid. With Q being positive, there is heat generation across the flow, which enhances the thermal boundary layer thickness.

ORCID® iDs

Santoshi Misra - https://orcid.org/0000-0002-8145-034X
Govardhan Kamatam - https://orcid.org/0000-0002-6707-3162

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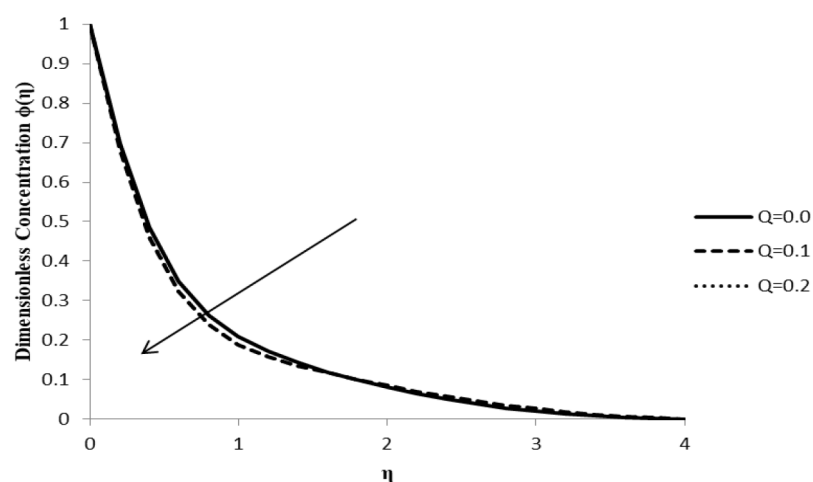
Figure 17: Influence of the heat generation/absorption coefficient Q on the concentration distribution $\phi(\eta)$ when n = 2.0, Pr = 2.0, Nb = Nt = 0.5, M = 0.0, Le = 5.0, F_w = 0.2, and ξ = 1.0.

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INFLUENCE OF CHEMICAL REACTION ON THE HEAT AND MASS TRANSFER OF NANOFLUID FLOW OVER A NONLINEAR STRETCHING SHEET: A NUMERICAL STUDY

SANTOSHI MISRA[*]
Department of Mathematics, St. Ann's College for Women
Mehdipatnam, Hyderabad, Telangana, INDIA
E-mail: sonyshukla29@gmail.com

K. GOVARDHAN
Department of Mathematics, GITAM University Hyderabad
INDIA

A numerical study on a steady, laminar, boundary layer flow of a nanofluid with the influence of chemical reaction resulting in the heat and mass transfer variation is made. The non-linear governing equations with related boundary conditions are solved using Adam's predictor corrector method with the effect of a Brownian motion and thermophoresis being incorporated as a model for the nanofluid, using similarity transformations. Validation of the current numerical results has been made in comparison to the existing results in the absence of chemical reaction on MHD flows. The numerical solutions obtained for the velocity, temperature and concentration profiles for the choice of various parameters are represented graphically. Variations of heat and mass transfer across a Brownian motion and thermophoresis are studied and analyzed.

Key words: nanofluid, Brownian motion, thermophoresis, chemical reaction, heat transfer, mass transfer.

1. Introduction

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The Effect of Chemical Reaction of a Nano Fluid Past a Permeable Stretching Sheet With MHD Boundary Layer Flow And Heat Transfer

K. Govardhan[1], Santoshi Misra[2, a)] and G. Narender[3]

[1]*Asst. Professor, Department of Mathematics, GITAM University Hyderabad, India.*
[2]*Department of Mathematics, St. Ann's College for Women, Mehdipatnam, Hyderabad, Telangana, India.*
[3]*Asst. Professor, Department of H & S (Mathematics), CVR College of Engineering, Hyderabad, India.*

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a)   Corresponding author: sonyshukla29@gmail.com

Abstract. A numerical evaluation and research for a boundary layer flow in a Magneto Hydrodynamic field of Nano fluid past a stretching sheet with the effect of chemical reaction, thermal radiation have been studied and analyzed systematically. The model involving the effect of Brownian motion and Thermophoresis Parameter on Nano fluid is assimilated during the analysis where transport equations are used for study. Similarity transformations are applied to convert the governing non-linear equations to a boundary value problem involving higher order ordinary differential equations. Adam's Predictor Corrector method is used to solve the equations numerically to find missing initial values to satisfy end conditions. Numerical results are obtained by studying the effect of various dimensionless parameters corresponding to different physical conditions for distributions of Velocity, Temperature, Concentration, Local Nusselt Number and Local Sherwood Number.

Keywords: MHD flow; Slip boundary conditions; Brownian motion; Thermophoresis; Chemical Reaction.

INTRODUCTION

Fluid Dynamics is an indispensable branch of Fluid Mechanics, dealing with the study of fluids and how forces affect them. Due to widespread industrial and engineering applications of MHD flows, its study has been the focus of great number of researchers. Nano Fluids in comparison to other fluids are preferred for research due to their novel properties, enhanced thermal conductivity and heat transfer rate. Sakiadis[1] introduced the study of boundary layer flow which was taken forth by Andersson [2].

The quality of result obtained in MHD flow problem depends on the heat and mass transfer rate. Keeping this in view, Kuznetsov AV and Nield DA [3]have studied the natural convective boundary-layer flow which was further extended by Khan WA and Pop I [4] keeping the surface temperature constant of the fluid. The study involving vertical plate phenomenon of flow and heat transfer was investigated at recent times by Ibrahim W and Shanker B [5]. The slip boundary condition was recently studied by Aminreza N [6] and Kalidas D [7]. Further research in this field has been done by eminent researchers which find major applications in the fields of industry and technology referred in articles [8], [9]. The present study focuses on the flow due to Nano fluids with regard to the velocity, temperature and concentration variations.

PROBLEM FORMULATION

Let us consider a two-dimensional steady state flow with V_w as the wall mass transfer, T_w as surface temperature, C_w as concentration, $u_w = ax$, as the stretching velocity of the sheet with 'a' being a constant. T_∞, C_∞ denote the ambient temperature and concentration of the fluid, B_0 being the transverse magnetic field.

The following are the governing equations:

Equation of Continuity:

$$\frac{\partial u}{\partial x} + \frac{\partial v}{\partial y} = 0 \tag{1}$$

Equation of Momentum:

$$u\frac{\partial u}{\partial x} + v\frac{\partial u}{\partial y} = -\frac{1}{\rho_f}\frac{\partial p}{\partial x} + v(\frac{\partial^2 u}{\partial x^2} + \frac{\partial^2 u}{\partial y^2}) - \frac{\sigma B_0^2}{\rho_f}(u) \tag{2}$$

$$u\frac{\partial v}{\partial x} + v\frac{\partial v}{\partial y} = -\frac{1}{\rho_f}\frac{\partial p}{\partial y} + v\left(\frac{\partial^2 v}{\partial x^2} + \frac{\partial^2 v}{\partial y^2}\right) - \frac{\sigma B_0^2}{\rho_f}(v) \tag{3}$$

Equation of Energy:

$$u\frac{\partial T}{\partial x} + v\frac{\partial T}{\partial y} = \alpha(\frac{\partial^2 T}{\partial x^2} + \frac{\partial^2 T}{\partial y^2}) - \frac{1}{(\rho c)_f}(\frac{\partial q_r}{\partial y}) + \frac{\mu}{\rho c_p}(\frac{\partial u}{\partial y})^2 + (\frac{\sigma B_0^2}{\rho c_p})u^2 + \tau\left\{D_B\left(\frac{\partial C}{\partial x}\frac{\partial T}{\partial x} + \frac{\partial C}{\partial y}\frac{\partial T}{\partial y}\right) + \frac{D_T}{T_\infty}\left[\left(\frac{\partial T}{\partial x}\right)^2 + \left(\frac{\partial T}{\partial y}\right)^2\right]\right\} \tag{4}$$

Equation of Concentration:

$$u\frac{\partial C}{\partial x} + v\frac{\partial C}{\partial y} = D_B\left(\frac{\partial^2 C}{\partial x^2} + \frac{\partial^2 C}{\partial y^2}\right) + \frac{D_T}{T_\infty}\left(\frac{\partial^2 T}{\partial x^2} + \frac{\partial^2 T}{\partial y^2}\right) - K_0(C - C_\infty) \tag{5}$$

Where u, v are the components of velocity along x, y directions, K_0 represents the Chemical reaction parameter.

The boundary conditions for equations (1) to (5) are as follows:
$$\left.\begin{array}{l} u = u_w + L\frac{\partial u}{\partial y}, v = v_w, T = T_w + K\frac{\partial T}{\partial y}, C = C_w \text{ at } y = 0, \\ u = 0, \ v = 0, \ T \to T_\infty, C \to C_\infty \text{ as } y \to \infty \end{array}\right\} \tag{6}$$

where $\alpha = \frac{k}{(c\rho)_f}$, $\tau = \frac{(c\rho)_p}{(c\rho)_f}$, $v = \frac{\mu}{\rho_f}$ The velocity components along x and y -axis are u and v respectively. The radiative heat flux q_r, is expressed as

$$q_r = \frac{-4\sigma^*}{3k^*}\frac{\partial T^4}{\partial y} \tag{7}$$

σ^* in equation (7) stands for Stefan-Boltzmann constant and k^* denotes the coefficient of mean absorption.
Expanding T^4 using Taylor's series expansion along T_∞ we obtain the following:
$$T^4 = T_\infty^4 + \frac{4T_\infty^3}{1!}(T - T_\infty)^1 + \frac{12T_\infty^2}{2!}(T - T_\infty)^2 + \frac{24T_\infty}{3!}(T - T_\infty)^3 + \frac{24}{4!}(T - T_\infty)^4 \tag{8}$$

Neglecting higher-order terms in equation (8) we get:
$$T^4 = T_\infty^4 + 4T_\infty^3(T - T_\infty)$$

Differentiating the above equation with respect to y we get: $\frac{\partial T^4}{\partial y} = 4T_\infty^3\frac{\partial T}{\partial y}$ \hfill (9)

Substituting equation (9) in (7) we get:

$$q_r = -\frac{16T_\infty^3 \sigma^*}{3k^*} \frac{\partial T}{\partial y}$$

Differentiating q_r with respect to y we obtain: $\frac{\partial q_r}{\partial y} = -\frac{16T_\infty^3 \sigma^*}{3k^*} \frac{\partial^2 T}{\partial y^2}$

SIMILARITY TRANSFORMATIONS

Similarity transformations are used to simplify the mathematical part of the problem which are the dimensionless quantities as follows:

$$\eta = y\sqrt{\frac{a}{v}}, \qquad \psi = \sqrt{av}xf(\eta), \quad \theta(\eta) = \frac{T-T_\infty}{T_w-T_\infty}, \quad \phi(\eta) = \frac{C-C_\infty}{C_w-C_\infty} \tag{10}$$

The velocity component of $\psi(x, y)$ is as follows :

$$u = \frac{\partial \psi}{\partial y} \quad \text{and} \quad v = -\frac{\partial \psi}{\partial x} \quad \text{which implies} \quad u = axf'(\eta), \quad v = -\sqrt{av}f(\eta) \tag{11}$$

Where prime shows differentiation with respect to η

We use similarity transformations to solve equations (2), (3), (4), (5) for the following:

$$f''' + ff'' - f'^2 - Mf' = 0 \tag{12}$$

$$\left(1 + \frac{4}{3}R\right)\theta'' + Prf\theta' - 2Prf'\theta + PrEcf''^2 + PrMEcf'^2 + PrNb\phi'\theta' + PrNt\theta'^2 = 0 \tag{13}$$

$$\phi'' + Lef\phi' - 2Lef'\phi + \frac{Nt}{Nb}\theta'' - LeK\phi = 0 \tag{14}$$

with boundary conditions

$$\left.\begin{array}{l} f'(0) = 1 + Af''(0), f(0) = S, \theta(0) = 1 + B\theta'(0), \phi(0) = 1 + C\phi'(0), \text{at } \eta = 0, \\ \qquad f'(\infty) \to 0, \theta(\infty) \to 0, \phi(\infty) \to 0 \text{ as } \eta \to \infty \end{array}\right\} \tag{15}$$

$$Pr = \frac{v}{a}, \quad = \frac{u_w^2}{c_p(T_w-T_\infty)}, \quad R = -\frac{4\sigma^*T_\infty^3}{k^*k}, \quad M = \frac{\sigma B_0^2}{\rho_f a}, \quad Nb = \frac{(\rho c)_p D_B(C_w-C_\infty)}{(\rho c)_f v}, \quad Nt = \frac{(\rho c)_p D_T(T_w-T_\infty)}{(\rho c)_f v T_\infty}, \quad Le = \frac{v}{D_B}, \quad K = \frac{K_0}{a}, \quad A =$$

$$L\sqrt{\frac{a}{v}}, B = k_1\sqrt{\frac{a}{v}}, C = k_2\sqrt{\frac{a}{v}}$$

f', θ and ϕ denote the dimensionless velocity, temperature and nanoparticle concentration which are differentiated with respect to the similarity variable η.

Skin Friction Coefficient C_f, Nusselt Number Nu_x and Sherwood Number Sh_x are as:

$$C_f = \frac{\tau_w}{\rho u_w^2}, \quad Nu_x = \frac{xq_w}{k(T_w-T_\infty)}, \quad Sh_x = \frac{xh_m}{D_B(C_w-C_\infty)} \tag{16}$$

$$q_w = -k\left(\frac{\partial T}{\partial y}\right)_{y=0}, \quad h_m = D_B\left(\frac{\partial C}{\partial y}\right)_{y=0} \tag{17}$$

By using the above equations, we get:

$$C_f\sqrt{Re_x} = -f''(0), \quad \frac{Nu_x}{\sqrt{Re_x}} = -1 + R\theta'(0), \quad \frac{Sh_x}{\sqrt{Re_x}} = -h'(0) \tag{18}$$

where $Re_x = \frac{xu_w(x)}{v}$.

NUMERICAL SOLUTION

An efficient Adam's Predictor Corrector method has been employed to study equations (12)-(14) and the values for various governing parameters are obtained using FORTRON programming. The first step of the solution

involves reducing the non-linear differential equations involving third order in f and second order in θ and $\emptyset$ to first order which is an easy technique to simplify the problem.

$$f''' = -ff'' + f'^2 + Mf' \tag{19}$$

$$\theta'' = -Pr\frac{(f\theta' - 2f'\theta + Ecf''^2 + MEcf'^2 + Nb\phi'\theta' + Nt\theta'^2)}{(1+\frac{4}{3}R)} \tag{20}$$

$$\phi'' = -Lef\phi' + 2Lef'\phi - \frac{Nt}{Nb}\theta'' + LeK\emptyset \tag{21}$$

By reducing the higher order equations into first order equations the problem is converted to initial value problem and the unknown value is found by giving an initial approximation. Assigning step size h =0.005 and η at its max to be 6, the solution is obtain for various parameters used and convergence is achieved with a change in the single parameter. The criteria for convergence is the approximation up to three significant digits. The results derived through computation are formulated into tables as shown below:

Table 1: Comparison of $f''(0)$ for different values of A when $S = M = 0, A = 5.0, Pr = 1.0, R = 0.5, Ec = 1.0, Nb = 0.5, Nt = 0.1, Le = 10.0, B = 1.0, C = 1.0$

A	Andersson H[2]	Ibrahim W [5]	Present Result
0.0	1.0000	1.0000	1.0005
0.1	0.8721	0.8721	0.8726
0.2	0.7764	0.7764	0.7769
0.5	0.5912	0.5912	0.5917
2.0	0.2840	0.2840	0.2844
5.0	0.1448	0.1448	0.1707

Table 2: $\theta'(0)$ and $\phi'(0)$ at $Le = 5, M = S = 1, Nb = Nt = 0.5, A = 1.0$ for $different$ $values$ of Pr, R, B, C, Ec

Pr	R	Ec	B	C	$-f'(0)$	$-\theta'(0)$	$-\phi'(0)$
1	0.2	0.2	1	1	0.5656	0.368	0.681
5	0.2	0.2	1	1	0.5656	0.547	0.648
7	0.2	0.2	1	1	0.5656	0.570	0.648
8	0.2	0.2	1	1	0.5656	0.577	0.643
9	0.2	0.2	1	1	0.5656	0.582	0.642
10	0.2	0.2	1	1	0.5656	0.587	0.642
5	0.5	0.2	1	1	0.5656	0.525	0.652
5	0.7	0.2	1	1	0.5656	0.511	0.655
5	1.0	0.2	1	1	0.5656	0.491	0.658
5	1.0	1.0	1	1	0.5656	0.386	0.687
5	1.0	2.0	1	1	0.5656	0.251	0.724
5	1.0	3.0	1	1	0.5656	0.114	0.761
5	1.0	0.2	0	1	0.5656	0.887	0.606
5	1.0	0.2	1	1	0.5656	0.491	0.658
5	1.0	0.2	2	1	0.5656	0.330	0.681
5	1.0	0.2	1	0	0.5656	0.401	2.496
5	1.0	0.2	1	1	0.5656	0.491	0.658
5	1.0	0.2	1	2	0.5656	0.505	0.379
5	1.0	0.2	1	3	0.5656	0.511	0.266

RESULTS AND DISCUSSION

Numerical computations involving various parameters describing the characteristics of fluid flow have been studied, analyzed and graphically interpreted.

Figure 1 shows the variation of concentration for different values of Lewis Number Le. With an increase in Lewis Number the concentration of the fluid and the boundary layer thickness decreases due to an increase in the mass transfer rate.

Figure 2 depicts the variation of chemical reaction K on the concentration of the fluid. As the value of K increases the concentration of the fluid also increases. An external chemical added to the fluid causes an increase in the fluid particles which further affects the movement of the fluid drastically increasing the concentration.

Figure 3 illustrates variation of $\theta'(0)$ for different values of Nb, with the influence of Thermophoresis parameter Nt. Heat transfer of the fluid decreases with an increase in Nb.

Figure 4 illustrates the variation of $\phi'(0)$ in response to a change in Nb. The mass transfer rate of the fluid decreases with an increase in Nb.
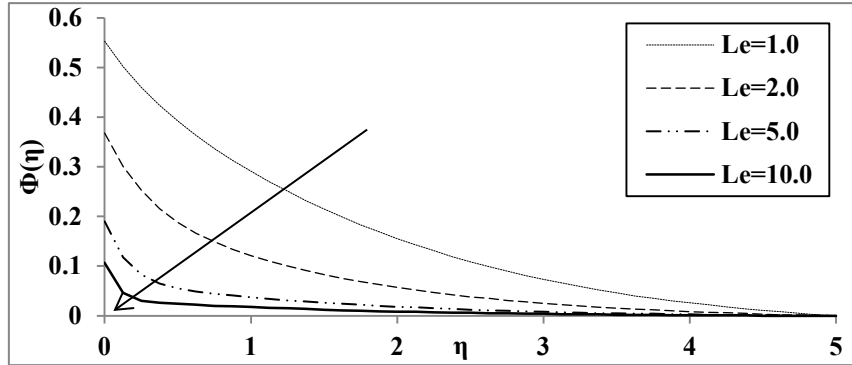
FIGURE 1: Concentration graph for Le when $M = S = Ec = Pr = A = B = C = 1, Nb = Nt = 0.5, R = 0.5$
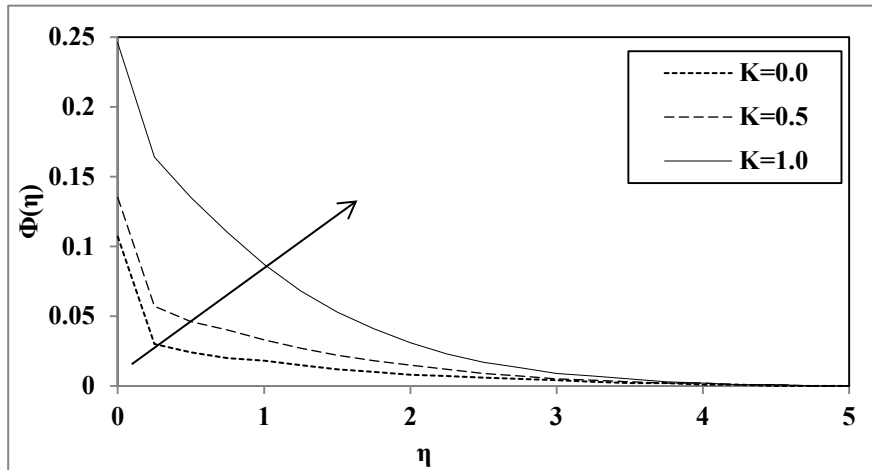
FIGURE 2: Concentration graph for K when $S = M = Ec = Pr = A = B = C = 1, Le = 10, Nb = Nt = 0.5, R = 0.5$
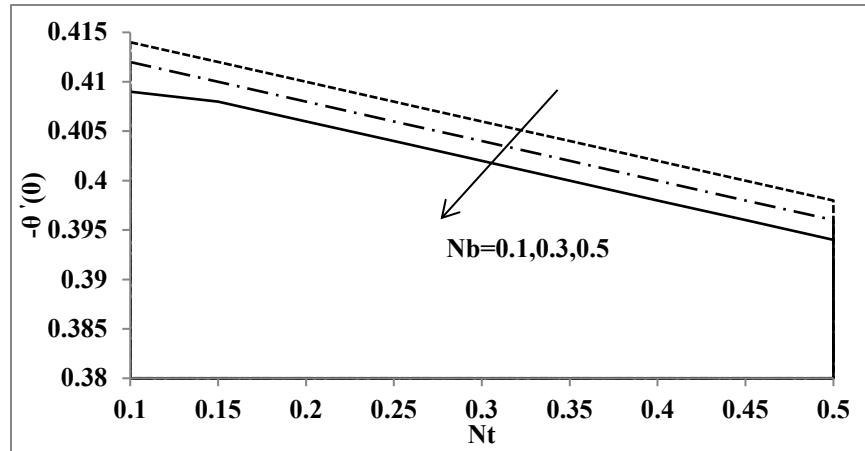
FIGURE 3: Variation of local Nusselt Number $-\theta'(0)$ *with Nt for different values of Nb when* $M = S = A = Pr = Ec = R = B = C = 1, K = 0, Le = 10$
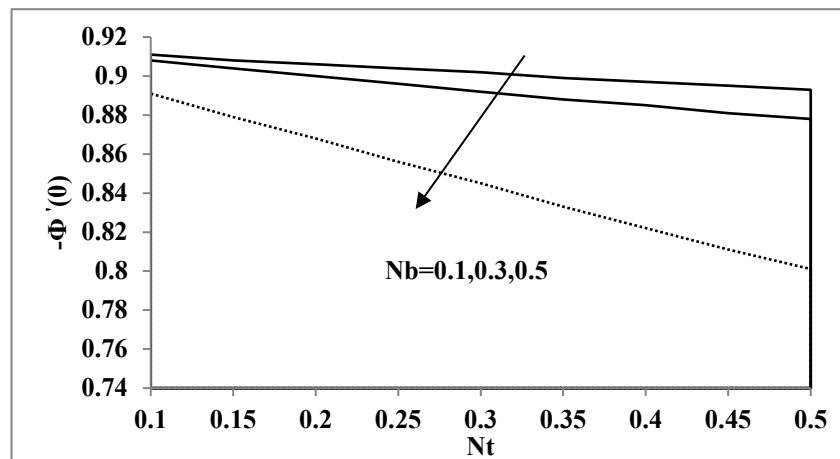
FIGURE 4: Variation of local Sherwood Number $-\phi'(0)$ *with Nt for different values of Nb when* $M = S = A = Pr = Ec = R = B = C = 1, K = 0, Le = 10$

CONCLUSIONS

The results in summary from the above discussion are:

- Lewis Number *Le* has considerable effect on the boundary layer and concentration of the fluid.
- An increase in the parameter *Nb* decreases the heat and mass transfer rates of the fluid.
- Chemical reaction parameter *K* has negligible influence on the temperature of the fluid but the concentration of the fluid increases with an increase in the value of *K*.

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"ఆధునిక తెలుగు సాహిత్యం - సామాజిక జీవన చిత్రణ"

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పెదనందిపాడు, గుంటూరు - 522 235, ఆంధ్రప్రదేశ్.

7. **Dr. J. VENKATA RAMANA**, M.A., M.Phil.PhD.
Asst.Professor, Dept. of Modern Indian Languages,
Madhurai Kamaraj University, Madhurai,
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Dept. of Telugu,
Regional Institute of Education (NCRT)
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Associate Professor, Dept. of Telugu,
Dravidian University, Kuppam, Chittor Dist.

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Asst.Professor, Dept. of Telugu,
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RAJAMUNDRY, East Godavari, A.P.

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P.A.S.College, PEDANANDIPADU-522 235,
Guntur, A.P.

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Asst. Professor, Dept. of Telugu,
Central University of Andhra Pradesh,
Ananthapuram District. A.P.

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HOD, Dept. of Telugu, C.R. Reddy College,
West Godavari District. A.P.

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Chebrole , Guntur, A.P.

17. **Dr. Guntupalli Gowri**, M.A., PhD.
Dept. of Telugu,
Govt. College for Women, Guntur - 522 002.

18. **Dr. Kakani Sudhakar**, M.A., M.Phil., PhD.
Dept. of Telugu,
S.S.N. College, Narasaraopet - 522 601.

19. **Dr. K. Pushpamma**, M.A., PhD.
HOD, Dept. of Telugu,
SVRM College,
Nagaram - 522 035.

20. **Dr. P. Srinivasa Rao**, M.A., M.Phil., PhD.
PDF Scholar, Dept. of Telugu,
Andhra University, Visakhapatnam.

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PDF Scholar, Dept. of Telugu,
Kakatiya University, Warangal.

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Guest Faculty, Dept. of Telugu,
Central University of Andhra Pradesh,
Ananthapuram District. A.P.

25. **Dr. M. Prasad**, M.A, M.A (Sat), PhD Diploma in Malyalam
School Assistant (Telugu)
Govt. High School,
Rapuru, SPSR Nellore.

26. **Dr. V. Triveni**
Head & Assistant Professor,
Dept. of Telugu Studies,
Telangana University, Dichpally, Nizamabad.

LEGAL ADVISER

Dr. A. GURAVAIAH, M.A., L.L.M., PhD.,

Principal & Research Guide, Dept., of Law, A. C. Law College, GUNTUR - 522 002, A.P.

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గమనిక : రచయితల అభిప్రాయములతో సంపాదక వర్గమునకు సంబంధం లేదు - ఎడిటర్.

తెలుగు సాహిత్యంలో గజళ్లు

- డా. ఉషా దివ్యప్రియ, తెలుగు శాఖాధ్యక్షులు, సెంట్ అన్న మహిళా కళాశాల, హైదరాబాద్.

ఆధునిక సాహిత్యంలో ప్రణయ భావాలతో పాటు సామాజిక చైతన్యాన్ని ప్రతిభావంతంగా ఆవిష్కరించిన కథ, నవల, నాటకం, పద్య కవిత వంటి ప్రక్రియల్లో గజల్ ప్రక్రియ విశిష్టమైంది. అరబ్బీలోను, పారసీ లోనూ ఉన్న కవితా ప్రక్రియ "కసీదా". చక్రవర్తులను రాజులను కీర్తించడమే కసీదా ప్రధాన వస్తువు. కసీదాలో ఒక భాగమైన ఆషిద్ విడిపోయి, క్రమంగా గజల్ అనే ప్రక్రియ ఏర్పడింది. అరబ్బీ సాహిత్యం నుంచి పారసీ సాహిత్యంలోకి గజల్ వర్ధిందని కొందరు, పారసీ నుంచి అరబ్బీకి వ్యాపించిందని మరికొందరు అంటున్నారు. పన్నెండవ శతాబ్దిలో మంగోల్ రాజుల కాలంలో ఇరాన్ సంస్కృతిలో భాగంగా ఈ గజల్ భారతదేశంలోకి ప్రవేశించింది. అమీర్ ఖుస్రూ (1253-1325) ఇండియాలో గజల్ రచన ఆరంభించారు. ఢిల్లీ, లక్నో నగరాలు కేంద్రాలుగా గజల్ ప్రక్రియ వర్ధిల్లింది. గజల్ అనే పేరు వినగానే అందరికీ గుర్తొచ్చేది ప్రేమ మాత్రమే. ప్రేమ, ఎడబాటు, వేదన, మర్మ తాత్వికతలు వస్తువుగా ఉన్న గజళ్లనే గాయకులు ఎక్కువగా పాడుతున్నారు.

ఈ గజల్ పేరు వెనక ఒక కథ ఉంది. ఒక అరబ్బు దేశంలో గజ్జాల్ అనే పేరుగల ఒక యువకుడు ఉండేవాడు. ఆతడు కవిహృదయం ప్రియురాలి గుర్తి, సౌందర్యాన్ని గుర్తి కవితలు రాస్తూ గడిపాడు. కేవలం ప్రణయ రచనలు చేయడమే కాక, ప్రణయ కావ్యాల్లో ఆతడు జీవితం వెళ్లబుచ్చాడు. ఆ రచయిత పేరు గజ్జాల్ కనుక ప్రణయ కవితకు గజల్ అనే పేరొచ్చిందంటారు ప్రముఖ కవి డా. దాశరథిగారు. గజల్ పదే పదానికి 'ప్రియునితో సంభాషణ' అనే అర్థమే ప్రచారమై ఉన్నది. కాని 20వ శతాబ్దిలో మహమ్మద్ ఇక్బాల్ (1873-1938), భారతి మోహని (1875-1951) లాంటి కవులు దేశభక్తి,

సంస్కరణ భావాలను ఈ ప్రక్రియ ద్వారా ప్రబోధించారు. ఆ తర్వాత కాలంలో ఫైజ్ అహమ్మద్ ఫైజ్, అలీ సర్దార్ జాఫ్రీలాంటి కవులు సామాజిక భావాలను గజల్లో చెప్పారు.

రెండు రెండు పాదాల ద్విపదల (ముక్తకాలు) సమూ రాయుమే గజల్. రెండు పాదాలను కలిపి "షేర్" అని, ఒక్కొక్క పాదాన్ని 'మిశ్రా' అని అంటారు. మొదటి షేరును 'మత్లా' అంటారు. మత్లా పాటలోని పల్లవిలాంటిది. ఇది పాటకుసీలో ఒక సర్వస్వమైన మానవ స్థితిని ఏర్పరచి గజలవి ఆనందించడానికి తన మానసిక సంసిద్ధని చేస్తుంది. ఒక షేర్లోని భావం ఆ షేర్తోనే పూర్తి కావాలి.

ఆంధ్ర సాహిత్యానికి షేక్స్పియర్ ఎండో నాథ్ ఉన్న కవిత్వానికి గాలిబ్ అంటివాడు. ఆ గాలిబ్ గీతాన్ని దాశరథి తెలుగులోకి అనువదించి ఆంధ్ర సాహిత్యానికి గజల్ ప్రక్రియను పరిచయం చేశారు. దాశరథిగారు గాలిబ్ గీతాలను తెలుగులో అనువదించే టప్పుడు ప్రతి పద్యాన్ని ఆకళించి చేసుకొని, ఆ పద్యానికి ఇతర భాషల్లో వివిధ వ్యాఖ్యాతలు, విమర్శకులు చెప్పిన అర్థాన్ని చదివి తనకు స్ఫురించిన అర్థాన్ని కీర్తించకని రాశారు.

"ప్రతిది మంచిదన్నా సాధ్యపడు కేమ్ము
సమయ సమాన ఎలో మన్నరమ సమ్మా"

మూలంలోని అర్థ్ని, ఇనిశాన పదాలకున్న భావ భేదాన్ని సమయ సమయాట అనే తెలుగుదనంతో వ్యక్తి కొందడం విశేషం. మానవుని సమాజ దేవంగా మందుకు సమీంది చేర్ పై పాటలున్నాయి.

"ఒటుపై ఇంత ప్రేమ ఎక్కడిది
మది మాందట ఇరుసుకృతికి పేటా?"

తెలుగు శాఖ, వినాయంధ అస్తిత్వ జాగ్ర్తి మహిళ కళాశాల, సికింద్రాబాద్.

700 | అంతర్జాల అంతర్జాత సంచిక

గుడిని కిడిమి దొరకున్న శక్తి గని
పరుగులెత్తి నేను అడయగలను?.."

వంటి ఇలాంటి షేర్లను చూస్తే హైందవేతర
వాతావరణాన్ని హైందవ వాతావరణంగా పరివర్తించడానిక
దాశరధిగారు ప్రయత్నించారని అర్థమవుతుంది. ఇక్కాది
అనువాదాలలోని భావ మార్గవం, శాక్ష్యకత, ప్రేమికుల
సహజ ఉద్వేగ ప్రవృత్తి, జీవన సత్యాలు తెలుగు
పాఠకులను విశేషంగా ఆకట్టుకున్నాయి.

"ఇన్నినాళ్లు తెలపమంటివి ఎందుకోరే నా పేర.."
అని మొదలయ్యే గజల్‌లో కవి దాశరధిగారు..

"పగబూని కకుసవాడు బంధించి ఉంచినాడు
మేనాటికైన గని ఆ నాడు పాడిది రారా.."

అని స్వాతంత్ర్యం కోల్పోయిన వ్యక్తి జాతీయతను
స్మురించేలా ప్రతిభావంతంగా కవిత్వీకరించారని విమర్శ
కులు పేర్కొన్నారు. ఇందులో ఆయన దేశభక్తి, జాతీయ
దృక్పథంకనిపిస్తుంది.

తెలుగు గజల్‌కు తొలుత ప్రవేశ పెట్టినవారు దాశరధి
గాఱితే, విశేష ప్రాచుర్యాన్ని తీసుకొచ్చినవారు
డా. సి. నారాయణరెడ్డిగారు. తెలుగు గజళ్ళు అనే గ్రంథాన్ని
1986లో తొలి తెలుగు గజల్ సంపుటిగా తీసుకొచ్చారు.
సినారె తన గజళ్లలో ఎక్కువగా సామాజక అంశాలు,
ప్రగతిశీల భావాలను వస్తువులుగా స్వీకరించినట్లు
కనిపిస్తుంది.

"పదుల కోసం పాటుపడని నరుని ప్రతుకు బతికినా?
మూగ నేలకు నీరందివ్వని వాన పడను బతికినా..?"

అన్న పాదాల్లో నెలకు మానవ ధర్మారోపణ, వాగుకు
ప్రాణి ధర్మారోపణలు గమనించదగినవి. అనావృష్టి
పీడనను మొగతనంతో కవి నిక్షేపించారు. వాగును నదిక
ప్రతీకగా గ్రహిస్తే, భీళ్లకు సస్యశ్యామలం చేయకుంటా
నాగరసంగమానిక ఉరకలెత్తి నడులది నిరర్థక జీవనమనేది
వ్యంగ్యం!

"ఎన్ని ఎన్ని కాలుగా వ్యక్తిత్వమెంతగ ఎదిగినా
అది చెడును పొడు పొడున కందుకుంటే మందిర"

భూగర్భ జల. బదలబదలు ఆర్ట్‌, & ప్రక్ సూరయ, బదలంచదరు.

అని వ్యక్తిత్వాన్ని శాఖలతో పోల్చడం ద్వారా ఇక్కర
వృక్షం చౌరిక ఉవమానమని వ్యంగ్యం చేశారు. మూలా
లను మరచిపోకుండా ఉంటేనే ఆధిక్యత భావం, అహం
తలకెక్కకుండా ఉంటాయి. దారిణకు మూలం మననం,
పొద్దు పొద్దున అనేది కష్ట వ్యవహారికంలోనూ, మాంత
లీకంగానూ అతికినట్లు ఒదిగిన ప్రయోగం.

ఒక వ్యక్తి మాట విలువ ఎంత అవనరమో కవి
సినారె ఇలా అభివ్యక్తీరించారు.

"ని మాటకు విలువ లేకుంటే అది మాట ఎలా
అవుతుంది?

తన మాటను పరువును దాచినే అది సైటు ఎలా
అవుతుంది?"

వ్యక్తి ఏ రకమైన వ్యక్తిత్వపు ముద్రదైనా ఇచ్చేది ఆతని
భావ వ్యక్తీకరణ పద్ధతే! మనో వాక్యాయ కర్మల మధ్య
నుంతే సమన్వయమే మాటకూ, వ్యక్తిత్వానికి ఉన్న
సంబంధం గాఢమైనది, లోతైనది! దముపు ఎలా మాట్లా
డాలో నేర్పుతుంది. జ్ఞానం ఎప్పుడు, ఎందుకు మౌనంగా
ఉండాలో తెలుపుతుంది. అంతర్యాన్ని ఎప్పుడు, ఎక్కడ,
ఎవరి వద్ద ఎంతవరకు బైటపెట్టాలి అనే వివేచన మీద
ఆ మాట ఆధారపడి ఉంటుంది. ఎప్పుడు, ఎక్కడ, ఎవరి
వద్ద ఎంతవరకు తన సౌందర్యాన్ని ప్రదర్శించాలో లేదా
దాయకోవాలో అనే నిర్ణయానిక సైటు సంకేతం!

అహంభావాన్ని విడనాడి మానవుడు నడవాలన్న
సూర్తితో సినారె గారు..

"తంపులాకై తంకు కొమ్మలు మెరిస్తే అది నవమాడం
అహంభావం పడగలెత్తితే అణుకుంటే మందిది.."

సతిపు నుంచి మనిసిని వేరుగా చేసేది బుద్ధి. తలకు
కొమ్ములు మొలిస్తే నతి లక్షణాలు సంక్రమించే ప్రమాదం
ఉందని, అహంభావం పొడు మీరతమే పదగలెత్తం
అన్నది అహంభావ తీవ్రతకు మూదన.

"కుదురుకొంటుందా నమాజం అమ్మ లేకుంటే?
"కళ్ల వేగం పెరుగుతుంటా కుమపు లేకుంటే.."

- సమాజం కూడా తన అదుపులో' కానుండే అన్ని సవ్యంగా ఉంటాయని అన్నారు.

"ఈ మాటున ఉన్న కేడును తెలుసుకుంటే మందిది కలవాలేనా జీవితంతం.. కలుసుకుంటే మందిది.."

అంటూ సామాజిక బాధ్యతను తెలియజేశారు. వ్యవస్థ లోని అవ్యవస్థకు కారణాలను అర్థేచేసిందడంతో పాటు వ్యక్తిత్వ వికాసానికి అనుసరించ వలసిన పద్ధతులు, వ్యక్తి నిర్వర్తించవలసిన నియమాలూ సినారె గజళ్ళలోని కవితా వస్తువులు! ఆ గజళ్ళ మీద ప్రేమే.

"నన్ను నేనే పాఠంగా పరిశీలించుకుంటను
నేమ్మలను నా మనిగీకి పరిశీలించుకుంటను.."

అంటూ ఆయన దేశ పాశంచింది! రాకరవి, సినారె.. పేరి జాటలో' వల్ల రాంబాబుగారు 'వల్ల గజళ్ళ' సంపుటిని రచించారు. పేరి రచనలోనూ ఎన్నో సామాజిక ప్రబోధాత్మక కావు, అనాడే వాస్తవ సద్యర్శాలు కనిపిస్తాయి.

"పైపైకి ఎదిగన్న నేల పైకి చూసో
"భూమి పైన కాళ్ళంది మందరచుస చేసో.."

పంటి ప్రబోధాత్మకమైన పంక్తులే గాక..

"అండేను సంతసష్ట ద్యత్తేలంత రాలు
కేంతంత అండైన అడ్గదుంటే రాలు.."

పంటి సేరేలు ప్రభుత్వం వారి కుటుంబ సంక్షేమ పథకాల ప్రచారానికి బాగా ఉపకరిస్తాయి.

"ఎవడు నారిని వాట పర్న ఫాలనివ్వను గడ్డిమొళ్ళ
"వెడం కొసం పంట సరిగ పెరవ పంటేను ఆసువడ్న"

–

"ఆసం రోసం అగ్గుతున్నది ఉడ్డ ఉపశే మొంకొస్నది
"మెళసవి స్వడయారు మసుషి వేడు.."

మొదలైన విచిత్ర, విలక్షణ ప్రయోగాలు పేరి సంపుటిలో ఉన్నాయి.

మరో గజల్ కవి సూరారం కంకర్ గారు 2007లో ఓగంధిక అనే గజల్ సంపుటిని వెలువరించారు. వారు చెప్పిన గజళ్ళలో'..

కన్ని గుండెను ఎంతగాలం చెప్పినా అవి మరచ్చ కన్ని రావును ఎంత మారం సెడిదిగా ఉది వేద్ధ.."

- బింబ, ప్రతిబింబ భావం స్ఫురింపే విధంగా ఒక లోకరీతిని పై మళ్ళారో అందంగా చెప్పారు.

"ఓటమే ఏ పెద్దమైనా పళ్ళు పడియబేమి కంకర్ అంటాడ్ ఏ అంత కేడు నాకు వేవే సేతువ్వు.."

మనిది తనకు తానే (పేరణకు గురి చేసే విదంగా ఈ పంక్తులున్నాయి.

"నీవ్వ నన్ను పిదిరాగ చేను పెంచ తెలిసింది వేట్టు వాడి పర్చ్యాస పిద పెంచ తెలిసింది.."

అంటూ ఆరంధిచెప్పే కంకర్ గారి కలం నుంచి గజళ్ళుగా జాలువారాయి.

డా. సిగరే ముంగడి 'ఆకరి రంగు' అనే గజల్ సంపుటిని ఆవిష్కరించారు. పేరు కవివ జల్ల రాయచోట వాసి. ఉద్యోగరీ గజళ్ళు, దుబాయులు రాసి సంకలనాంగా పెలు పరించారు. పట్టుదలతో, స్వయంకృషితో తెలుగు భాష మీద పట్టు సాధించారు. పేరి 'ఆకరి రంగు' సంకలనంలో 52 గజళ్ళున్నాయి.

"నేత్యం తగిన కళ్ళు పెమలరు ఎదని పద్చని రాళ్ళంటరు.."

–

"స్ఫురాతి నేత్రాలంద్ద్మి వి ప్రాంతి గేత్రాంద్ద్మి.."

–

"నన్ను పిన్ను కాశీరను కినివేట్ లోరను.."

అంగ దూదంది..

"గత సరళ అన్న నిన్పు రాసి ఆ లోలో పెమసలుంత ఉపేలు.."

అని అకల లోలింతో' పెరిగె మనిష పిర్ళ కబడె భావాన్ని కవి దర్శించారు.

"నన్ను వ్యక్తం చేయగలవా?
అస్తిత్వం నీయగలవా.."

—

"నేనూ చేపల తినగలను
రాధ నాకేం చేయగలదా..?"

—

"అనుకొన్న చెలుకుదుస్తు
పాలుపంచు చెయగలవా..?"

అంటూ అద్భుత వరవిజ్ఞార్ని సృష్టించిన జయాద్

ఇద్ద మాతృభాయియా తెలుగులో దక్షిది కవిత్వం
రాయడం విశేషం. నిజానికి ఇద్ద మాతృభాగా కలిగిన
వారు రాసిన మొదటి తెలుగు గజల్ సంపుటి ఇదే! తన
గజల్లో ముఖ్యం సమాజం మీద, ఇయటి విద్యేవ
రాయల్ని, అంతర్గత సమస్యల్ని, రాయలిసమల్లో ఆవా
వృష్టిని, కరువును, ప్యక్షింజంను సున్నితంగా దృగించ
జేసిన కవి ఉయన!

ఇక గజల్ రచయిత్రుల విషయానికి వస్తే
డా. ఎమ్.డి.డి. శ్యామలగారు తొలి గజల్ కవయిత్రిగా
పేరొందారు. ఆమె సమ్యళ్ళే అనే గజల్ సంకలనాన్ని
తెచ్చారు. వారు రాసిన కొన్ని పంక్తులు..

"ఏమి చెప్పగలను నేను అమ్మ గురించి?
అంటింటా అమ్మ నిన్ను జప్పా గురించి.."

—

"నిద్ర నేల నేను నింగి కలశారం రమ్మంది
నిన్ను పడలి పాలోను పాడిమ్మని కోరింది"

పై పంక్తులలో ఎంతో ఆర్ద్రత ఉంది. ఇలాంటి
గజళ్ళు ఏరి రచనల్లో అంతకొనవచ్చు.

డా. అద్దేపల్లి రామమోహనరాఫుగారు తెలుగు
గజళ్ళలో రాసిన ఈ పంక్తులను చూద్దాం.

"లోకం కంటె నేనే ఎక్కువ లోకం నాలో అన్నప్పుడే
దేవుడి కంటె నేనే ఎక్కువ మనిషి నేనే అన్నప్పుడే"
- ఈ పాదాల్లో మనిషి అస్తిత్వానికున్న విలువను
తెలిపే చక్క అందంగానూ అర్థం, & ప్రశ్న కూడా, పదించింది.

తెలియజేస్తున్నారు. ఇలా గజళ్ళ రచనకు పూనుకున్న పలు
రచయితల పంసలలో డా. రెంటాల శ్రీవేంకటేశ్వర
రాఫుగారు రాసిన గజళ్ళు కూడా సాహిత్య లోకంలో
ప్రముఖ్యాన్ని సంతరించుకున్నాయి. సివారె గారి గజళ్ళె
ఉయన ఈ ప్రక్రియలో కృషి చేయడానికి కారణమని
చెప్పుకున్నారు.

"చేదసంటే వాది నీరు చెప్పినాంది
నావసంటే ప్రతిధనున్న పది వాంది.."

అనే గజల్లో ప్రతి మనిషి ప్రతిభావంతుడు కావాలంటే
నిత్యమూ తన మెరుపును సదును పెటుకుంటాలంటారు.

"ఓటమి వాడ్లుతామని & రెంటాల
ఇరి ఏదక అనుదవాల అది వాంది.."

వంటి వాక్యాల్లో పోరులో గెలిచినా, ఓడినా అను
భవాల నుంచి పాఠాలు నేర్చుకోవాలని చెప్పడం మనం
గమనించదగినది.

అలాగే నేటి సమాజంలో మొబైల్ పోన్ అధివత్యం
ఎంతగా విస్తరిల్లిస్తుందో ఉయన గజళ్లోనే చూద్దాం.

"పోరు గంది పోను వాని తీసు చూస్తే కలవరం
అంత అక్షరాన్ని చెమ్మనిస్తుందా కాఫరు"

- ఇందులో పోరు గంది అనే మాట పోనుకు సంబం
ధించి సార్థకమవుతూనే ఉంది. కాఫరం చెయ్యనివ్వడం
అనే అంశం ద్వారా పోనుకు, ఉత్తరానికి నవత్సీకత
వ్యంగ్యమవుతుంది.

కేవలు సాహిత్య వర్మైన గుర్తింపు కోసం రాసి
ప్రక్రియ కాదు గజల్. మనసు ఉవసీకలంలో తేనిన
భావాల్ని ఎవర మెప్పు కోసమో గుదిగుచ్చితే ఆ గజల్ ఏ
అమధూనని కలిగించలేదు. ఎందుకంటే గాయపడని
గుండెలో గజల్ మొలవదు. గజల్ రాయడం కోసం ఏ
కష్టో ఎదుర్కొ గాయపడతక్కరలేదు. వ్యక్తిగత, సామాజక,
సాంస్కృతిక, రాజకీయ స్థితిగతుల వల్ల ఎంతో కొంత
మానసిక సంఘర్షణను అనుభవించని వ్యక్తులు సారా

రీతిగా ఉండదు. వ్యక్తి జీవితానికి, లోకానికి మధ్య సామరస్య నైమనస్యం మిత్ర శత్రు నైరుధ్యం ఎరుక కలుగుతుంది. ఆ గాయం కల్గించే దుఃఖం నుండి తనకు తాను సాంత్వన చేకూర్చుకుని గజల్ రాయడానికి ప్రయత్నించినప్పుడు జీవన సంఘర్షణ స్వరూపం పరిణాలు అనుభవంలోకి వస్తాయి. ఆ మానసిక స్థితి నుండి

అద్భుతమైన గజల్ ఉద్భవిస్తుంది. అలా ఉద్భవించిన కాలడ్డి...

"ఏ మనసు వారధి ఏముందో ఏనాడో పెనవేసు తెలుసుకో

ఎవరెవరి వంతన హైఅతిశ కనుగొని ఆరాటమింతే.."

అంటూ గజల్ కవి గళమెత్తుతాడు!

ఉపయుక్త గ్రంథాలు :

1. డా. సూరారం శంకర్ - 'సుగంధిక' (గజల్)
2. చల్లా రంబాబూ 'చల్లా గజల్'
3. డా. ఎమ్.బి.డి.శ్యామల - 'మహాలేఖ'
4. డా. సాగర్ జయూద్ - 'అక్షర రంగు'
5. డా. సిహార - 'శోభ తెలుగు గజల్'
6. డా. దాశరథి - 'గాలిబ్ గీతాలు'
7. డా.పెన్నా శివరామకృష్ణ - 'తెలుగు గజల్ రూబాయిలు'
8. డా. అద్దేపల్లి రామమోహనరావు 'తెలుగు గజల్'
9. డా. రెంటాల శ్రీవెంకటేశ్వరరావు గజల్
10. తెలుగు గజల్ - వికీపీడియా

❖   ❖   ❖

తెలుగు రాష్ట్ర, ప్రభుత్వ కళాశాల అధ్య. & ప్రశ్న కళాశాల, విజయనగరం.

704 | తెలంగాణ సాహిత్య సదస్సు

Language Communication and Literature

"If you talk to a man in a language he understands, that goes to his head. If you talk to him in his language that goes to his heart."
— Nelson Mandela

The very essence of communication in a language is *comprehension*.

Language, as mentioned by Encyclopaedia Britannica, "is a conventional mode of symbols, either spoken or written by people to express their emotions to make interaction simple and comprehensible." To communicate effectively, it is not enough to have well organized ideas expressed in complete and coherent sentences and paragraphs. One must also think about the style, tone and clarity of his/her writing, and adapt these elements to the reading audience. Literature can be referred to as any written or oral form, predominantly considering any art form in areas mostly such as poetry, novel or drama.

The relationship between Language and Literature, one can describe as, 'a work form in any language which is the outcome of any research or creativity. It is essential in language, especially, to keep in mind, the grammar and terminology in formal usage whereas in literature it is important to focus on all areas of knowledge such as chronology of events etc. These forms of literature use words in a very influential, effective and thought-provoking manner. It is a record of what one has seen, experienced, thought or felt in life, which has an instant and enduring interest.

Literature is a written document of the writer of the same people and culture of the society. Whether the types of Fiction or Non-Fiction, genres of Novel, Drama, Short Story, Essay, Poetry or any other form of writing. Literature reflects and influences of the races of the countries.

In literature, creative writing is used as a means to express the intellectual thoughts and ideas, but language is just a tool of expression. Further, it is regarded as a graciousness of written work. Literature is classified according to historical periods, or their adherence to certain aesthetic features, or genre.

As a matter of fact language can do without literature but literature cannot do without language. Literature enthusiasts apply all the language related parts of speech and figures of speech to make their work more notable and effective. This makes it very obvious that one should know all the nuances of language to bring out a flawless literary work!

Be it any language, a great and perfect work is always acceptable. Here the aspect of communication plays a very important role. Here are some ways that communication problems can happen:

The speaker doesn't express him/herself clearly enough.

The speaker uses language that the listener doesn't understand.

The listener does not understand the speaker.

The listener is not paying enough attention to the speaker.

There are ways to prevent communication errors, as both a speaker and a listener.

Preparation and practice are important to improve your communication skills in English.

In any language, there are these extremely important points to remember when you're communicating with someone.

1. **Listen**. Listening is the most important aspect of communication. When you *listen*, you actually understand analyse and interpret the speaker in contrast to *hearing*.

2. **Clarity.** It can be difficult to express some ideas clearly, but if you're trying to prevent miscommunication, it's important to say exactly what you mean. Be clear and to the point.

3. **Queries.** Communication is a two-way process, which means you can't be the one doing all the talking. To make sure your listener is *engaged* (interested in what you have to say)and understanding you, ask questions. Many a times because of the language barrier, you're never sure if people really understood what you meant, so just ask them to repeat what you said. Also ask questions that will clarify (or clear up) any misunderstandings or any information you're missing. The types of questions you ask will vary based on the conversation you are having.

4. **Body language.** Another important aspect of communication is your body language which speaks almost as loudly as you. The way you sit, the way you hold your hands, even which direction you look at—all these things can change the meaning of the words you speak. For native speakers, most body language is ***intuitive***. That means you do it without thinking about or having to learn it. Not all of it, however, is universal.
That is, not all body language and gestures mean the same thing in different cultures.

5. **Empathy.** *Empathy* is the ability to understand what someone else feels, and it's an important part of communication in any language. Showing empathy is important for listening well. You can't just listen to a person's words, you need to understand what they're saying, and try to understand what they mean.

Literature and language are neither same nor contradictory in nature; rather literature is highly dependent on language whereas language independent as discussed. Literature is an aesthetic subject which deals with the study of work and styles of various authors and writers. The whole literature is based on the language in which it is written.

Since English has become an international important language, more and more people are studying it. "The study of literature allows people to develop new ideas and ethical standpoints, and can help individuals to present themselves as educated members of society. Studying literature can be enriching, eye-opening experience"

Traditionally, the role of Literature was not taken into consideration in English Language Teaching programs due to the importance given only to functional language.

Today, Literature in second language classes has a very important linguistic input for students and it is a valuable source for learners' motivation.

The moment one hears the name ***Shakespeare***, the name can bring back scary memories of dull assignments from their school days which is misconception. Teachers need not be afraid to use literature in language teaching.

In everyday life, literature tends to be something unique by itself. If one is a voracious reader they can read out a simple poem or a short fiction, then the idea of bringing literature into your language classroom!

But teaching literature in the target language can be much more exciting than it seems at first glance. There's no other medium that provides the same vantage point on the world of language and culture than literature. Not only does it reveal insider information about people, history, words and expressions… but it also reveals deeper truths about human nature that transcend language barriers.

So it's worth being fearless, putting a brave front and taking the literary approach after all. One can be sure that once we get a few pointers about why and how to teach literature, we can be just be eager and ready to share that excitement with students.

Usually, students who study only English Language emphasising on reading and writing skills, sometimes fail to see the point of studying English literature, especially if they have no plans to study English or Translation at a University.

Literature can introduce students to a range of aspects, open new vistas not only of the English Language but also of the English culture.

There are parts of the English culture that are "encapsulated" by English Literature.

This is quite evident when studying the works of Shakespeare or other writers, poets and playwrights of previous centuries.

As teachers are the *basic pillars* to reach students through literature trying to attract them to this area that can be interesting to anyone and everyone.

We as teachers should think about unique ways to keep their students interested and engaged. Also we must consider that we are the ones who can make students get closer to literature so that they can realise that it is not as abstract and dry as they think, trying to avoid their frustration or disinterest.

When studying Literature, learners can improve not only language such as vocabulary, but also the language that can be used for specific purposes.

The study of Literature can provide them with a fresh and creative angle with which they can approach their studies in particular and their lives in general.

English Literature makes the learners hone their creativity and imagination, interacting with the text themselves, and playing different literary roles. Literature must be presented as an authentic task, so that the learners can get through the texts discovering their different learning styles.

They can live literature not only read but also memorize it. Plays can be represented so that students can be immersed in a different time and into a different person.

By following certain histories or eras presented through English Literature, it is possible to understand how contemporary Western culture has developed into the present. Reading new words and new phrases can increase any one's lexicon.

"The study of literature offers many ways to improve literacy: it gives access to language, reading, and writing, a shared culture and one's own self."

Through the reading of literary texts, students can develop their autonomy and independence, making hypothesis and guessing unfamiliar words. The use of authentic literary texts gives learners the opportunity to connect to real world issues at the time they can learn true values for their lives.

There are specific ways in which students can expand their language, culture and personal growth from the experience of learning literature in a second language:

- **Literature offers experiences that can only be accessed through the target language.**

  The comical play on words in a scene of Shakespeare won't have any meaning if explained out of context. Learning experiences give students a fore front to history and culture which would be impossible to replicate otherwise.

- **Literature gives students a unique understanding of the target culture.**

  Teaching idioms from a textbook is not memorable. But reading an idiom in a conversation between two strong characters will surely imprinted in the students' minds. They can also witness life through the eyes of soldiers, preachers, writers in a way that gives insight into the people and events that shaped the culture.

- **Literature makes seamless connections between the language and other subjects.**
  We all know that language is more relevant to students if they can connect it to other disciplines like art, history, math or instruction in their native language. Sometimes language teachers need to explicitly spell out the connections between the target language and these other disciplines. But literature makes these connections effortless, allowing to teach to the whole person rather than targeting language solely.

- **Literature provides better understanding of the universal nature of language.**
  Literature brings that point to life. Students will see examples of metaphors, symbols, puns and analogies that make them think about similar constructions in their native language and the universal truths behind them, connecting language and personal growth in a meaningful way.
  Literature has been proven as a great tool to approach English language and English culture. Even though there are students and teachers that consider it as too abstract or theoretical, we, as teachers and students in the past have experienced the situation and we can say that it works. It gets you closer to the language, culture as well as customs and it is always a way of learning how to write and spell correctly. Teachers should make literature interesting for students.

  In the contemporary world where technology has progressed leaps and bounds and the global connect has contracted in the form of e- mails and social media; Communication has played a vital role in globalization under one common platform which is *English language.* The internet has, to a great extent, bridged the gap, not only between people but nations. It has made communication accessible and literary work popular, especially when literature was not getting its due!

K. Saritha , Lecturer in English
 e- mail ID : sarithasamuel6@gmail.com
contact no:9866094540

Rashmi Pulizala, Lecturer in English
e- mail ID : rashmi2016march@gmail.com
contact no: 9676692418

Department of English
St. Ann's College for Women
Mehdipatnam
Hyderabad
Telangana
500 028

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Evaluation of Antimicrobial and Antioxidant Activity of *Acanthus ilicifolius* Leaf Extract

G. Beulah, Deepthimahanthi Divya[1], K. Govinda Rao, N.S. Sampath Kumar[2] and G. Simhachalam*

Department of Zoology and Aquaculture, Acharya Nagarjuna University, Nagarjunanagar-522 510, India
[1]Department of Zoology, St. Ann's College for Women, Hyderabad-500 028, India
[2]Department of Biotechnology, Vignan's Foundation for Science, Technology & Research, Vadlamudi-522 213, India
*E-mail: chalamgp@yahoo.co.in

Abstract: Mangroves posses potential bioactive molecules because their ecosystem is always under stress leads to the production of diversified compounds such as antimicrobial, antioxidant properties, anti-cancer etc. The antimicrobial and antioxidant potential of the *Acanthus ilicifolius* leaf extract was investigated. The antimicrobial screening by agar diffusion method confirmed that the aqueous extract showed the maximum activity against *Bacillus* sp., and petroleum ether extract showed against *Staphylococcus* sp. and *E. coli*. By using DPPH free radical scavenging method, antioxidant activity of *A. ilicifolius* was determined and the same was compared with ascorbic acid. The tested extract showed that the highest DPPH scavenging ability with the half maximal inhibitory concentration (IC_{50}) value 116.75 µl ml^{-1}. Overall, the petroleum ether extract of *A. ilicifolius* showed good results revealing higher antibacterial and free radical scavenging activity and also as a potential source for herbal drug formulation and nutraceutical.

Key words: Antibacterial, Antioxidant, *Acanthus ilicifolius,* Mangroves, Aqueous extracts

The active constituents of plants identified as having high levels of antimicrobial and antioxidant potent molecules (Shaheena et al 2019, Nikunz et al 2016, Raju et al 2019). Among the plants mangrove is a rich and underexploited ecosystem with great diversity for discovery of novel bioactive compounds (Eswaraiah et al 2020). Mangrove forests are incredibly important ecosystems occur worldwide in the tropics and subtropics (Ayyakkannu et al 2019) and habitat to a large variety of fish, crab, shrimp species and provide high nutritional food to the coastal communities around the world (Nazeer et al 2014). Pathogens are infectious agents to the fish and are always present in aquaculture as well as in wild fisheries are the main reason for the shortage of fish meat and there by nutrient inadequacies. The fish pathogens include *Clostridium botulinum, Staphylococcus aureus, Salmonella* sp*, Escherichia coli, Vibrio* sp*, and Aeromonas* sp etc that are naturally living in the fish habitat (Agnieszka 2018). Now-a-days oxidative stress also plays an important role in shaping fish's responses to the change in surrounding and their survival (Kumar et al 2019). Oxidation process is one of the most important path for producing free radicals in organ systems (Kollati et al 2017, Singh et al 2019) and Antioxidants are the substances that prevent the damage to the cell caused by free radicals (Kumar and Nazeer 2012, Shaker et al 2017). Most of the mangroves like *Avicennia officinalis*, one of the dominant species in India (Ajit et al

2016) showed promising antibacterial activity against many fish bacterial species (Mulia et al 2018). The information on the antimicrobial, antioxidant potentials of *A. ilicifolius* is limited. So, the present study was designed to test the possible antimicrobial and antioxidant activity of leaf extracts of *A. ilicifolius*.

MATERIAL AND METHODS

Sample collection and extraction: The leaves of the mangrove plant *Acanthus ilicifolius* were collected from Gilakaladindi (16°8'50" N and 81°9'37" E) Machilipatnam, Andhra Pradesh. The plant species was identified and confirmed by the herbaria of BSI, Hyderabad. The leaves were cleaned in the running tap water and shade dried. The dried leaves further subjected to a fine coarse powder with the help of blender. Leaf extracts were prepared using Soxhlet apparatus using acetone, diethyl ether, petroleum ether and water as solvents. The extracts were collected and stored in sterile containers.

Phytochemical screening: Phytochemical screening (Thirunavukkarasu et al 2010) was carried out by using different tests as per the standard test methods for tannins (Bromine water), alkaloids (Mayer's), saponins (Frothing), flavonoids (Alkaline reagent), glycosides, terpenoids, steroids (Salkowaski**),** phenols (Ferric chloride),reducing sugars (Fehling's), proteins (Biuret test),cardiac glycosides (Keller-Killiani test), phytosterols (Salkowaski**)** and

anthraquinones (Sravani et al 2015).

Antimicrobial assay: Acetone, diethyl ether, petroleum ether and water extracts of *Acanthus ilicifolius* leaf were tested against *Staphylococci cusaureus, Bacillus thuringiensis, Escherichia coli* by agar well diffusion method. Mueller-Hinton agar (MHA) plates were inoculated with an inoculum size of 106 colony forming units (c.f.u) ml^{-1} of bacteria. Into the agar medium with diameter of 8 mm wells were made and filled with 1 mg ml^{-1} of leaf extract. The plates were then incubated for 24 hrs. Amikacin an antibiotic at a concentration of 50 µg m^{-1} was used as a positive control and the solvent was treated as negative control. After incubation, the diameters of the growth inhibition zones were measured in mm (Guntur et al 2018).

Antioxidant assay: The radical scavenging activity of *Acanthus ilicifolius* was done by DPPH (2-diphenyl-2-picrylhydrazyl) radical method. Different concentrations of extract were mixed with 5 ml of a DPPH methanol solution (40 µg ml^{-1}). The reaction mixture was mixed thoroughly and incubated for 30 min at room temperature in dark. The absorbance of the mixture was measured at 517 nm by UV-Vis spectrophotometer and free radical inhibition (%) was calculated using

% of DPPH RSA= Mean Abs (control-sample)/control x 100

RESULTS AND DISCUSSION

Phytochemical screening: The qualitative estimation of the leaf extracts of *Acanthus ilicifolius* in acetone, diethyl ether, petroleum ether and water showed the presence of phytochemicals like terpenoids, tannins, saponins, flavonoids, glycosides, steroids, phenols, proteins, alkaloids, phytosterols, anthraquinones, cardiac glycosides and reducing sugars (Table 1). *A. ilicufolius* is reported as rich source of long chain alcohols, terpenes, steroids, triterpenoids and saponins (Arumugam 2015). These biologically active compounds act by different mechanism and show antimicrobial action viz., glycosides, saponin and alkaloids serve as defense mechanisms against predation by many microorganisms, insects and herbivores with a distinct mechanism (Vijaya et al 2017). Flavonoids are complex molecules capable of solubilizing extra cellular proteins within the bacterial cell walls and also exhibit antioxidant properties (Vicente et al 2018). Steroids have antibacterial properties act on membrane lipid and cause liposomal leakage (Barrett et al 2018). Tannins intervene with the protein synthesis by binding with proline rich proteins (Fraga et al 2020). Plant phenolic compounds can act as antioxidants (Sulaiman et al 2012).

Antibacterial activity: The petroleum ether and aqueous extracts were selected for assessing anti microbial activity

(Table 2). Petroleum ether extract exhibited highest potential of antibacterial activity against *S. aureus* (3 mm) and *B. thuringiensis* (0.3 mm) than aqueous extract. Antibacterial activity of *A. ilicifolius* root extract against *Enterobacter cloacae* (19.66 mm), *Escherichia coli*, *Bacillus subtilis* and *Staphylococcus aureus* with inhibition zone of 16.66 mm each (Sharief et al 2017). The solvents used for extraction always play a major role in inhibitory effect along with the parameters adopted (Sepahpour et al 2018). Ravi et al (2011) reported the antimicrobial activity of *A. ilicifolius* leaf extracts against *Staphylococcus* where in petroleum ether extract from the present study showed better zone of inhibition.

Antioxidant activity: Petroleum ether extract inhibited high DPPH radical with varying concentrations compared to aqueous extract; the 125 µg ml^{-1} concentration itself inhibited 43.43 % of radicals which is nearly equal to that of standard Ascorbic acid (49.98%) where in aqueous extract needed 250 µg ml^{-1} concentration. Among the two extracts petroleum ether showed 116.75 µl ml^{-1} and water had 208.59 µl ml^{-1} inhibitory effects (Table 3). The presence of phenolics and alkaloids contribute directly to the overall antioxidant activity (Velmani et al 2016, Jara et al 2017) due to their neutralizing

Table 1. Qualitative estimation of secondary metabolites of *Acanthus ilicifolius* leaf extract

Secondary metabolites	Acetone	Diethyl Ether	Petroleum Ether	Water
Tannins	-ve	+ve	+ve	-ve
Saponins	+ve	+ve	+ve	+ve
Flavonoids	+ve	-ve	+ve	-ve
Glycosides	+ve	-ve	-ve	+ve
Terpenoids	-ve	-ve	+ve	-ve
Steroids	+ve	+ve	+ve	+ve
Phenols	-ve	-ve	+ve	+ve
Proteins	-ve	-ve	-ve	-ve
Alkaloids	+ve	+ve	+ve	+ve
Phytosterols	+ve	+ve	+ve	+ve
Anthraquinones	-ve	-ve	-ve	-ve
Cardiac glycosides	+ve	+ve	+ve	+ve
Reducing sugars	-ve	-ve	+ve	+ve

+ve: Presence, -ve: Absence

Table 2. Antibacterial property of leaf extracts of *A.ilicifolius*

Extract	Diameter of zone of inhibition (mm)		
	S. aureus	*B. thuingiensis*	*E. coli*
Petroleum ether	3.0	0.3	0.2
Water	1.3	1.9	0.4

Table 3. Inhibition of DPPH radical and IC_{50} for plant extracts of *A. ilicifolius* (Mean ±SD)

Concentration (µl ml⁻¹)	% inhibition by plant	
	P. Ether extract	Water extract
31.25	34.39± 0.98	09.67±1.35
62.5	43.43±0.85	30.80±1.04
125	57.46±0.64	41.60±0.87
250	68.06±0.48	64.73±0.53
500	88.76±0.16	91.90±0.12
IC_{50}	116.75	208.5

ability against free radicles (Arumugam et al 2015). Chakrabarti et al (2008) reported strong reducing power of *C. decandra* bark extract is due to the presence of high phenolic content in its stem. *In vitro* antioxidant studies (Reddy et al 2016) revealed that the methonolic extract of *Lumnitzer arecemosa* and *Exocecaria agallocha* exhibited good radical scavenging ability.

CONCLUSIONS

The petroleum ether and aqueous extracts of *A. ilicifolius* were investigated for their phytochemical constituents, anti bacterial and anti oxidant activity. The Petroleum ether extract showed high anti microbial and anti oxidant activity. This is a basic report that provides sufficient evidence for carrying out further research on the selected plant by phytochemical purification and characterization of the extract to identify potential novel bioactive compounds to assess their therapeutic applications in aquaculture industry.

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Phytochemical Screening of *Avicennia marina* Extracts and its Evaluation for Antioxidant and Antimicrobial Activities

Deepthimahanthi Divya, G. Beulah[1], K. Govinda Rao[1], N.S. Sampath Kumar[2] and G. Simhachalam[1*]

Department of Zoology, St. Ann's College for Women, Hyderabad-500 028, India
[1]Department of Zoology and Aquaculture, Acharya Nagarjuna University, Guntur-522 510, India
[2]Department of Biotechnology, Vignan's Foundation for Science, Technology and Research, Vadlamudi-522 213, India
[*]E-mail:chalamgp@yahoo.co.in

Abstract: In the present study, the mangrove plant *Avicennia marina* was screened for presence of phytochemical and evaluated for antibacterial and antioxidant activities. The leaves of *Avicennia marina* were collected from Gilakaladindi mangroves, Machilipatnam, Andhra Pradesh, shade dried, extracted secondary metabolites successively with Acetone and Diethyl ether using soxhlet method. The antioxidant activities of *A.marina* were assessed by DPPH radical scavenging and FRAP assays with varying concentration. The zone of inhibition of *A.marina* leaf extract on bacteria *viz., Klebsiella pneumonia* (0.8 mm and 0.5 mm)*, Pseudomonas aureginosa* (1.2 mm and 0.5 mm)*, Staphylococcus aureus* (1.8 mm and 0.7 mm) and *Bacillus thuringiensis* (0.2 mm and 0.4 mm) authenticated the antimicrobial activity. The leaf extract of *A. marina* has greater antimicrobial and antioxidant properties hence it can be used in the treatment of infectious diseases caused by antibiotic resistant pathogenic microorganisms.

Keywords: Antibacterial activity, Antioxidant activity, *Avicennia marina,* Mangrove plant

Mangroves are salt-tolerant plants and spread in the inter-tidal region in the tropical and subtropical zones, and widely distributed over the east and the west coasts of India (Ayyakkannu et al 2019). Mangroves are the world's rich and productive ecosystem with variety of flora and fauna (Ajit et al 2016). They also possess various chemical compounds that could be used as natural drugs for many epidemic diseases (Behrooz Alizadeh Behbahani et al 2018). All these plants are well adapted to grow and flourish in harsh environmental conditions and are periodically submerged by tidal water (Packialakshmi and Kanimozhi 2014). Mangrove wetland serves as spawning grounds for many estuarine fishes and migratory birds (Thomas et al 2017).

Most of the mangrove plants such as *Bruguiera* sp.*, Avicennia* sp.*, Rhizophora* sp.*, Suaeda* sp.*, Exocoecaria* sp., etc known for the presence of antibacterial, antifungal and antiviral compounds. Studies have also shown that the mangroves have been used for centuries as a good remedy for many infectious diseases, because they contain high therapeutic components (Sravani et al 2015) and medicinal plants obstinately provide inexpensive treatment both in recent and folk systems of medicine (Revathi et al 2014). Mangrove plants containing secondary metabolites like alkaloids, phenolics, saponins, flavonoids, steroids and terpenoids have been characterized and tested successfully for toxicological, pharmaceutical and ecological importance. Increasing rate of antibiotic resistant microorganisms is one

of the severe problems of the world and therefore, new drugs have to synthesize from medicinal plants having antimicrobial and antioxidant properties (Sahoo and Dhal 2009). Natural drugs derived from plants are easily available, safer and effective.

The traditional system of natural medicine is often used to provide first-line treatment for many infectious diseases in fish and many other aquatic organisms (Kumar and Nazeer 2012, Kollati et al 2017). Fishes are usually infected by pathogenic microorganisms such as *Pseudomonas, Lactococcus, Enterococcus, Aeromonas* and *Streptococcus* can cause infectious diseases like columnaris, ich, gill disease, fish dropsy and fin rot disease and considered as one of the main reason of fish mortality (Pandey et al 2012). Current strategy for controlling the diseases in fish by interfering the life cycle of a parasite is mainly based on chemotherapeutic agents, but these chemicals appeared are not sufficient to prove the remedial action to control the parasitic infection.

Avicennia marina is commonly called as white or grey mangrove present nearly in all the coastal areas of India. It belongs to the family Avicenniaceae and grows as a shrub up to a height of ten meters. It is a medicinal plant used mainly against many diseases like rheumatism, paralysis, asthma and ulcer. The fruits, leaves and bark of *A.marina* possess antibacterial, antifungal, antiviral properties against different pathogens. The presences of phenolic compounds shown

divulge biological activities like anti-inflammatory, anticarcinogenic activities and antioxidant activity (Nikunz Naidu et al 2016, Dhayanithi et al 2012). Among several other mangrove species, three types of *Avicennia* sp. such as *A. marina A. alba*, and *A. officinalis* are common mangrove plants that found in the coastal areas, studies on *Avicennia officinalis* proved to contain various phytochemicals, anti-diabetic and free radical scavenging activities (Zhou et al 2018). The aim of the current research work is to investigate the novel constituents present in *A.marina* to treat infectious bacterial diseases in fish by carrying out detailed study on screening of phytochemicals in the selected plant extracts and its evaluation for antibacterial and antioxidant properties.

MATERIAL AND METHODS

Collection and identification of plant material: The leaves of *Avicennia marina* were collected from the mangroves of "Gilakaladindi", Machilipatnam, Andhra Pradesh which extends from 25- 27 ' N and 55-65'E. Collected leaves were washed, shade dried and ground to fine powder using motor and pestle prior to analysis of biological activity. Genus and Species of the plant material was identified by Botanical Survey of India.

Preparation of extracts: The solvent extracts of *A. marina* were prepared using acetone and diethyl ether as 80% solvent and sterile water (450 ml) for 4 h at 30°C using soxhlet apparatus. The extracts were stored in room temperature until further usage.

Phytochemical screening: Preliminary screening for phytochemical constituents of *A. marina* leaf extracts were carried out using qualitative tests to identify the alkaloids, flavonoids, steroids, terpenoids, saponins, glycosides, tannins, reducing sugars and phenols using standard protocols (Harborne 1984).

Antimicrobial assay: The antimicrobial assay of *A. marina* leaf extracts were examined by agar well diffusion method against *Klebsiella pneumonia* (MTCC 3384), *Pseudomonas aureginosa*, (MTCC 1688) *Staphylococcus aureus* (MTCC737) and *Bacillus thuringiensis* (MTCC 1953). Mueller Hinton Agar medium (MHA) was poured in to the petriplates by using an inoculum with a size of 10^6 colony forming units (c.f.u)/ml of bacteria (Perez et al 1990). The wells are made MHA plates with a borer of 8mm. Then the extracts at a concentration of 1 mg ml^{-1} were used for evaluating the antibacterial assay. Amikacin, as an antibiotic was used as a positive control at a concentration of 50 µg ml^{-1}. The prepared plates were incubated at 37ºC for 12 h for initiating the growth of the bacteria. After incubation, the zonal inhibition was observed and the wells were measured and tabulated.

Minimum inhibitory concentration (MIC): The minimum inhibitory concentration (MIC) was determined by the broth dilution method (Chattopadhyay et al 1998). Muller-Hinton broth is used and the crude extracts are diluted in twofold serial dilution by taking antibiotic (Amikacin mg ml^{-1}) as +ve control. Microorganisms were directly suspended in saline solution from a 24 h old suspension of Mueller–Hinton broth. The suspension turbidity was adjusted to match 0.5 McFarland standards which correspond to 2.4 × 108 cfu/ml. For broth dilution tests, 0.1 ml of the standardized suspension of bacteria (2.4 × 108 cfu ml^{-1}) was added to each tube at a final concentration of 0.005–5.12 mg/ml, and incubated at 37 °C.

Antioxidant Assays

DPPH (1, 1-diphenyl-2-picrylhydrazyl) free radical scavenging capacity of *A.marina* extracts was determined and compared with ascorbic acid as positive control (Lu and Foo 2000). Freshly prepared DPPH solution was mixed with extracts in 1:1 ratio and observations were recorded using spectrophotometer at 517 nm after 30 mins of incubation. The radical scavenging assay percentage is calculated as:

% of DPPH RSA= Mean Abs (control-sample) / control x 100

Ferric reducing antioxidant power (FRAP) activity of the given extracts was measured according to the method proposed by Benzie and Strain (1996). FRAP reagent was prepared by mixing in 25 ml acetate buffer (30 mm; pH 3.6), 2.5 ml TPTZ solution (10 mm) and 2.5 ml ferric chloride solution (20 ml). The mixture was incubated for 15 min at 37 °C before use. Ascorbic acid was employed as a standard in this assay, and its calibration curve was obtained by using its concentrations ranging from 15.62 ul ml^{-1} to 500 ul ml^{-1} in methanol. To 2.85 ml FRAP reagent in a test tube, 150 µl plant sample (1mg ml^{-1}) or standard was added. The mixture was incubated for 30 mins in the dark, and its absorbance was measured at 593 nm. The results were recorded as µg of ascorbic acid equivalents (AAE) per ml.

RESULTS AND DISCUSSION

Extraction and photochemical studies: The selected plant extracts of *Avicennia marina* was found to be negative for phenols, proteins, and flavonoids, a moderate level of terpenoids and steroids were detected. Both the solvent extracts found to be positive for glycosides, cardiac glycosides, phytosterols and reducing sugars (Table 1). Previous studies reveal that *Avicennia marina* and *Avicennia officinalis* contain higher concentrations of phytochemicals like alkaloids, terpenoids, flavonoids and phenols. The extracts of *A.marina* contains saponins and proteins and those are absent in *Avicennia officinalis* (Ramanathan 2012).

(Raffat et al 2017) proved that *A. marina* seeds contain coumarins, flavonoids, saponins, sterols, tannins and glycosides and carbohydrates with very low amounts of carbohydrates. Earlier workers also reported that acetone, ethyl acetate and *N, N* dimethyl formamide (DMF) are very effective in extracting various antioxidants (Shaheena et al 2019).

Antibacterial and minimum inhibitory concentration (MIC): The antibacterial activity of *A. marina* extracts has showed significant antibacterial activity against different pathogenic bacteria namely *Klebsiella pneumonia*, *Pseudomonas aureginosa*, *Staphylococcus aureus* and *Bacillus thuringiensis* with different concentrations and results are shown in (Table 2). The acetone and diethyl ether extracts of *A.marina* has highest inhibitory activity against *K. pneumonia* (0.8 & 0.5 mm)*, S. aureus* (1.8 & 0.7 mm) and *P. aureginosa* (1.2 & 0.5) and lowest inhibition against *B. thuringiensis* (0.2 & 0.4 mm). Amikacin at 50 µg ml^{-1} exhibited highest concentration in acetone extract against *K. pneumonia* species*.*

The results indicated that the extracts of mangrove plant *A.marina* reduced the growth of all tested organisms and also it is observed that by increasing the concentration of leaf extracts the diameter of inhibition zone is also increasing, however the eloquent inhibitory concentration was observed in *K. pneumonia* (375 µg ml^{-1}). The methanol leaf extracts of *A. marina* exhibited highest antimicrobial activity (21 mm) against *p. aeruginosa* reported by (Thamizharasan and Anbusaravanan 2016). In comparison the acetone leaf extracts of plant *Ipomoea tuba,* showed the highest zone of inhibition against *Micrococcus luteus* with concentration of (200 µg) and least inhibitory effect was found against *M. luteus* at the concentration (25 µg). Earlier workers were reported in acetone, ethyl acetate and methanol leaf extracts of *A. officinalis* showed antimicrobial activity with (21-25 mm) zone of inhibition against various pathogenic bacterial strains such as *S. aureus*, *K. pneumonia, P. aeruginosa, A. tumefaciens*, *S. mutans and E.coli.* (Bakshi and Chaudhuri 2014). Therefore, the studies revealed the importance of selected mangrove plant *A. marina* extracts to control and inhibit the growth of pathogenic bacteria and is used as a natural drug to reduce the infectious diseases in fish and other organisms (Nazeer et al 2014).

Free radical scavenging activity: Antioxidants provide a vital role in maintaining the oxidative resistance (Kumar and Nazeer 2012). According to the results, the test extracts of *A. marina* exhibited better free radical scavenging activity and showed a notable dose dependent inhibition of DPPH activity (Table 3) with the IC50 concentrations at 294.48 µl ml^{-1} and 373.63 µl ml^{-1}. The percentage of inhibition is relatively

increased in the concentrations (31.25-50 µl ml^{-1}) of leaf extracts than the standard ascorbic acid 49.98±0.39*.* Previous studies reveal the maximum scavenging activity 95.62% in leaf extract of *Lumnitzera racemosa* at 200 ug ml^{-1} concentration and minimum scavenging activity 37.78% found in leaf extract of Suaeda nudiflora at 50 ug ml^{-1} concentration reported by (Eswaraiah G et al 2020). (Packia Lincy et al 2013) reported that the IC50 values in DPPH and ABTS radical scavenging activity in methanol extracts of *A.*

Table 1. Phytochemical analysis in *Avicennia marina* leaf extracts

Name of the test	Acetone extract	Diethyl ether extract
Tannins	+ ve	+ ve
Saponins	+ ve	+ ve
Flavonoids	- ve	- ve
Glycosides	+ ve	+ ve
Terpenoids	- ve	+ ve
Steroids	+ ve	- ve
Phenols	- ve	- ve
Proteins	- ve	- ve
Phytosterols	+ ve	+ ve
Cardiac glycosides	+ ve	+ ve
Reducing sugars	+ ve	+ ve

Table 2. Antibacterial activity and MIC in *Avicennia marina*

Name of the organism	Acetone extract		Diethyl ether extract	
	Zone of inhibition (mm)	MIC µg ml^{-1}	Zone of inhibition (mm)	MIC µg ml^{-1}
Klebsiella pneumonia	0.8	375	0.5	128
Staphylococcus aureus	1.8	187	0.7	140
Pseudomonas aureginosa	1.2	120	0.5	180
Bacillus thuringiensis	0.2	140	0.4	127

Table 3. DPPH radical scavenging assay values at IC50 concentrations (Mean ±SD)

Concentration (ug ml^{-1})	% of inhibition in *A.marina*	
	Acetone extract	Diethyl ether extract
31.25	06.90±1.40	01.79±1.47
62.5	27.65±1.08	09.50±1.36
125	38.20±0.92	23.56±1.15
250	48.68±0.77	44.23±0.83
500	70.25±0.44	06.81±0.58
Ascorbic acid	49.98±0.39	49.98±0.61
IC 50 Value	294.48	373.63

Table 4. FRAP test for *A.marina* with acetone and diethyl extracts (Mean ±SD)

Concentrations (ug ml^{-1})	AAE (ug ml^{-1})	
	Acetone extract	Diethyl ether extract
15.62	04.5±0.09	1.50±0.08
31.25	24.66±0.15	10.50±0.11
62.5	34.00±0.18	22.50±0.15
125	43.16±0.21	32.00±0.18
250	51.33±0.24	42.66±0.21

marina showed increased values (13.24-27.96 ug ml^{-1}) at 1 ug ml^{-1} concentration.

The reducing power of leaf extracts could be estimated from their ability to reduce TPTZ-Fe (III) complex to TPTZ-Fe (II) (Bidve et al 2019). The present study *A. marina* leaf extracts showed moderate amount of ferric reducing antioxidant activity at different concentrations (15.62 ug ml^{-1} – 250 ug ml^{-1}), compared to the standard drug ascorbic acid (Table 4). However, in comparison of two extracts of *A. marina*, the activity shown by FRAP in acetone is relatively higher (0.45-51.33 ug ml^{-1}) than the diethyl ether extract (1.50-42.66 ug ml^{-1}). Earlier studies reveal the reducing power of ferric in *A. marina* leaf extracts at different concentrations (0.04 and 0.15 mg ml^{-1}) were closely related to concentrations (7 and 0.5 mg ml^{-1}) of synthetic antioxidant, TBHQ reported by Molaee et al (2017). Therefore, the study indicates the mangrove plant *A.marina* is rich in antioxidants. (Najwa Ahmad Kuthi and Norazah Basa 2019) reported in the methanol extracts of *Pellacalyx axillaris* exhibited highest FRAP with equivalent values at varying concentrations (1.0 mm, 2.69 and 2.97 ug ml^{-1}).

CONCLUSION

The present research work indicated the potential properties of selected mangrove plant *Avicennia marina* leaf extracts as a bio-control for infectious bacterial fish diseases, and confirmed its antibacterial activity. Phytochemical studies proved the presence of alkaloids, tannins, saponins, phytosterols, terpenoids in phytochemical evaluation. Studies also revealed that, the extracts have potent antioxidant properties. However, further research is needed to confirm its effect against live infected fishes.

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A STUDY ON CHALLENGES AND PROSPECTS OF FORENSIC ACCOUNTING IN INDIA

K. Pranathi[1], C. UdayaSree[2]
[1]Lecturer in Commerce, St. Ann's College for Women,
Santosh Nagar, Mehdipatnam.
Email id: pranathi.goud@gmail.com
[2]B.Com III year Student, St. Ann's College For Women,
Santosh Nagar, Mehdipatnam.
Email id: udayacumsali@gmail.com

ABSTRACT

Forensic accounting is a rapidly growing area of accounting, where Forensic Accountants deter, detect and investigate frauds in financial reporting. Forensic accounting is a combination of Accounting, Auditing and Investigation skills. Initially, forensic accountants were appointed by government agencies such as, the CIA, the FBI, and the IRS, to uncover and investigate leading frauds. Later on they became financial detectives employed by management to uncover fraudulent financial reporting and misappropriated assets. They help the companies in accomplishing their organization's objectives, with a systematic, disciplined approach to evaluate and improve the effectiveness of risk management, control and governance processes. Opportunities for forensic accountants are increasing rapidly in recent years due to a rise in white collar crime. The objective of the paper is to study the Challenges faced by the forensic accounting investigator in fraud detection on the basis secondary data collected from various sources.

Keywords: Forensic accounting, fraud, Forensic Accountants, Investigation tools, Challenges and Prospects.

1. INTRODUCTION

FORENSIC

The word forensic comes from the Latin word forēnsis, meaning "of or before the forum." Means

➢ Relating to the use of science or technology in the investigation and establishment of facts or evidence in a court of law
➢ It is a scientific tests or techniques used in connection with the detection of crime.

WHAT IS FORENSIC ACCOUNTING?

Forensic accounting utilizes accounting, auditing and investigative skills to conduct an examination into the finances of an individual or business. Forensic accounting provides an accounting analysis suitable to be used in legal proceedings. Forensic accountants are trained to

look beyond the numbers and deal with the business reality of a situation. Forensic accounting is frequently used in fraud and embezzlement cases to explain the nature of a financial crime in court.

DEFINITIONS OF FORENSIC ACCOUNTING

According to AICPA (1993): "Forensic accounting is the application of accounting principles, theories, and discipline to facts or hypotheses at issues in a legal dispute and encompasses every branch of accounting knowledge."

HISTORICAL PERSPECTIVE OF FORENSIC ACCOUNTING

Maurice E. Peloubet first time used the term Forensic Accountant in 1946 in his essay "Forensic Accounting: Its Place in Today's Economy." Archaeological studies disclose that, during 3300-3500 BC, accountants of Egypt, were involved in the prevention and detection of fraud. During 18th century close relationship developed between accountancy and legal profession. Many amendments to financial statement disclosure can be attributed to frauds in the corporate. In 1930s an American Eliot Ness was credited to bring down gangster Al Capone, but his case was based on the investigative work done by Elmer Irey, an accountant with the Internal Revenue Service that ensured Capone's conviction for tax evasion. He was probably America's first high-profile forensic accountant.

FORENSIC INVESTIGATION

Forensic investigation is the gathering and analysis of all crime-related physical evidence in order to come to a conclusion about a suspect. Investigators will look at blood, fluid, or fingerprints, residue, hard drives, computers, or other tec\nology to establish how a crime took place.

FORENSIC ACCOUNTING CAN SOMETIMES BE REFERRED TO AS FORENSIC AUDITING

PURPOSE CAN BE

- ➢ A forensic audit can be conducted in order to prosecute a party for fraud, embezzlement or other financial claims
- ➢ In addition, an audit may be conducted to determine negligence.

FRAUD AUDITING

A fraud audit is a detailed examination of the financial records of a business, with the intent of finding instances of fraud. This procedure is more detailed than a normal audit, since some types of fraud involve such small amounts of money and other assets that they might fall below the standard materiality threshold. The task of the auditor is to gather evidence regarding a

fraud, which may also result in acting as an expert witness during subsequent legal proceedings.

Fraud auditors often go outside the books of accounts to find fraudulent transactions. This may include reviewing receipts, not only from the company, but from customers as well. Any inconsistencies in these numbers could help uncover an act of fraud. These auditors also interview employees, customers and sometimes clients to find out if a fraud has taken place.

WHAT IS FRAUD?

Fraud is a type of criminal activity, defined as:

The Companies Act, 2013 defines Fraud in relation to affairs of a company or anybody corporate, to include,

- ➢ any act
- ➢ omission,
- ➢ concealment of any fact
- ➢ abuse of position committed by any person or any other person with the connivance in any manner
  - ➢ with intent to deceive
  - ➢ to gain undue advantage from, or
  - ➢ to injure the interests of,
  - ➢ the company or
  - ➢ its shareholders
  - ➢ or its creditors or
  - ➢ any other person

Whether or not there is any wrongful gain or wrongful loss;

- ➢ "wrongful gain" means the gain by unlawful means of property to which the person gaining is not legally entitled
- ➢ "wrongful loss" means the loss by unlawful means of property to which the person losing is legally entitled.

2. LITERATURE REVIEW

- ➢ Apostolou, Hassell, and Webber (2000) identified forensic accounting as the use of accounting, auditing and investigative abilities to provide expert support in legal matters. Forensic accounting is a branch of accounting which deals with present, existing or projected disputes and it is therefore suitable for legal assessment and provides a guarantee.
- ➢ Zysman (2004) stated that Forensic Accounting utilizes accounting, auditing and investigative skills when conducting any kind of investigation. Equally critical is the ability

> to respond immediately and to communicate financial information clearly and concisely in a courtroom setting.

> Bhasin (2007) finds that the services of forensic accountants are in great demand in the areas which include criminal investigation, settlement of outgoing partner; settlement of insurance claims, detection of employee fraud and case relating to professional negligence.

> Mehta and Mathur (2007) posited that forensic accounting involves a financial detective with a suspicious mind, a financial bloodhound, someone with a sixth sense that enables reconstruction of past accounting transactions and an individual who looks beyond the numbers.

> Mazumder (2011) remarked that law enforcement personnel in recent years have become more aware of white-collar crimes and have lacked the training and expertise in combating such crimes. They are better trained at combating violent or personal behavior crimes, but now it has the responsibility to expand its knowledge and expertise into the economic crimes area.

> Ms. ShrutiGarg (2014) Forensic Accounting and its Relevance in Indian Context". She explained the Forensic Accounting and its importance in Indian Economy. As per her opinion, A forensic Accountant deals with analysis, interpret, summarize and showed the financial data and business issues. But for the completion of these duties forensic Accountant must be aware and have knowledge about the legal concept and procedure. Basically Forensic Accountant deals with the criminal investigation, shareholder and partnership disputes, Employee fraud investigation etc.

> NishaChaturvedi (2015), "Forensic Accounting in India (Future Prospects for its Application".Under this study she examined the various uses of Forensic accounting and its role to solving the financial problems and how to reduce the financial frauds India.

> Lakshmi P., Ganesh Menon (2016), they proved the forensic accounting as a checkmate for corporate Fraud. As per their study forensic accounting helpful to overcome the corporate financial crimes or scams.

3. OBJECTIVES OF THE STUDY

> To study the challenges faced by forensic accountants in fraud detection.
> To understand the conceptual framework of financial fraud.

4. RESEARCH METHODOLOGY

> The study is explanatory in nature and the sources of data is secondary data which is collected from different journals, research papers, related magazins and other related websites on Forensic Accounting.

CORPORATE FRAUD

'Corporate Fraud refers as a violation of the Internal Revenue Code and related statutes committed by large, publicly traded corporations and/or by their senior executives' - The Internal Resources

Service, Department of the USA of the Treasury. In addition, corporate frauds have gradually become very complex in nature.

CORPORATE FRAUD CAN BE CLASSIFIED IN THREE BROAD AREAS

- ➢ **Financial fraud or Accounting** fraud consists of falsifying financial information by fudging the books thereby misleading the investors and is usually perpetuated by management. capitalising expenses, side deals, swap transactions, channel stuffing, accelerated revenues and deferred expenses are the most popular accounting schemes.

- ➢ **Self-dealing by corporate insiders** is mostly related to misappropriation of corporate assets by senior executives such as loans granted to senior management that are never intended to be repaid, failure to disclose forgive loans, reimbursed personnel expenses and extra ordinary personnel expenses charged to the company. In addition, it may be insider trading, misuse of corporate property for personal gain, kickbacks and individual tax violations related to self-dealing.
- ➢ **Obstructive conduct** is falsifying testimony to regulators, erasing computer files, shredding documents, creating or altering document to support illegal conduct.

MAJOR FRAUDS AND SCAMS IN INDIA

Recently, forensic accounting has become an indispensable tool for investigation in India due to rapid increase in white-collar crimes and our law enforcement agencies do not have the expertise or time to uncover such stock market fraud or bank fraud or cyber fraud. Scams like Satyam (2009), Commonwealth games (2010), Railgate scam (2013), Tatra Truck scam (2011) and most recently coal block allocation scam (2012) and Chopper scam (2013) in India had improves the scope for forensic accounting in India. According to the Corruption Perception Index 2016 Report released by Transparency International, India's rank is 79th among 176 countries affected with corruption.

FRAUDS AND SCAMS	YEAR REPORTED	LOSSES SUFFERED (RS.)	FRAUDS AND SCAMS	YEAR REPORTED	LOSSES SUFFERED (RS.)
Harshad Mehta Securities Market Scam	1991	5,000 Crore	Commonwealth Games Scam	2010	2,342 Crore
Fodders Scam (CharaGhotala)	1992	980 Crore	Adarsh Housing Society Scam	2010	NA
C. R. Bhansali Scam Sahara	1995	1,200 Crore	India Pariwar Investor Fraud Scam	2010	25,000 Crore
The UTI Scam	2001	4,800 Crore	Tatra Truck Scam	2011	750 Crore
Ketan Parekh Securities Market Scam	2001	1,250 Crore	Coal Block Allocation (Coalgate Scam)	2012	1.856 Billion

Stamp Paper Scam	2005	600 Billion	Railgate Scam	2013	90 Lakhs
Satyam Scam	2009	10,000 Crore	VVIP Chopper deal Scam	2013	362 Crore

Forensic accounting has played a major role in all the above scams. It has been used as an investigative tool (rather than a preventive tool) where the documents pertaining to the scams are sent to the forensic laboratory. Many scams could have been detected and prevented in the early stages if forensic auditing is made mandatory in India.

PROCESS OF FORENSIC ACCOUNTING

Below are four steps that forensic accountants follow when investigating financial crimes or issues.

Map out the investigation: The first step involved in investigative accounting is preparation. A good forensic accountant will start with the end in mind and will create a master plan to help guide his or her research and analysis.

THE INVESTIGATIVE PLAN INCLUDES TASKS LIKE THESE

- ➢ Meeting with the client to understand and capture his/her view of the dispute
- ➢ Probing deeper to understand all aspects of the dispute
- ➢ Locating and organizing all relevant financial documents
- ➢ Identifying anomalies and inconsistencies
- ➢ Postulating motives and seeing if any evidence supports them

GATHER EVIDENCE TO SUPPORT THE CASE

Evidence is the most important outcome of investigations. Forensic accountants have a specialized skill set which allows them to analyze complex and opaque financial issues. One can imagine how much more complex the process becomes when a perpetrator has purposefully covered his or her tracks and "cooked the books."

In order to find irregularities, investigative accounts use various tools and techniques, which include the following:

- ➢ Accounting skills needed to read financial records
- ➢ Deep analysis needed to turn up clues, identify patterns, and make the numbers tell the truth
- ➢ Applying computer-assisted auditing techniques
- ➢ Testing internal financial controls in order to find loopholes that may have allowed fraud to occur
- ➢ Interviewing individuals involved in the case
- ➢ Observing suspects in order to detect suspicious behavior and evidence of fraud

➢ Interrogating suspects in order to get them to admit their misconduct

PREPARE THE REPORT

Once the forensic accountant has gathered all necessary evidence, he or she must lay it all out in a clear and orderly fashion. Providing a comprehensive report may seem like a boring detail, but the data collected and explained within it could mean the difference between a positive outcome at the trial or a lack of evidence.

The report must hold up even when the opposing party seeks to discredit or disprove it. A typical forensic accounting report provide answers to questions like these:

➢ Was there fraud or misconduct? If so, what type?
➢ When did the fraud or misconduct happen (or not happen)?
➢ How did the misconduct happen and how was it concealed?
➢ Who were the responsible parties?
➢ What were total loss and damages caused by the misconduct?

The report may also help to prevent further fraud by revealing the tactics or loopholes used by the perpetrator and recommending improvements to internal auditing controls.

TESTIFY IN COURT

Forensic accountants gather evidence and may also serve as testifying experts in trials. Their expert testimony gives the report greater credibility and context because they can explain the data and findings in clear, concise language.

The world of accounting has its own terminology and slang, and a skilled forensic accountant can help translate dense language into layman's terms.

TECHNIQUES OF FORENSIC ACCOUNTING FOR EXAMINING FRAUDS

Some of the techniques involved in Forensic Accounting to examine the frauds are:

BENFORD'S LAW

It is a mathematical tool and is one of the various ways to determine whether variable under study is a case of unintentional errors or frauds. Use of parametric test called the Z test is carried to measure the significance of variance between two populations i.e. Benford's percentage numbers for first digit and observed percentage of first digit for a particular level of confidence. Benford's Law is not affected by scale invariance and is of help when there is no supporting document to prove the authenticity of transactions.

DATA MINING TECHNIQUES

It is a set of assisted techniques designed to automatically mine large volumes of data for new, hidden or unexpected information or patterns. Data mining techniques are categorized in three ways: Discovery, Predictive modeling and Deviation and Link analysis. It discovers the usual knowledge or patterns in data, without a predefined idea or hypothesis about what the pattern may be i.e. without any prior knowledge of fraud. .It explains various affinities, association, trends and variations in the form of conditional logic. In predictive modeling, patterns discovered from the database are used to predict the outcome and to guess data for new value items. In Deviation analysis the norm is found first, and then thoseitems are detected that deviate from the usual within a given threshold (to find anomalies by extracted patterns). Link discovery has emerged recently for detecting a suspicious pattern. It mostly uses deterministic graphical techniques, Bayesian probabilistic casual networks. This method involves "pattern matching" algorithm to 'extract' any rare or suspicious cases.

RATIO ANALYSIS

Another useful fraud detection technique is the calculation of data analysis ratios for key numeric fields. Like financial ratios that give indications of the financial health of a company, data analysis ratios report on the fraud health by identifying possible symptoms of fraud. Three commonly employed ratios are:

> ➢ Ratio off the highest value to the lowest value
> ➢ Ratio of highest value to second highest value
> ➢ Ratio of current year to previous year.

Using ratio analysis, a financial expert studies relationships between costs and some measures of production, such as units sold, dollar of sales or direct labor hours. For example, to arrive at overhead costs per direct labor hour, total overhead costs might be divided by total direct labor hours. Ratio analysis may help a forensic accountant to estimate expenses.

THEORY OF RELATIVE SIZE FACTOR

It highlights all unusual fluctuations, which may be routed from fraud or genuine errors.RSF is measured as the ratio of the largest number to the second largest number of the given set. In practice there exist certain limit (e.g. financial) for each entity such as vendor, customer, employee, etc. These limits may be defined or analyzed from the available data- if not defined. If there is any stray instance of that is way beyond the normal range, then there is a need to investigate further into it. It helps in better detection of anomalies or outliners. In records that fall outside the prescribed range are suspected of errors or fraud. These records or fields need to relate to other variables or factors in order to find the relationship, thus establishing the truth.

MAJOR THREATS TO THE ORGANISATION

➢ **Fraudulent Financial Reporting:** Financial statement fraud is deliberate mis-representation, misstatement or omission of financial statement data for the purpose of misleading the reader and creating aσ false impression of an organisation's financial strength.

➢ **Related Party Transactions:** The most frequent type of transactions that require regulatory action areσ concessionary loans to related parties, payments to company officers for services that were either unapproved or non-existent, transfer of funds through overvalued purchases of assets investments and sales of goods or services to related entities in which the existence of the relationship was not disclosed.

➢ **Procurement Fraud:** It refers as the unlawful manipulation of the procurement process to acquire goodsσ /services obtain an unfair advantage, avoid an obligation or cause a loss to public property during the procurement process by public servants, contractors or any other entity involved.

➢ **Tax Evasion and Money Laundering:** Money laundering is a criminal offence aimed at presenting wealth of illicit origin or the portion of wealth that has been illegally acquired or concealed from the purview of tax and other authorities, as legitimate, through the use of methods that hide the identity of the ultimate beneficiary and the source of the ill-gotten profits.

➢ **Fraudulent expense claims:** This is the easiest means of stealing some money from an organisation.σ Employees inflate their expense reimbursements and derive gains from the company.

➢ **Misappropriation of Assets:** Asset misappropriation schemes include both theft of company assets, suchσ as cash or inventory and the misuse of company assets such as using a company car for a personal trip.

➢ **Payroll Fraud:** It is a theft of cash from a business via the payroll processing system and it can occur inσ various ways like advances not paid back, buddy punching, ghost employees, pay check diversion, pay rate alteration, unauthorised hours etc.

KEY CHALLENGESFACED BY FORENSIC ACCOUNTANTS IN INDIA

➢ Forensic accounting is developing field of financial fraud detection. There is acute shortage of qualified accountants with adequate technical knowledge of forensicissues in India.

➢ In India, most of the financial fraud cases involved politicians, so it is crucial to find evidences against them.

➢ Indian judicial system still follows age old British judicial system. It is expensive to bring the matter to court and hire expert advocates.

➢ Due to liberalization and fast moving economy, more and more investors from foreign countries invest in India and so, it is difficult to sue financialfraudster from other countries.

➢ Because of continuous adoption of new techniques of Information and Technology by fraudster, it is difficult to Forensic Accountant to cope up with them.

- ➢ Forensic accounting is an expensive field compared to other investigativefields.
- ➢ It is not mandatory for companies to appoint forensic accountant in companies.
- ➢ There is no specific guideline or act on forensic accounting in India.
- ➢ Limited ability to demonstrate ethical behaviour or compliance with an ethics programme.
- ➢ Lack of segregation of duties

PRECAUTIONARY MEASURES

- ➢ Formulating a strong anti-fraud programme like Leadership setting the tone, Fraud prevention, Ethics Code and Whistleblower or complaint mechanism, Training/awareness programmes to employees
- ➢ Enhanced focus on identifying fraud risks
- ➢ Continuous monitoring using data analytics
- ➢ Due diligence on third parties

5. CONCLUSION

The review article examine several issues including the nature of fraud, forensic accounting, as well as scams associated. To conclude Forensic accounting as a discipline is an interesting area and can be highly useful to both the society and the investigator. On the basis of this finding, the paper concludes that forensic accounting services provide firms with the necessary tools to deter fraudulent activities

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Bringing Real-World Microbiology Experiences to Undergraduate Students in Resource-Limited Environments

Y. Aparna[1], K. Anuradha[1], Ch. Jyothi[2], K. Sri Manjari[3] and Anil Kumar Challa[4,5]*

[1] Bhavan's Vivekananda College of Science Humanities and Commerce, Secunderabad, India, [2] St.Ann's College for Women, Hyderabad, India, [3] University College for Women, Osmania University, Hyderabad, India, [4] Dr. Reddy's Institute of Life Sciences, Hyderabad, India, [5] Department of Biology, The University of Alabama at Birmingham, Birmingham, AL, United States

Undergraduate microbiology curriculum should be amenable to periodic changes to incorporate new developments and ideas. The curriculum should be used not merely as a way to disseminate facts but also as a way to allow students to experience the process of science. In the context of undergraduate microbiology education in Osmania University (Hyderabad, India), existing curriculum does not explicitly allow students to engage in deeper understanding of concepts and understanding of the process of science, both in lecture and laboratory courses. The assessment methods that are currently used are limited in scope as they only test factual recall and superficial understanding of the subject and very minimally assess critical thinking skills. Another factor hampering innovation in the broader context of undergraduate education is the unavailability and inaccessibility to adequate resources. To address the issue of resource-limitations in implementing activities that expose undergraduate students to real-world microbiology experiences, a collaboration between a research institute and two teaching colleges was formed. This collaboration involved teacher and student workshops on exploring microbial diversity using 16S rRNA analysis with a view of blending novel research questions with technical skills in the undergraduate microbiology lab. This effort is an example of educators providing students with authentic experiences and, helping them gain critical knowledge and research skills in microbiology even under resource constraints, and students demonstrating motivation to participate in similar activities in the future. The collaborative effort described here can be a broadly sustainable model to improve overall undergraduate education in relatively resource-limited environments.

Keywords: microbial diversity, undergraduate academic success, resource limitations, faculty professional development, student workshops, primary research articles

INTRODUCTION

The goals of undergraduate biology education can range from enabling students to gain broad knowledge and experiences that will prepare them to be become socially conscious citizens who can effectively contribute to the needs of the community (Ferren and Anderson, 2016; Hatcher, 2011; Association of American Colleges and Universities, 2007; Penn, 2011; Zai, 2015). Learning

spaces should be engaging, enriching and empowering in order to help students achieve these goals. Real world experiences are inherently rich in context, have the power to engage and are relevant. Bringing these real-world experiences into classrooms can significantly improve the quality of teaching and learning (Alberts, 2005).

Basic science education in India at the undergraduate level, unlike technical and professional education, predominantly happens not on University campuses but in over 10,000 colleges affiliated to degree granting Universities under the purview of the University Grants Commission (Government of India and University Grants Commission [UGC], 2019). With a greater emphasis on didactic classroom instruction, and constrained by resource limitations, it is believed that most of these colleges are unable to provide adequate training to their students to meet the needs of academia and industry. In addition, existing problems with the current higher education system in India pointed by Saberwal (2019), pose a challenge to the introduction of newer ideas and methods, especially in biology education. In a meeting report on "Policy framework for catalyzing excellence in science education and research in India" (Lakhotia et al., 2013), the authors state that "...a rejuvenation of the existing undergraduate and postgraduate science education system together with an integration of teaching with high-quality research is also desperately needed" and point to a steady decline of the infrastructure, quality of faculty and the research capacity of the higher educational system as a whole for over three decades. One of their specific recommendations for improving the current state of undergraduate education is to recognize good teaching practices and development of open-ended laboratory work/research projects by students.

While a top down approach via policy statements and national plans have their role in transforming undergraduate education, true transformation is only possible when individual colleges and informed teachers are empowered to take action. Under the purview of University Grants Commission of India (UGC), which is charged with coordination, determination and maintenance of standards of higher education in India, out of all educational institutions in India, about 750 colleges are autonomous allowing them to design and implement small courses or programs by themselves and supplement the University prescribed syllabus. This autonomy allows administrators and teachers the freedom to experiment and pursue alternative pedagogies to develop innovative and exemplary teaching practices and create rich open-ended learning experiences for students.

While extended research internships in an apprentice model are highly desired to provide rich and comprehensive experiences to students, they are not always possible and scalable due to resource constraints. Studies show that well designed shorter experiences can have equally enriching experiences for students (Wei and Woodin, 2011; Frantz et al., 2017). Here, we describe a program developed through a collaboration between a research institute and teachers at two private autonomous colleges to expose undergraduate students majoring in microbiology to real-world microbiology experiences in Hyderabad, India.

PROFESSIONAL DEVELOPMENT PROGRAM – CATALYST FOR TRANSFORMATION

A series of teacher (faculty development) workshops developed by the Center for Advancement of Research Skills (CARS) at Dr. Reddy's Institute of Life Sciences (Hyderabad, India) initiated a dialog between its researchers and undergraduate educators in the local colleges to create specific interventions that would result in changes with respect to student engagement and learning outcomes in college classrooms. Typically, faculty professional development workshops are focused either on discipline-specific content or exclusively on pedagogy. The CARS workshop series made a deliberate attempt to blend these two aspects and placed emphasis on teachers sharing their skill and experiences with their students. The workshops, which were conducted at the research institute, involved teachers assuming student personas to experience a research project while discussing ideas in pedagogy and potential challenges in classroom implementation. The final aspect of the workshops was to create classroom modules or co-curricular activities that they could implement in their respective institutions. A 3-day hands-on workshop on "Exploring Microbial Diversity" was organized for educators with an explicit goal of enabling them to plan and implement student programs that align closely to the existing microbiology curriculum in their respective colleges. Subsequent to the workshop, the research institute provided continuous support to the teachers, as needed, throughout the duration of the student programs in the colleges.

CLASSROOM IMPLEMENTATION

The current undergraduate degree programs in the sciences are offered as 3-year programs in India. Students opt for specific combinations of subjects upon enrolling into college and continue with the same combination for 3 years. For example, students interested in microbiology can select combinations such as Microbiology, Botany, Chemistry (MBC) or Microbiology, Biochemistry and Chemistry (MBiC), Microbiology, Genetics, Chemistry (MGC), or Microbiology, Zoology, Chemistry (MZC). The syllabus for the courses taught in the above programs is framed and prescribed by the Boards of Studies at the degree-granting University. While University constituents and affiliated colleges are mandated to strictly follow these syllabi, the autonomous colleges (which are a focus of this study) have some flexibility in modifying their curricula and syllabi. This also includes the control of 20–30% of student grades within the college, through internal assessments. This autonomy allowed us the freedom to – design and implement the "Exploring Microbial Diversity" workshop for undergraduate students in two colleges.

Following the teacher workshop, we designed a laboratory workflow that is likely to succeed during classroom implementation, keeping in view the academic semester schedules, alignment with the syllabus and available material resources in the colleges. There were differences between the two colleges that resulted

TABLE 1 | Outline of the collaborative program development in two phases.

	Phase I		Phase 2	
			College 1	**College 2**
		Microbiology majors	100 × 3 years = 300	90 × 3 years = 270
		Women	154 (51%)	270 (100%)
		Men	146 (48.6%)	0 (0%)
	Exploring Microbial Diversity by 16S rRNA profiling			
Activity	Teacher workshop		Student workshop	Student workshop
Organizer	Research Scientist		College Teachers (5)	Research Scientist + Teaching Assistant
Venue	Research Institute		Bhavan's Vivekananda College	St. Ann's College for Women
Participants	College Teachers (4)		BSc II year students (30) 23 women; 7 men	BSc I and III year students (15) 13 I year; 2 III year
			6 students per team	3 students per team
Duration	3 days (6 h per day)		1 week (4 h per day)*	2 weeks (2 h per day)**
Assessment	Implementation of student workshops in the colleges		Informal conversations, self-reported student feedback	Poster presentation at the research institute, self-reported student feedback

*Phase I included an undergraduate teacher workshop followed by Phase II where student workshops were conducted at two colleges. * The workshop was split into two sessions of 2 h, in the morning prior to regular classes and in the afternoon after formal college hours; **the workshop was conducted at the end of the formal college hours.*

in unique challenges for classroom implementation. Apart from structural constraints, there were inclusivity issues with respect to student enrollments into the workshop. Since the activities would be conducted beyond the official college hours, only those students who could and chose to stay beyond regular class time could enroll in the workshop (**Table 1**). With regards to the instructional team, two undergraduate instructors from one college who participated in the teacher workshop trained three additional instructors at their own institution to strengthen the program with adequate resources by all means. All the five instructors who were conversant with the laboratory workflow conducted a 3-day trial run at the college to test and ensure proper functioning of chemicals, reagents and equipment. Critical reagents and consumables were supplied by the research institute while the colleges provided the basic laboratory infrastructure. With respect to instruments, micropipette sets, a miniPCR[TM] thermocycler, a blueGel[TM] gel electrophoresis unit and a PCR microcentrifuge were made available on loan from the research institute. They were extensively used in one college while the other college did not need all of them to run the workshops.

EXPLORING MICROBIAL DIVERSITY IN THE LABORATORY

One of the specific goals of this program was to integrate practical laboratory experiments prescribed in the University syllabus into a research project, changing the focus from laboratory techniques to investigating authentic, real-world biological questions (**Supplementary Table 1**). This program was designed as a short and intense 1- to 2-week workshop focused on exploring bacterial diversity using 16S RNA T-RFLP workflow (Schütte et al., 2008). Similar workflows can be seen in successful programs such as Small World Initiative and Tiny Earth

(Basalla et al., 2020), and Urban Barcode Project (Henter et al., 2016). Students at both colleges worked in teams to explore bacterial diversity in an environmental sample of their choice. They were required to provide reasons for their choice of samples brought for analysis; the samples included raw unpasteurized milk, spoiled fruits and vegetables, cockroach gut, water and soil from a lake and a polluted local river (Musi), iron rust, compost pit, cow dung and effluent from a local chemical industry. All microbial cultures were handled under aseptic conditions and proper lab safety measures were ensured starting from inoculation of cultures to their eventual disposal by autoclaving; safety considerations were discussed before each step of the workflow was executed. After practicing the micropipetting technique and making serial dilutions, students streaked the samples on LB media plates to isolate single bacterial colonies and use those single colonies to perform PCR amplification of the 16S rRNA gene locus using Universal primers (27F and 1492R; Frank et al., 2008). PCR amplicons thus obtained were analyzed using either agarose or polyacrylamide gel electrophoresis (PAGE) before and after digestion with restriction endonucleases such as AluI, DpnI, MboI, and TaqI, which are frequent (4 bp) cutters. The banding patterns corresponding to different bacterial colonies were compared to infer diversity. A brief introduction to genome databases and bioinformatics tools enabling sequence analysis was also given toward the end of the program. *In silico* restriction digestion of 16S rRNA gene sequences from different bacterial species using NEB/web cutter[1] allowed the students to compare their experimental results with computational analysis. This workflow allowed us to cover several molecular methods and techniques that undergraduate microbiology students benefit from learning and are part of the prescribed syllabus.

Despite the short duration, students were not only able to go through the complete workflow but were able to collect

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[1]http://nc2.neb.com/NEBcutter2/

FIGURE 1 | Stacked bar graph showing student rating the impact of college courses and "Exploring Microbial Diversity" workshop on their research and scientific attitudes and skills. **(A)** Rating of college experiences by students who did not participate in the workshop (control) and **(B)** rating of college and workshop experiences by student participants. 1 corresponds to the lowest rating and 5 to the highest rating.

data and document their findings. The collected data included geographical location and nature of samples, serial dilutions and the corresponding density of bacterial colonies on media plates, presence of PCR amplified 16S rRNA gene fragment after gel electrophoresis, observed RFLP patterns, and simulated RFLP patterns using gene sequences from genomic databases. Documentation of their findings became especially important since students had to present the summary of their work at the conclusion of the activity.

Alongside the content of the student activity, several pedagogical considerations were deliberated during the teacher workshop and implemented in the colleges. We made teamwork an essential component so as to enable peer interactions and enhance peer learning. Moreover, the entire activity was based on experiential, hands-on laboratory work beginning from sample collection, planning and executing individual steps of the experiments, and ending with comparing and discussing the results. Therefore, student interactions became essential for successful completion of the experiments and thus were ensured. During the course of the laboratory work, we emphasized on the process of science alongside the execution of instructions and protocols. Questions regarding the underlying principles and logical reasons behind various steps in the experimental protocol were raised at appropriate times.

IMPACT OF THE STUDENT WORKSHOPS ON STUDENT LEARNING

To understand and assess the impact of this program, we collected self-reported student feedback at the end of the workshop using a questionnaire with response options in the Likert-scale and open-ended questions. The questions aimed at understanding students' views on different aspects of the curriculum, teaching and learning practices, and gain of cognitive skills, both in their regular college courses and the workshop. Another set of first- and second-year students who did not attend the workshops were given a similar set of questions focusing only on

their college experiences, and their anonymous responses were used as "controls." A total of 41 students from the two colleges who participated in the workshops (91% response rate) and 102 students from the "control" group completed the questionnaire.

With regards to rating the impact of college courses and the workshop on academic experiences, especially developing science identity and process skills, there is a clear distinction in the number of students who gave a rating of "5" (highest) attributed to regular courses/classes and the "Exploring Microbial Diversity" workshop; 49% of the workshop participants rated the workshop experience at "5"; 31.7% of the workshop participants rated the impact of their college experience at "5"; only 17.65% of the students in the control group rated the impact of college courses at "5" (**Figure 1**).

The questions focused on three broad categories of students' learning experiences; key questions from each category included (a) Emphasis, how much either the college or the workshop emphasized on memorization, analysis, synthesis and application (**Supplementary Figure 1**); (b) Impact, how either the college or the workshop helped them to think independently and analyze concepts (beyond memorization), actively engaged them with academic learning, enhancing their interest in the subject, and gave them full autonomy to plan and execute lab experiments (**Supplementary Figure 2**); and (c) Involvement, how well they prepared for regular college courses and for the workshop by reading study material, writing extensive notes, reading beyond the prescribed material, and discussing course material with classmates (**Supplementary Figure 3**). The analysis of all the responses generally indicated that the students gained more from the workshop experience, when compared to their experiences in regular classes. However, the responses do not allow us to draw specific conclusions with regards to differences between student "involvement" with various activities (**Supplementary Figure 3**).

When asked to comment (as free responses) on the differences they noticed between regular college classes and the workshop, students felt that the workshop enabled them to learn things which they were not able to do and analyze in

regular courses, made them to ask questions, think independently, understand concepts better and to apply them effectively. Many students mentioned that they would look forward to more such workshops during their undergraduate studies. One student comment captures the essence of many of these responses – "*Without doubt the "Exploring Microbial Diversity" workshop was better than classes as it involved doing something we do not usually do in classes. Practical experience is the best teacher. But I think a little emphasis on research papers for reference should be included as part of the [laboratory] practicals, and if possible, in classes, as that will not only intensify the research component of the subject but also will give fundamental exposure to scientific literature.*" An analysis of the responses represented in the stacked bar graphs, in conjunction with the free responses, suggests that the workshop experience had a positive impact on most of the students with respect to their engagement with discipline-specific content and also science process skills.

AN OUTSIDE PERSPECTIVE ON STUDENT PERFORMANCE

Students from one of the colleges (**Table 1**) had an opportunity to present their work to scientists (principal investigators, postdoctoral researchers, graduate students and research associates) at the research institute. This was the only interaction they had with the students. Judging from the presentations, in spite of the variation in the quality, the scientists felt that the students were well prepared, interested, enthusiastic, and demonstrated an ability to understand and critically analyze primary research and experimental findings.

TEACHER PERSPECTIVES

The teachers were aware of the limitations and shortcomings related to both material and time resources. Nevertheless, they were encouraged by the fact that we were able to create and implement these pilot programs to engage students, give them an opportunity to explore research questions and enable them to gain deeper insights into real-world microbiology. The key learnings from the first implementation of the "Exploring Microbial Diversity" workshops are that material limitations can be overcome with creative program design in collaboration with the research institute, and time limitations can be overcome by scheduling the programs around regular college hours.

DISCUSSION

There are several well documented microbiology education programs aimed at providing authentic experiences to undergraduate students (Jordan et al., 2014; Henter et al., 2016; Staub et al., 2016; Basalla et al., 2020). However, in India there are strong perceptions about the difficulty of implementing such programs to systematically improve undergraduate programs. These perceptions are strengthened in the light of

poor infrastructure and inadequate facilities in colleges, and time constraints imposed by rigid curricular outcomes. Most of these challenges are likely to be common and widespread in similar academic settings, therefore we need to examine practical ways to overcome these challenges by creating focused college faculty development programs aimed at fostering collaborative programs with research institutes by way of sharing of their resources.

The pilot study described here provides a model for redesigning undergraduate microbiology education in resource-limited environments such as small, private, autonomous, University-affiliated colleges in India. The program focused on inquiry and problem solving in an environment that fosters teamwork and collaboration amongst students. In an academic system rife with intense competition, this is an alternative to expose students to working in teams and enhancing their learning experiences. It is vital that any of these efforts aimed at improving undergraduate STEM education should include rigorous designs and assessments that will help us evaluate learning outcomes of students and thereby the effectiveness of programs (Linn et al., 2015).

The success of high-standard abbreviated "nanocourses" that provide high-impact learning experiences to students (Bentley et al., 2008) provide templates upon which shorter, workshop-style courses can be developed and implemented that address the constraints of time and material resources. Scaffolding authentic research across the curriculum (Sieg et al., 2019) by way of short workshops can be an effective way to leverage academic autonomy and overcome the said constraints. With either internal support from college administration or external support through collaboration with research institutes, teachers can design workshops that cater to the knowledge and skills needs of students while spreading their efforts to implement these workshops. If limited physical presence in colleges is imposed, lecture/discussion-based instruction can happen using synchronous or asynchronous online platforms, while the in-person time can be spent in laboratories to gain important hands-on skills. Scientists in research laboratories are also likely to get involved and collaborate with college teachers to organize such short student programs.

The most significant factor that made this pilot study feasible is the autonomy (even in the limited sense) the participating colleges had in terms of creating and implementing small programs, which allowed the undergraduate instructors to engage in professional development at a research institute. The research institute's participation was rooted in their interest in ensuring that these students who are prospective recruits are well trained to fuel their future scientific research programs. The active collaboration between research institutes like Cold Spring Harbor Laboratory, Howard Hughes Medical Institute's (HHMI) Janelia Farms, and undergraduate teaching institutes have given rise to exemplary educational innovations. Similar partnerships, on a smaller scale, can be built and nurtured to support educational activities in local undergraduate institutions. In fact, these smaller partnerships are likely to be stronger and become successful because of greater connectedness. In India, research laboratories belonging to the Council for Scientific and Industrial Research

(CSIR), Department of Biotechnology (DBT), Indian Council for Medical Research (ICMR), Indian Council for Agricultural Research (ICAR) are spread across the country and have a wide variety of microbiology-related research programs, which can provide modest support through knowledge- and resource-sharing. This support can result in deep and far-reaching impact on the quality of undergraduate microbiology education.

As long as colleges are willing to find creative ways to use even a limited amount of autonomy at their disposal to innovate in their classrooms, and research institutions with greater resources at their disposal are willing to become enthusiastic support-ers and enablers of high-quality educational experiences, there is immense opportunity for students to engage in authentic learning experiences.

CONCLUSION

With India unveiling a new National Education Policy in 2020, there is a promise to revamp the entire education landscape in the country. As alluded to earlier, while policies do have a top-down influence on the system, true change is possible when the teachers and colleges are developing and practicing exemplary work at the grassroots. The collaborative model showcased here has given the necessary impetus to the teachers to continue providing rich authentic, real-world microbiology experiences to undergradu-ate students. We hope that our modest effort and its positive impact on student experiences will inspire and motivate several such local initiatives in resource-limited settings.

The "Vision and Change in Undergraduate Biology Education: A Call to Change" document has recommendations about utilizing innovative pedagogy and the integration of authen-tic research experiences into individual courses and biology programs overall to ensure that all undergraduates can experi-ence the processes, nature, and limits of science. However, there are several undergraduate institutions with limited resources that are unlikely to provide such experiences to their students. Our Perspective article showcases a collaborative model where a research institute collaborated with two teaching colleges to devise and implement a small program that was effective in providing an authentic research experience to undergraduate students that can be sustainable. The model can be used by other undergraduate institutions with either perceived or real resource limitations.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

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ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Research Committees of Bhavan's Vivekananda College and St.Ann's College for Women. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

AKC designed the programs and study. YA and KA contributed equally to the implementation of the program. YA, KA, ChJ, KSM, and AKC contributed toward preparation of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2020.589405/full#supplementary-material

Supplementary Figures 1–3 | Summary of student responses to the questionnaire. The left panels (in cream box) represents data from the control group (n = 102). The second and third columns correspond to responses we obtained from students who participated in the "Exploring Microbial Diversity" workshop (41/45 students completed the questionnaire, 91% response rate); second column corresponds to student/workshop participants experiences in regular college courses, while the third column corresponds to their experiences in the workshop. All data are represented as percentages. Student responses relating to emphasis on memorization, analysis, synthesis, and application (**Supplementary Figure 1**); impact of college courses or workshop on independent thinking, analysis (beyond memorization), active engagement, enhancement of academic interest, and academic autonomy (**Supplementary Figure 2**); student involvement with content (**Supplementary Figure 3**).

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Determination of oil and grease present in the Hussain Sagarlake, Hyderabad, Telangana, India

Anitha and S. Kedarini

ST. Ann's College for Women, Mehdipatnam, Hyderabad, Telangana, India

ABSTRACT

Oil and grease includes fats, oils, waxes, and other related constituents generally found in wastewater. If these compounds are not removed before discharge of treated wastewater, oil and grease show impact on biological life in surface waters and can cause thick films. The aim of the present study is to estimate the amount of oil and grease present in the Hussain Sagar Lake, tank bund, Hyderabad, Telangana. Samples were was collected early in the morning. For this study chemicals like Petroleum ether, Sulfuric acid and Ethyl alcohol were used. Presence of oil and grease was taken as an important criteria to check the quality of water. According to the General standards for discharge of environmental pollutants and ENVIS, CPCB the oil and grease concentration should not exceed 10 mg/L max.

Introduction

Oil and grease means mixture of substances which are commonly used in anthropogenic activities. Industry and daily activities.vehicles, illegal dumping, spills, and disposal of cooking oils are some of the most common sources of oil and grease. Oil and grease can reduce oxygen levels in water which can cause serious threat to aquatic animals. Compounds like petroleum hydrocarbons found in oil and grease can affect human kidneys, livers, and blood and increase the risk of cancer.

The presence of oil and grease is an important parameter for water quality. In water surface films and shoreline deposits are the result of oil and grease which leads to environmental degradation, and also shows impact on human health.

Depth is 32 feet. It is one of the tourist spot in the Hyderabad. This raised interest to check its water quality by taking a parameter like oil and grease.

There are three methods for estimation of grease and oil in the water they are (i) the partition-gravimetric method (ii) partition infrared method, and (iii) the solvent extraction method. In Partition-Gravimetric method, dissolved oil and grease is extracted from water by intimate contact with trichlorotrifluoroethane; petroleum ether (40/60) or hexane.

High initial viscosity is the characteristic feature of grease.Srilankan standard Institute is recommending 0.2 mg/L as a maximum allowable limit for Oil and Grease contamination (petroleum product) in Drinking water (SLS 614, 2013). But in 2014, Ministry of health gazetted a standard for Bottled water and they recommending 2.0 mg/L as a maximum permissible limit.

Materials and Methods

Water sample was collected from the Hussainsagar lake, Tankbund, Hyderabad, Telangana. 250 mL of water sample was taken in volumetric flask which

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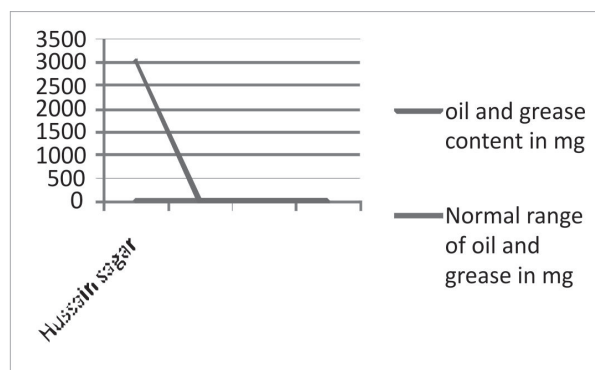
Fig. 1.

capacity is 500 mL. 10 mL of concentrate sulphuric acid and 50 mL of petroleum ether was added to it. Shake the solution properly and then transfer it to the separatory funnel. Allow the funnel to stand for 30 minutes without any disturbance. After 30 minutes 2 distinct layers are formed, the upper layer is petroleum ether layer and lower is water layer. Filter it and collect petroleum ether in pre weighed glass beaker (W_1). Place the whole content in the water bath until the petroleum ether gets evaporated. Allow it to cool and note down the (weight W_2).

Results and Discussion

The oil and grease content in the sample can be calculated as, Oil and Grease (mg/L) = ($W_2 − W_1$) 1000/volume of the sample
where,
W_1= Internal weight of the beaker (g)
W_2 = Final weight of the beaker (g)
    The amount of oil and grease present in the water sample is calculated by using the above formula
    Approximately 10% of Oil and grease are present in wastewater oils of animal orvegetable produce

similar effects to oils spills (Fondriest Environmental Inc). Presence of oil and grease in water in high content shows toxic effect on aquatic life, mainly in seawaters. From the above study it was observed that the amount of grease and oil present in the lake is very high and it was shown in the Fig. 1. According to the General standards for discharge of environmental pollutants and ENVIS, CPCB, India the oil and grease concentration should not exceed 10 mg/L max.

Conclusion

The presence of oil and grease is an important parameter for water quality. In water surface films and shoreline deposits are the result of oil and grease which leads to environmental degradation, and also shows impact on human health. In this study it was observed that the amount of grease and oil present in the lake is very high it exceeded the limit.

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Plant Protocol / Environmental Science Methodology

ప్రభుత్వ డిగ్రీ కళాశాల

సదాశివపేట, సంగారెడ్డి జిల్లా, తెలంగాణ.

భావవీణ ప్రత్యేక సంచిక

భారతీయ సాహిత్యం – చతుర్విధ పురుషార్థాలు

అంతర్జాతీయ సదస్సు

తేది : 08-02-2020

ప్రత్యేక సంచిక – ప్రధాన సంపాదకురాలు :

డా. ఎస్. బేబిరమణి

శాఖాధిపతి, తెలుగు విభాగం,
ప్రభుత్వ డిగ్రీ కళాశాల, సదాశివపేట,
సంగారెడ్డి జిల్లా, తెలంగాణ.

జ్ఞానాదేవతుకైవల్యమ్

- శ్రీదేవి కాశవఝల, శాఖాధిపతి, సంస్కృత విభాగం, సెయింట్ ఆన్స్ మహిళా కళాశాల, మెహదీపట్నం, హైదరాబాద్.

ఆచారాత్ జాయతే ధర్మః ధర్మాదర్థః వర్ధతే ।
అర్థాత్ కామమవాప్నోతి కామాత్ మోక్షముచ్యతే ॥

నిరంతర సదాచారమే ధర్మానికి మూలము. నిరంతర ధర్మాచరణ వలన అర్థము (సంపద) సిద్ధిస్తుంది. ధర్మ బద్ధముగా సిద్ధించిన అర్థమును వినియోగించి ధర్మ బద్ధములైన కోరికలను నెరవేర్చుకోవలెను. ఈ విధమైన ఋజు ప్రవర్తనము మోక్షసాధనకు మార్గమును సుగమము చేయును.

మోక్షము అనగా ఏమి? :

ఈ జగత్తు పాంచభౌతికమైనది. జీవపరిణామక్రమాన్ని గమనించినట్లైతే-

'ఆకాశాద్వాయుః । వాయోరగ్నిః । అగ్నేరావః ।
అద్యఃపృథివీ । పృథివ్యా
ఓషధయః । ఓషధీభ్యోన్నమ్ । అన్నాత్పురుషః । "

అనగా 'ఆకాశము నుండి వాయువు । వాయువు నుండి అగ్ని । అగ్ని నుండి జలము । జలము నుండి పృథ్వీ । పృథ్వీ నుండి ఓషధులు । ఓషధుల నుండి అన్నము । అన్నము నుండి పురుషుడు వృద్ధి చెందారు'. దీనిని అనుసరించి అవసరమైన అన్ని విషయాలు అమరిన తరువాతే జీవుని ఉత్పత్తి జరిగింది. ఐతే ఈ జీవుడు పుట్టుకకు ముందు ఎక్కడ ఉన్నాడనే ప్రశ్న ఉదయిస్తుంది. దానికి సమాధానంగా జీవుడు ఆత్మ స్వరూపంగ పంచ భూతాలలో లీనమై విశ్వాంతరాళాలలో సంచరిస్తూ కర్మ విపాకం కలిగిననాడు పంచభూతతత్వాన్ని కలిగిన భౌతిక దేహంలోకి ప్రవేశిస్తాడు. అనంతరం తన పుట్టుకకు కారణమును మరచి విషయలోలుడై సుఖదుఃఖానుభవాన్ని పొందుతాడు. ఈ స్థితి నుంచి తిరిగి పుట్టుకకు ముందున్న స్థితిని అనగా ఆత్మస్వరూపాన్ని తెలుసు కోవడమే మోక్షము.

మోక్షము గురించి భిన్నాభిప్రాయాలు ప్రచారంలో ఉన్నాయి. మోక్షము అంటే మరణానంతరము పొందునది అని, స్వర్గప్రాప్తియని జన్మరాహిత్యమని సామాన్యుల అభిప్రాయము. అయితే శ్రుతులననుసరించి స్వస్వరూప జ్ఞానమును కలిగి ఇంతర నిష్కళంక ఆత్మానంత ప్రాప్తియే మోక్షమని తెలియుచున్నది. స్వస్వరూప జ్ఞానమును పొందుటకు జీవుడు మొదట అంతర్ముఖుడై అవిద్యతో కూడిన శరీరమును, దానికి సంబంధించిన సుఖదుఃఖ భావాలను గుర్తెరిగి, వాటిని లక్షింపకుండా ఉండడానికి సాధన చేయాలి. ఈ స్థితిలో అశాశ్వతమైన సుఖ దుఃఖముల అనుభవము అవిద్య (అజ్ఞానము) వలన కలుగుతున్నదని గ్రహించగలగడమే మోక్షసాధనకి మొదటి సోపానం. అనంతరం ఆ సుఖదుఃఖ భావములు మనసునకు అంటకుండా సాధన చేయవలసి ఉన్నది. దానికై అవిద్యా నాశనము ముఖ్యము. అవిద్య నశించి నపుడే స్వస్వరూపజ్ఞానము కలుగుతుంది. వీరిని సుఖ దుఃఖాలు, ప్రియాప్రియాలు, మానావమానాలు ఆనం దాన్ని కాని బాధను కాని కలిగించవు. తామరాకుపై నీటిబొట్టువలె ఉంటారు. ఈ విధమైన స్థితిని నిరంతరం అభ్యసిస్తే అది మోక్షమార్గమును సూచిస్తుంది. అందుకే 'జ్ఞానాదేవతుకైవల్యమ్' అన్నారు పండితులు.

మోక్షమునకు మరియొకపేరు 'భూమా'[2], అనగా ఆత్మజ్ఞానము కంటే అన్యమైన విషయమందు ఆసక్తిని కలిగి ఉండకపోవటం.

'యత్ర నాన్యత్పశ్యతి నాన్యత్ శృణోతి, నాన్యద్విజానాతి స 'భూమా' యోవైభూమా తదమృతమ్.

ఏ జీవుడైతే అన్యమును చూడడో, వినడో, ఆ స్థితి 'భూమా', అదే అమృతతుల్యం. ఒక్కమాటలో చెప్పా లంటే అద్వైత భావన. జగద్గురు ఆదిశంకరులు చెప్పి

నట్టుగా 'అహం బ్రహ్మాస్మి', 'తత్వమసి' 'సర్వంఖల్విదంబ్రహ్మ' అనే మహావాక్యాలను అనుసరించి మోక్ష మార్గము వైపు అడుగులు వేయవచ్చు. దీనికై సాధన చతుష్టయమును ఆచరించుట ముఖ్యము. అవి

1. వివేకము, 2. వైరాగ్యము, 3. షట్ సంపత్తులు, 4. ముముక్షుత్వము

వివేకము :

శాశ్వత అశాశ్వత జ్ఞానము వివేకము ద్వారా పొంద వచ్చు. జననమరణాలు ఈ భౌతికదేహానికి చెందినమే గాని ఆత్మకు కాదు. అనే జ్ఞానమును కలిగి ఉండడమే వివేకము. సాధువచన శ్రవణము, వేదాంత సాహిత్య అధ్యయనము ద్వారా వివేకమును సాధించవచ్చు.

వైరాగ్యము :

లౌకిక వ్యవహారముల పట్ల ఆసక్తి లేకపోవడం వైరాగ్య మైనను అది శాశ్వతము కాదు. వివేకము వలన జనించిన వైరాగ్యమే శాశ్వతమైనది. అనగా ప్రాపంచిక విషయాల మధ్య ఉంటూ వాటిపట్ల అనాసక్తిని కలిగి వుండటం. దీనికి ఎంతో సాధన అవసరం.

షట్ సంపదలు :

1. శమము, 2. దమము, 3. ఉపరతి, 4. తితిక్ష, 5. శ్రద్ధ, 6. సమాధానము- ఈ ఆరు సంపదలు జీవి మనో నిగ్రహణకు, క్రమశిక్షణ సాధనకు సహకరించె గుణ ములు. ఏటి సాధనతోనే ఏకాగ్రత సిద్ధించి, ధ్యాన మగ్నులమవుతాము.

శమము : శమము అనగా అంతరిన్ద్రియ నిగ్రహము, కోరికలను నశించపజేయడము వలన వచ్చిన ప్రశాంతత.

దమము : ఇది హేతుబద్ధమైన బాహ్యేన్ద్రియ నిగ్రహము.

ఉపరతి : ఇంద్రియ సుఖాల నుండి మనసును మరల్చి, మోక్షసాధన పైన మనసును లగ్నం చేయడం.

తితిక్ష : సుఖదుఃఖాలను సమభావనతో స్వీకరించే శక్తిని కలిగి ఉండడమే తితిక్ష.

శ్రద్ధ : ఉపనిషత్తులచే ప్రామాణికంగా ప్రతిపాదింప బడిన విషయాలపై విరుద్ధభావన లేక గౌరవం కలిగి ఉండటం. ప్రామాణికమైన గురూపదేశ శ్రవణం మరియు వేదాస్త్రాది గ్రంథ పతనం వలన కలిగితే శ్రద్ధ.

సమాధానము : బ్రహ్మపై మనస్సును నిలిపి మనసు లోని ఆందోళనలను పారద్రోలి మానసిక అస్థిరతల నుండి విముక్తిని కలిగిస్తుంది.

ముముక్షుత్వము :

లౌకిక (అనగా వృద్ధాప్యం, రోగం, సుఖదుఃఖాలు, జననమరణాలు) విషయాలపై మనస్సును నిలుపకుండా ఉండగలగటం. దీనిని సాధించడానికి పరిపూర్ణ జ్ఞానియైన గురూపదేశం అవసరం. గురువు సహకారముతో అంతర్ము ఖుడై ఆత్మసాక్షాత్కారాన్ని చేసుకోవడానికి తీవ్రకృషి చేయాలి. ఆత్మసాక్షాత్కారాన్ని పొందడమే ముము క్షుత్వము.

ఈ సాధన చతుష్టయమును ఆచరించుట ద్వారా బ్రహ్మను తెలుసుకొనవచ్చు. 'బ్రహ్మవిద్బ్రహ్మైవభవతి'' - బ్రహ్మను తెలుసుకున్నవాడు బ్రహ్మ అవుతాడు. ఆ స్థితియే మోక్షము.

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GOVT DEGREE COLLEGE • SADASIVPET

ఆ స్టైల్ లో వరిగెనా వాసి సంకలన కర్తకు వాసికను ఇస్తారికి రి.1000 (కన్నా రూ) ని దారాలును చెలించారి.

వసగించ సభాపుడు తెలుగు,
ఇంగ్లీష, హిందీ, ఆర్థ కాంపర్
drkolliramani@gmail.com లేను
వసగించారు.

PHONE No: 8332932330

Bank Details :

Account Payee : S. BABY RAMANI
Account No. : 62011228955
IFSC Code : SBIN020106
SBI – ICRISAT Branch,
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ఇశ్వరిలో తెలియసాగిన ధర్మబద్ధమైన
నయము నిరంధనకు లేవడి ఇందాలి.
అన్నిటి మానన జీవితానికి సార్థకత
లభిస్తుంది. ఆ నయము నిరంధనకే
చతుర్విధ పురుషార్థాలు. అవి ధర్మార్ధ
కామ మోక్షాలు.

చతుర్విధ పురుషార్థాలు

ధర్మము : మనుసునెట్లైనో మనుసు
వరిపెన ధర్మాలు నది. అవి నత్యము,
అహింస, ఇంద్రియ ని(గ్రహం,
అస్తేయము (దొంగతనము), అపరి(గ్రహము
(ఆమితేముగా ఇతరులనుండి
(గ్రహించులు), కృతి (ధైర్యము), శమము
(మ(ప్తేది, శాంతి, క్షమ, ఓర్పు.

అర్థము : ధర్మబద్ధముగా ధనమును
అర్జించుట

కామము : ధర్మబద్ధమైన కామమును
అనురించుట

మోక్షము : జననమరణాలో చిక్కుకోడిన
ఆత్మ వరమాత్మలో ఐక్యమగుట. ఇట్టి
ధర్మార్థ కామ మోక్షాలను గూర్చి అందరు
సమ(గ్రింగా తెలుసుకోవాలని ఈ సదస్సు.
లక్ష్యం.

భారతీయ సాహిత్యం – చతుర్విధ పురుషార్థాలు

అంతర్జాతీయ సదస్సు

తేది : 08-02-2020

ప్రత్యేక సంచిక – ప్రధాన సంపాదకురాలు :

డా. ఎస్. బేబిరమణి

శాఖాధిపతి, తెలుగు విభాగం,
ప్రభుత్వ డిగ్రీ కళాశాల, సదాశివపేట,
సంగారెడ్డి జిల్లా, తెలంగాణ.

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धर्म कर्म नीती

- डा. बि.दि.वि. क्रिश्न वेणि, अध्यापक, संस्कित विभाग, सेन्ट आन्स महिला कलाशाला, मेहदिपट्नम, हैदराबाद.

परित्राणाय साधूनां विनाशाय च दुष्कृताम् ।

धर्म संस्थापनार्थाय संभावमि युगे युगे ॥ (गीता 4.8)

स्वयं भगवान कृष्ण ने कहा है ,जब जब धर्म का हानी होगा तब तब मै स्वयं इस धरती पर अवतार लेकर धर्म को पुनः स्थपान करता हु ।

हमार समाज इतना धर्म प्रवण है और हमारे धर्म मे समाज एवं यतोचित व्यवहार का इतना ध्यान रखा गया है कि धर्म तथा समाज व्यवहार नीती को पूर्णतः अलग करना संभव नहीं है धर्म तथा समाज व्यवहार के अंतर्गत आनेवाली बहुत सी बाते एसी है ,जो आचार एवं धर्म मे रखी जा सकती है । धर्मोरक्षति रक्षितः धर्म को तुम रक्षा करो, धर्म तुम्हारी रक्षा करेगा ।

"धारयेती धर्मः" अर्थात् धारणकरने (आचरण) योग्य नियमों को हम धर्म कहते है। धर्म सम्पूर्ण जीवन की पद्दति है

Man is social animal.मानव एक सामाजिक प्राणी है । समाज में विभिन्न परिस्थितियाँ के साथ उसे एक दुसरे के संपर्क में आना व रहना पड़ता है । धर्म और कर्म मानव जीवन के दो पेहलू है। किसी वस्तु या व्यक्ति की वह वृती जो उसमे सदा रहे ,उसमे कभी अलग न हो ,उसे धर्म कहा गया है । जैसे आँखों का धर्म है देखना । यहाँ धर्म का अर्थ स्वभाव नित्य नियम है ।

उदा -सत्य ,क्रोध ,परोपकार ,दया ,क्षमा आदि धर्मी एवं आचार नीती मे होते हुए भी समाज एवं व्यवहार से अलग नहीं है । वैसे ही शील ,विनय तथा निराभिमान आदि समाज नीति होते हुए भी धर्म से जुड़े है ।

धर्म कर्म काण्ड से समीप है। स्मृतियों मे आचारों प्रथमो धर्म: कहा गया है। और वर्ण तथा आश्रम के अनुसार उसके अवस्था का निदेश किया गया है। ब्रह्मचारी के लिये गुरुसेवा,स्वाअध्याय- अर्थात् व्यक्तिगत नियमों का पालन कैसे करे, इस दशा मे धर्मा को सीखने के लिये , तथा सिखो से धर्मा को आचरण किस प्रकार करे ,गृहस्थ के लिये दान -धर्मा का आचरणतन तन ,मन ,धन से सम्पूर्ण रूप से व्यक्तीगत,पारिवारिक   तथा समाज के प्रति नियमों का पालन करना,-- वानप्रस्थाश्रम मे निस्वार्थ भावन से पारिवारिक धर्मा को वर्तमान पीडी को सौंप कर मार्गदर्शन करना ही उतामोत्तम धर्म है। सन्यासाश्रम मे वर्तमान पीडी को तथा आनेवाले पीडी को धर्मपथ पर चलने के लिये आदेश देना , प्रथम कर्तव्य है ।

मुनु स्मृति के अनुसार धर्म के दसलक्षण इस प्रकार है

धृति:,क्षमा दमोऽस्तेयं शौचमिन्द्रय निग्रह:

धीविध्य   सत्यम्क्रोधो दशकं धर्म लक्षणं ।।मनु स्मृति -6 .82

पुराणों मे भी धर्म पर प्रकाश डाला गया है । महाभारत मे अनेकानेक धर्म सूक्ष्मो को विवेचन किया गया है ।   "परोपकाराय इदं शरीरं"धर्म को आचरण करने के लिये राजा शिबी ने एक कपोत को बाचने के लिए उसका वजन का अपना जांघ का मांस खण्ड दिया है ।   तथा भीम ने अपने आप को बकासुर को आहार के रूप मे समर्पित होने के लिये , सक्तु प्रस्थस्य महत्वम् मे "अतिथि देवो भाव:"धर्म का विवेचन इस प्रकार किया कि कुरक्षेत्र भूमि मे धर्मराज से पहेली ही एक उच्छ वृती गरीब ब्राह्मण परिवार द्वारा जो सात दिन लगतार आहार न मिलने पर 8 वा दिन जो केवल मुट्ठी भर दाना से परिवार के चार सदस्य खाने के लिये एक पल पहले जो अतिथि कुटिया मे प्रवेश करने से वे अति सन्तुष्ट हो कर, उस अतिथि को सत्कार किये है । प्राणजाते समय मे भी कर्ण ने अपने कवच कुण्डलो को दान किया, इतना ही नहीं बड़ो का आदर करने के लिये पांडव अपने राज्य को भी छोड़ दिये है ।मित्र धर्म को निभाने के लिये भगवान कृष्ण ने राजा जब सुधामा को स्वयं स्वगत किया है । कर्ण अपने प्राण भी छोड़ दिया वीर शूर

होने पर भी सत्य और धर्म पालन करने के लिये आज्ञात वास मे अपने आप दास बनकर समय बिताये है ।

पद्मा पुराण मे ब्रह्मचर्य ,सत्य ,तपस्या ,दान ,नियम,क्षमा ,पवित्रता ,अहिंसा ,शन्ति ,अस्तेय कहे गए है । मत्स्य पुराण आदि सनातन पुराणो आदि मे अद्रोह ,अलोभ ,दम,दया,ब्रह्मचर्य ,सत्य,अक्रोध ,क्षमा ,और धृति सनातन के मूल तत्व है ।

मानव कर्मशील प्राणी है। इस विस्तृत कर्म स्थल मे उसका जीवन चक्र न केवल सजग रहता है,स्वप्न मे भी निरंतर गतिशील रहता है । कर्म करने के बिना एक क्षण भी नहीं जी सकता है ।

धर्म -समय परिस्तिथियों के अनुसार परिवर्तन एवं परिवर्धन होते हुए समाज को तथा व्यक्ति को अवसर करने की क्षमता को प्रदान करने वाला नियम हि धर्म है ।धर्म मनुष्य को विवेचन शक्ति प्राधान करति है।

धर्म- कर्म का दर्पण है । कर्म - धर्म का पूरक है । धर्म कर्म निभाना वाला मानव ही जीवन मे सार्थकता पाप्त करता है ।

धर्म अनेकानेक है । शैशव ,बाल कौमार,युवा,प्रौडा ,वृद्ध अवस्थाऔ के अनुसार धर्म के पालन करना अनिवार्य है ।

धर्म निभाने के लिए अन्तः प्रेरणा की आवश्यकता है । धर्म के अनेकानेक पेहलु भी है - पारिवारिक ,सामाजिक,आर्थिक ,राजनैतिक,आदि ।

लेकिन कुछ नित्य नूतन धर्म है । उदा मातृ देवो भवः,पितृ देवो भवः, माता पिता सेवा ही परम धर्म मानकर श्रवण कुमार ने अपना प्राण त्याग करते समय भी सेवा तत्पर रहा , आचार्य देवो भवःअर्थ है गुरु की आदेश का पालन करना प्रथम कर्तव्य है,इसलिय एकलव्य ने गुरु आदेश के अनुसार अपनी दाया हाथ की अंगूठी काट करके दे दिया है । अतिथिः देवो भवः---- । वेद और उपनिषद मे जो धर्म का उपदेश दिया गया है उन नियमो को आचरण करने से व्यक्ति कालातीत पूजनीय हो जाता है ।

धर्म उपदेश देने मात्र से नहि , आचरण करना भी अनिवार्य है । सत्य वचन पालन के लिये 14 वर्ष वनवास किया है । यहाँ तक की धर्म का पालन के लिया सीता को भी परित्याग किया है

भगवान श्री राम ने कभी किसी से धर्म आचरण करने के लिये नहि कहा गया ,स्वयम् आचरण किये है ,इसलिये भगवान राम को मर्यादा पुरुषोतम श्री राम कहा जाता है ।। राजा नल महाराज सत्य वचन निभाने के लिये राज्य को छोड़ कर ,पत्नी पुत्र सहित अनेक दुखो को सहना पड़ा । अंत मे पत्नी को छोड़ कर शमशान वाटिका मे काम करना पड़ा,लेकिन धर्म को कभी नहीं छोड़ा है ।

निष्कर्ष —किसी भी स्थिती मे मनुष्य धर्म विमुख (भ्रष्ट) नहीं होना चाहिए । यदि ऐसा कोई विमुख हो तो ,उसे दण्ड देना का आदेश दिया जा सकता है । इसका मुख्य उद्देश्य यह है कि धर्म व्यक्ति और समिष्टि की रक्षा करना है । इसलिये हो सके तो कष्टों को सहकर भी आचरण एवं अनुकरण करना चाहिए । धर्म को निभाते हुए कर्म करना और अर्थ को संचयन करते हए कामनाओ का तुष्ट करते हए मोक्ष को प्राप्त करना है ।

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Sri Vinayaka Kumar, Vice Principal

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CHAIRMAN OF THE SEMINAR   CONVENOR

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PRINCIPAL   HEAD OF THE DEPT, TELUGU

Bank Details :

Account Payee : S. BABY RAMANI

Account No. : 62011228955

IFSC Code : SBIN020106

SBI – ICRISAT Branch, Patancheru.

PHONE NO: 8332932330

drkolliramani@gmail.com

భారతీయ సాహిత్యం – చతుర్విధ పురుషార్థాలు

అంతర్జాతీయ సదస్సు

తేది : 08-02-2020

ప్రత్యేక సంచిక – ప్రధాన సంపాదకురాలు :

డా॥ ఎస్. చేబరమణి

శాఖాధిపతి, తెలుగు విభాగం,

ప్రభుత్వ డిగ్రీ కళాశాల, సదాశివపేట,

సంగారెడ్డి జిల్లా, తెలంగాణ.

పోతన భాగవతంలో మోక్ష మార్గం

- డాక్టర్ ఉషాలి విష్ణుప్రియ, తెలుగు శాఖాధ్యక్షులు, తెలుగు విభాగం, సెంట్ ఆన్స్ మహిళా కళాశాల, మెహదీపట్నం, హైదరాబాదు.

కర్మ, జ్ఞాన, భక్తిమార్గాలలో సకల జనులకు సులభ మయినది భక్తి మార్గం. తద్వారా ముక్తిని పొందడాన్ని భాగవతం బోధిస్తుంది. మానవ జీవిత లక్ష్యం మోక్షాన్ని సాధించడం. మోక్షము జ్ఞాన రూపంలో ఉంది. చిత్తశుద్ధి ఉన్నవారికి మాత్రమే జ్ఞానం లభ్యమవుతుంది. ఆత్మజ్ఞానం లేకుండా ఎంత భక్తిని సంపాదించినానా మోక్షం రాదు. పుణ్యం మాత్రమే వస్తుంది. ఆ పుణ్య ఫలంతో ఊర్ధ్వ లోకాలకు పోయి అక్కడ సుఖభోగాలను అనుభవించి పుణ్యము తీరిపోగానే తిరిగి భూలోకంలో మరల జన్మించవలసిందే. "మోక్ష సాధన సామగ్య్రాం భక్తి దేవ గరీయసి" అని మోక్ష సాధన సామగ్రిలో భక్తికి గొప్ప స్థానం ఇచ్చారు ఆది శంకరులు. భక్తితో అంటే ఆత్మ జ్ఞానంతో ఆ మహావిష్ణువును ఎవరు స్మరిస్తారో వారికి ఆ దేవదేవుడు మోక్షాన్ని ప్రసాదిస్తాడు. తనలోకి ఐక్యం చేసు కుంటాడన్నమాట. ఆంధ్ర మహా భాగవతంలో పలు ఘట్టాల్లో మోక్షానికి మూలం భగవన్నామ స్మరణే.

భగవంతుడు సర్వాంతర్యామి. ఎచ్చోట ఉన్నా మన మొరలు విని మనల్ని రక్షించే దేవదేవుడతడు. దానికి ఒక గొప్ప ఉదాహరణ గజేంద్రమోక్ష ఘట్టమే. విర్ర వీగుతూ వనవిహారానికి బయలు దేరిన ఒక మత్తేభము దాహార్తితో సరస్సులోకి దిగింది. దప్పిక తీర్చుకోవడంతో సరిపెట్టుకోక తన బృందంతో కలిసి ఆ కాసారాన్ని కకా వికలం చేసింది. ఆ మదగజం ఆగడాలన్నిటిని మడుగు లోని ఒక మొసలి గమనించసాగింది. కొంత సేపటికి అదను చూసి ఒక్కసారిగా ఆ కరిరాజుపై విజృంభించింది. హుంకారం చేస్తూ కుప్పించి దాని కాళ్ళను పట్టుకుంది. కరి మకరి ఒకదానిపై మరొకటి దాడి చేసుకోసాగాయి. తొలుత ఎంత ఆత్మ విశ్వాసంతో అలుపెరుగకుండా సమరం సాగించిన ఆ కరీంద్రుడికి క్రమేణా ధైర్యం

సన్నగిల్లింది. గుండెల్లో భయం పొడచూపింది. అప్పుడు దానికి ఆ పరమాత్మ మనస్సులో మెదిలాడు.

"ఎవ్వనిచే జనించు జగమెవ్వని లోపలనుండు లీనమై... శరణు వేడెదన్"

ఈ లోకం ఎవరి వల్ల పుడుతుందో, ఎవరితో కలిసి ఉంటుందో, ఎవ్వని లోపల లయమవుతుందో, ఎవరు పరమాత్ముడో, ఎవరు ఈ విశ్వానికి మూలకారణమో, ఎవరు పుట్టడం గిట్టడం పెరగడం లేకుండా ఉంటాడో, ఎవరు అన్ని తానై ఉంటాడో అటువంటి ప్రభువైన భగవంతుణ్ణి నేను శరణు వేడుతానని నిర్ధారించుకుంది.

ఆపద కాలంలో ఆ ఆశ్రిత పాలకుడు గుర్తుకు రావా లంటే గత జన్మల్లో ఎంతో పుణ్యం చేసుకోవాలి. గజేం ద్రుడు గత జన్మలో ఇంద్రద్యుమ్నుడు అనే మహారాజు. అగస్త్య ముని శాపం మూలంగా తమోగుణం కలిగిన మత్తేభంగా జన్మనెత్తాల్సి వచ్చింది. అయినా పూర్వ జన్మ సంస్కారాలు చిత్తం నుంచి చెదిరిపోలేదు. ఆ ఫలితంగానే కమలనాభుడు తన ఆత్మలోకి వచ్చాడు.

"కలడందురు దీనులయెడ కలడందురు పరమయోగి... కలడో లేడో"

దేవుడు ఆర్తులైన వారి వెంట ఉంటాడంటారు. ఉత్తములైన యోగుల చెంత ఉంటాడంటారు. అన్ని దిక్కులలోనూ ఉంటాడని అంటారు. 'ఉన్నాడు ఉన్నాడు..' అనే దేవుడు మరి ఉన్నాడో లేడో అని సందేహించాడు. గజేంద్రుని మొర ఇలా విన్నప్పుడు విష్ణువు కాపాడాలని బయలుదేరి వెనుదిరిగి వచ్చేశాడు. గజేం ద్రుడు ఆ పరమాత్మను సందేహించినందు వల్లే శ్రీమహా విష్ణువు స్పందించలేదు. 'కలుగడే నా పాలి కలిమి..' ఇక సందేహించడమెందుకు? 'కలిమి లేములు లేక కలుగు వాడు..' అని తీర్మానించుకున్నాడు. కరుణాంతరంగుడైన

కమలలోచనుడు భక్తులు కలిగినవారా మరి లేనివారా అని చూడకుండా కాపాడుతాడన్న నమ్మకంతో ప్రార్థించాను.

ప్రాణాపాయ స్థితిలో ప్రతి ఒక్కరూ గజేంద్రుని వలె నిరాసక్తులై ఉంటారు. కాని గజేంద్రుని వలె ఎంతమంది శ్రీహరిని ప్రార్థించగలరు? ఆపత్కాలాల్లో అందరూ ఆ పరమాత్ముని వేడగలరా? కనుక నిరంతరం భగవన్నామ స్మరణ చేయడం అభ్యసిస్తే ఆపద సమయంలో భగవంతుడు స్మరణకు వస్తాడు.

పోతన భాగవతంలో సప్తమ స్కంధంలో -

"ఎట్టి వారికైనా నేకాంతులకునైనా వచ్చి చొరగరాని వాసుదేవు

తత్త్వమందు జేరి ధరణీపడహితుడై, ఎట్లు సొచ్చె మునివరేణ్యా! నేడు"

ధర్మరాజు నారదుణ్ణి ఒక ప్రశ్న వేశాడు.. ధర్మ మర్మజ్ఞా! నాకొక పెద్ద సందేహం కలిగింది. మహానుభావుడైన వాసుదేవునిలో ప్రవేశించగలగడం ఎటువంటి పుణ్యాత్ములకైనా వీలు కాదే, ఎటువంటి తేజస్సునకైనా ప్రవేశించే శక్తి ఉండదే, అటువంటిది శత్రువైన శిశుపాలుడు ఆ మహాసౌభాగ్యమును ఎలా పొందగలిగాడు స్వామీ.. విష్ణువును నిత్యం నిందించడమే కదా! మరి ఇది ఎలా సాధ్యమైంది?"

ఈ ప్రశ్నకు నారదుడు..

"అలుకనైన జెలిమినైన గావంబుననైన... వేరుసేయడతడు"

'ధర్మనందనా.. కోపంతో గాని, కోరికతో గాని, స్నేహంతో గాని, మోహంతో గాని, శత్రుత్వంతో గాని, ప్రీతితో గాని, భీతితో గాని, ఏ విధంగానైనా నరే.. హరిని ఎమరకుండా స్మరిస్తున్నట్లయితే సర్వ భూతాత్మకుడైన విష్ణుదేవుని సన్నిధానము అభ్యమవుతుంది. ఆ స్వామి ఎవరిని ఎప్పుడూ పరునిగా చూడడు..' అని మరలా ఇలా అన్నాడు.

"వైరానుబంధంబయిన జేరిన చందమున... నా చిత్తమునన్"

ఆ విష్ణువును విశృంఖలమైన విరోధంతో చేరటం కంటే భక్తితో చేరడమే కష్టమనిపిస్తుంది. అనురాగంతో చేసే ఆరాధనకంటే, ద్వేషంతోనే భగవంతుణ్ణి పొందవచ్చేమో నని నాకు అనిపిస్తున్నది అని నారదున్నాడు. అధికమైన కామంతో గోపికా భామలు, భయంతో కంసుడు వెనుదీయని వైరంతో శిశుపాలుడు, దంతవక్రుడు మొదలైన రాజులు, అనుబంధం పెంచుకున్న యాదవులు మొదలైన వారు ఆ స్వామి అనురాగానికి పాత్రులవడం సాధ్యమైందంటే నిరంతరమైన స్మరణం వల్లే!

సనక సనందనాది మునీంద్రులు ఎంతో నిగ్రహం కలవారు. ఒకసారి వారిని శ్రీహరి సన్నిధికి పోనియక ద్వారపాలకులు అడ్డుకున్నారు. దాంతో వారు వెంటనే కోపానికి గురయ్యారు. ఆ ద్వారపాలకులను రాక్షసులై పుట్టండని శపించారు. ఈ హఠాత్పరిణామానికి ద్వార పాలకులు పదవీ భ్రష్టులై దుఃఖంతో పశ్చాత్తాపంతో మునులను శరణు వేడారు. వారి దుఃఖం చూసి ఆ మహాత్ముల సుకుమారమైన మనసు ద్రవించింది. "మీకు మూడు జన్మలలో వైర భక్తితో భగవంతుని సాన్నిధ్యం సంప్రాప్త మవుతుంది.." అని శాపానికి ఉపసంహారం చెప్పి వళ్ళి పోయారు. అలా ఆ వైకుంఠ ద్వార పాలకులిద్దరూ మొదటి జన్మలో హిరణ్యకశిపుడు, హిరణ్యాక్షులుగా జన్మించారు. రెండవ జన్మలో కైకసి అనే రాక్షసిక రావణ, కుంభ కర్ణులై జన్మించారు. మూడవ జన్మలో సాత్వతికి శిశుపాల, దంతవక్రులుగా జన్మించారు.

"వక్రంబెతులేక పాయని మహావైరంతో... సంఘాతులై"

ఆ ఇద్దరూ ద్వేషాయుధులతోనే పుట్టయి. హరిని మాషిచూట పుట్టుకతోనే ప్రారంభమైంది. ఎవరిక కృష్ణ పరమాత్మ వారి శిరస్సులను ఖండించి వేశాడు. అలా వారికి మోక్షాని ప్రసాదించాడు. ప్రణాద ఘట్టంలో కూడా ప్రహ్లాదుడు

హిరణ్యకశిపుడికి పునర్జన్మ లేని ముక్తి మార్గమును ఇలా వివరించాడు..

"సంసార జీమూత సంఘంబు ఎచ్చునే... దానవేంద్రా!"

'దానవేంద్రా.. పెనుగాలి విసురు లేకుండా కారు మబ్బుల గుంపులు విడిపోవు. అలాగే చక్రధారి సేవ చేయ కుండా సంసార బంధాలు తొలగిపోవు. వర్షం పడకపోతే అడవులలో రగులుకొన్న అగ్ని ఆరదు. అలాగే హరి సేవ అనే అమృతం పడకపోతే మనిషికి తాపత్రయాలు చల్లారవు. బడబాగ్ని ప్రజ్వరిల్లితే సముద్రాలు కూడా ఇంకి పోతాయి. ఆదే విధంగా విష్ణు చింతన ఉంటే పాపాలు పటాపంచలైపోతాయి. సూర్యుని కిరణాలు విడిపోవు. హరిభక్తి అనే అంజనం లేకుండా నిర్మలమై నిరుపమానమై పునర్జన్మ లేని ముక్తి పెన్నిధిని అందుకోవడం ఎవరికీ సాధ్యం కాదు. ఆఖరికి ఆ బ్రహ్మకు కూడా ఇది తప్ప మరో మార్గం లేదు. దానవేంద్రా.. అన్ని శాస్త్రములు చది వాను. కథలూ గాథలూ అనేకం తవ్వి చూశాను. ఈ సంసార సముద్రంలో భార్య పుత్రులు తిమింగలాలు. కామ క్రోధ మద మాత్సర్యాలు పొంగి పొరలే కెరటాలు. ఇటువంటి ఘోరమైన సముద్రాన్ని దాటాలంటే అనవసర మైన వాదాలతో, అతి తెలివితేటలతో సాధ్యం కాదు. హరిభక్తి అనే నౌక ఒక్కటే దీని దాటించగలదు. ఇతర ఆలోచనలు, చదువులా, పాండిత్యాలా ఎందుకూ పనికి రావని ప్రహ్లాదుడు ముక్తి మార్గం చెప్పాడు. ఇంకొక మాట కూడా అన్నాడు.. సంసారంలో కొట్టు మిట్టాడే వారు ఎన్ని టికీ ఆ మహావిష్ణువును చేరుకోలేరు అని కూడా ఉద్బోధిం చాడు. మోక్షాన్ని పొందాలంటే నిరంతరమైన భగవంతుని స్మరణం వల్లనే సాధ్యమవుతుందని చెప్పాడు.

గోపికా వస్త్రాపహరణ వృత్తాంతం ఒక గొప్ప ఘట్టం. కృష్ణుడు ఎందుకు అవతరించాడంటే భూదేవి గోరూప మున బ్రహ్మదేవుని వద్దకు వెళ్లి రాక్షస ప్రవృత్తి గల రాజుల వలన తనకు కలుగుతున్న ఉపద్రవమును విలపిస్తూ విన్నవించింది. బ్రహ్మ విష్ణుమూర్తిని ప్రార్థించి ఇలా ఆదేశించాడు.. "దేవాధిదేవుడు తన సర్వోత్కృష్ట శక్తులతో

నవా త్వరలో భూలోకమున శ్రీకృష్ణరూపమున అవతరించ నున్నాయి. ఆయన భూలోకమున ఉన్న శిక్షణా, శిష్టరక్షణ అను కర్తవ్యములను నిర్వర్తించు వరకు దేవతలు కూడా ఆయనకు తోడ్పడుటకు భూలోకమున ఉండవలెను కావున దేవాధిదేవుడు అవతరించేలోప్పు యదువంశమున దేవతలు వెంటనే జన్మించవలెను.. అని అన్నారు.

భగవంతుడు స్వయంసంపూర్ణుడు. ఆది అంతము పుట్టుక చావు లేనివాడు. మనం అనుభవిస్తున్న దుఃఖం నుంచి పారమార్థిక ఆనందం వైపు దానిని చూపడానికి అద్భుతమైన తన సంకల్పానుసారం గతంలో అనేకమార్లు అనేక రూపాల్లో అవతరించినట్లే, ఈసారి శ్రీకృష్ణుని రూపంలో అవతరించాడు. 'కృష్ణ' అనే శబ్దానికి 'అందరిని ఆకర్షించేవాడు' అని అర్థం! గోపికలెవరు? అన్న ప్రశ్నకు గోపికలందరూ వేదవిద్యాంసులు అన్నది సమాధానం! వెనకటి జన్మలో రామావతారం కాలంలోమునులు, ఋషులూ శ్రీరామచంద్రునితో కలిసి మెలిసి ఉన్నారు. ఆయన సాహచర్యంలో నిత్యం గడపాలనే విన్నపస్ని రాముని ముందుంచగా, కృష్ణావతారమున మీరు తిరిగి జన్మింతురని, అప్పుడు మీ అభీష్టన్ని తీరుస్తానని శ్రీరాముడు అభయమిచ్చాడు. ఆవేద విద్యాంసులే కృష్ణావతారం కాలంలో బృందావన గోపికలై జన్మించారు.

'గోపికా వస్త్రాపహరణము':

కృష్ణుడు తమకు భర్త అవాలని గోపికలు హేమంత ఋతువు ప్రారంభం నుంచి యమునా నది తీరమున ప్రతి రోజూ తెల్లవారు జామున కాత్యాయని దేవిని పూజిం చారు.

"కంటివి మా హృదయంబులు కంటివి మానవ
లజ్జగంటివి వలువల్
గంటివ నెట్లు జేసెదొ కంటపుగద విను
నెటిగంటిమి కృష్ణ"

మాసాంతమున ఒక రోజు గోపికల కోరికలు గ్రహించి కృష్ణుడు ఒడ్డున ఉన్న వస్త్రాలను అపహరించి ఓ చెట్టు

మీదకు ఎక్కడు. వస్త్రాపహరణమన్నది బాహ్యానికి ఓ మెఱ మాత్రమే! గోపికల వస్త్రాపహరణమంటే వారి చిత్త వృత్తులను తీసివేయడం అని అర్థం! వారిని అలానే ఒడ్డు మీదకు రమ్మన్నాడు. అంటే నగ్నదేహాలతో రమ్మని అనడమేమిటి? అన్న భౌతికార్థముతో కాకుండాఆందులోని అంతరార్థం గ్రహిస్తే బాహ్యాలంకరణల మీద, దేహం మీద, కామ మోహ మమకారములు అన్నీ వదులు కోవాలన్న పరమాత్ముని భావన అవగతమవుతుంది. అలాగేవచ్చిన గోపికలను రెండు చేతులూ ఎత్తి నుదుటున తాకించి, నమస్కరించమంటాడు ఆ కృష్ణుడు. నుదుటున ఉన్నది ఆజ్ఞా చక్రము! ఆ స్థానము ఆత్మను పరమాత్మతో కలుపుతుంది. ఇక్కడే జ్ఞాన నేత్రం తెరుచుకొని ఆత్మ దర్శనమవుతుంది. ఈ చక్రాన్నే దివ్య నేత్రమని, జ్ఞాన చక్షువని అంటారు. కృష్ణుడు వీరిని మేల్కలపడానికే చేతులను జోడించమంటాడు. ఈ చేతుల జోడింపు వలన

జరిగినది ఏమిటనగా, "జీవాత్మ పరమాత్మల కల యిక"!వజ్ర వైదూర్యాలు, కామ మోహాలు, మమకారాల అడ్డు తొలగించుకోమని, అలా తొలగించుకొని వస్తేనే తనలో లయమవుతారని కృష్ణుడు ఈ గోపికా వస్త్రాపహరణ లీలలో ముక్తి యొక్క అంతరార్థాన్ని తెలియజేశాడు!

నారదుడు ధర్మరాజునకు మోక్ష ప్రాప్తికి సంబంధించిన విషయాలను ఇలా ఉటంకించాడు.

"అసంకల్పాజ్జ యేత్కామం క్రోధం కామ వివర్జనాత్ అర్థానర్థే క్షయాలోభం భయం తత్త్వా వమర్శనాత్"..

సంకల్పములను వదలి వేయుట ద్వారా కామమును, కామమును త్యజించుట ద్వారా క్రోధమును, ప్రాపంచిక వస్తు సంపదలయందు అనర్థమును చూచుట ద్వారా లోభమును, తత్త్వ విచారణచే భయమును జయించ వలయును.. అని మోక్ష ప్రాప్తి గురించి ధర్మరాజునుద్దేశించి నారదుడు ఉపదేశం చేశాడు!

ఉపయుక్త గ్రంథాలు :

1. మోక్ష సాధన - కూరపాటి ఆంజనేయులు

2. మోక్ష సాధన రహస్యం - శ్రీ విద్యాప్రకాశానందగిరి స్వాములు

3. ఆంధ్ర మహాభాగవతము - బమ్మెర పోతన

4. శ్రీ పోతన భాగవత మధురిమలు - సంకలనం: బాలగంగాధర్ పట్నాయక్

❖ ❖ ❖

ప్రభుత్వ డిగ్రీ కళాశాల, సదాశివపేట.

Impact of Mobile games- A Case study on PUBG

Mrs.J.Saujanya[1] & Mrs.N.Supriya[2]

[1]*Faculty of Commerce, St.Ann's Degree College for Women, Mehdipatnam, Hyderabad*
[2]*Faculty of Commerce, St.Ann's Degree College for Women, Mehdipatnam, Hyderabad*

ABSTRACT

We are living in an era of rapid transformation in technology due to continuous progress of computer and internet technology. Today, new technology is emerging in a new way, where artificial intelligence and motion graphics is upgrading every day. One such example is PUBG, a mobile game also knows as player unknown's battleground where hundreds of players play together. PUBG is a multiplayer shooter game where up to one hundred players fight in a 'battle royal' and the last person or team alive wins.

This paper focuses on the impact of PUBG game among teenagers. The study aims to find out the addiction among teenagers with the help of an online questionnaire. The questionnaire is prepared in two sets, one is being circulated to the people who play PUBG and another questionnaire is circulated to their parents to find out their opinion on PUBG.

Keywords: Technology, Teenagers, PUBG

1. INTRODUCTION

A video game is an electronic game that involves human interaction with a user interface to generate visual feedback on a video device which is any type of display device that can produce two or three dimensional images. In the early 2000s, mobile games gained popularity in Japan's mobile phone culture, years before the United States or Europe. By 2003, a wide variety of mobile games were available on Japanese phones, ranging from puzzle games and virtual pet titles that utilized camera phone and fingerprint scanner technologies to 3D games with exceptionally high quality graphics.

The launch of Apple's App Store in 2008 radically changed the market. First of all, it widened consumers' opportunities to choose where to download apps; the application store on the device, operator's store or third party stores via the open internet, such as GetJar and Handan go. The Apple users, however, can only use the Apple App Store, since Apple forbids the distribution of apps via any other distribution channel. Secondly, mobile developers can upload applications directly to the App Store without the typically lengthy negotiations with publishers and operators, which increased their revenue share and made mobile game development more profitable. Thirdly, the tight integration of the App Store with the device itself led many consumers to try out apps, and the games market received a considerable boost.

In 2013, Japan was the world's largest market by revenue for mobile games. The Japanese gaming market today is becoming increasingly dominated by mobile games, which generated $5.1 billion in 2013, more than traditional console games in the country. China is the largest market for mobile gaming, by both revenue and number of players. Until July 2015, video game consoles were banned in the country. While personal computers were still used for gaming, the ban led to a large growth in the use of mobile phones for gaming that has persisted even after the ban was lifted. Tencent Games is the largest publisher of mobile games in the country, and due to the size of its player base within China, is known as the largest video game company in the world, measured by revenue. Tencent published King of Glory (known in Western markets as *Arena of Valor*), a multiplayer online battle arena that had a 200 million user base from China alone before expanding the game out into other markets.

Market analysis firms identified that mobile gaming global gross revenues exceeded that of either personal computer or console games in 2016, earning around US$38 billion, and remained one of the fastest growing sectors of the video game market.

PUBG

Player Unknown's Battlegrounds (***PUBG***) is an online multiplayer battle royale game developed and published by PUBG Corporation, a subsidiary of South Korean video game company Blue hole. The game is based on previous mods that were created by Brendan "Player Unknown" Greene for other games, inspired by the 2000 Japanese film Battle Royale, and expanded into a standalone game under Greene's creative direction. A battle royale genre of game is the type of video game that mixes survival, exploration and scavenging items for survival in the game with the "Last man standing" game play. In the game, up to 100 players parachute onto an island and scavenge for weapons and equipment to kill others while avoiding getting killed. The available safe area of the game's playable area decreases in size over time, displacing the surviving players into tighter areas to force encounters against each other. The last player or team which eliminates the others get a chance to win the round, for which they are rewarded with experience and bonus points which includes points for the number of people killed in each round. It was officially launched in December 2017 and can be played on both gaming consoles and smart phones. The game has been downloaded over 100 million times on Google.

Online video gaming has become one of the world's most popular leisure time activities among youth. Research has consistently shown that gaming can bring many positive benefits including therapeutic, medical, health, cognitive, and educational benefits. However for a small minority, gaming can be problematic and potentially addictive.

Objectives of the study

The following are the objectives of the study:

1. To find the level of awareness of PUBG-mobile game.

2. To study the impact of PUBG-mobile game on teenagers.
3. To analyze the parents point of view on PUBG-mobile game.

Research Methodology

The Primary data was collected using Questionnaire. Two sets of questionnaires were made in which one was given to the teenagers and the other questionnaire to the parents. The questionnaires consists of a number of questions involves both specific and general question related to influence of PUBG-mobile game. The secondary data was collected from journals and websites for the study.

Sample Design

Sample Element   :   Teenagers and parents.
Sample Size   :   130 samples
Sample Media   :   Questionnaire
Sample Analysis   :   Excel.

Literature Review

- Anand (2007) suggests that video game usage is related to a decrease in SAT scores as well as GPA. In his study, a survey was distributed to college-age students that survey addressed which types of games players preferred as well as time management questions designed to assess how the participants allocated their time between school, work, video games, and other recreational activities. This data was then related to reported GPA and SAT scores. The results indicate that video games may have an adverse effect on GPA and SAT scores.

- Mary Kekatos (2019) states in his article paper that Nearly ALL parents think their teenagers are gaming too much and worry the habit disrupts sleep and schoolwork - but three-fourth of parents still think there's a 'positive impact' on their children's lifestyles..

- Jeremy Spirogis In his study, it was stated that 71 per cent moms and dads think that video gaming is not beneficial to kids and while 44 per cent admitted they had attempted to ban video games as once children get accustomed to playing it is hard to eliminate all of them.

Findings

➢ From the research, it has been found that 70% of the people know about PUBG and its features.

➢ 83% teenagers who play PUBG are above the age of 12 years play PUBG.

➢ 86% of the teenagers plays PUBG for less than 1 hour and 1-2 hours.

➢ 48% of the parents are satisfied by the time spent by their children on PUBG where as 42% of the parents are not satisfied and the rest do not know whether they are satisfied or not.

- ➢ Most of the parents do not have to stop their child from playing PUBG.
- ➢ 66% of the parents don't think that PUBG has any effects on the health of their children.
- ➢ 27% of the teenagers play PUBG for fun, followed by 21% play to relax their mind and 20% of them play to make new friends.
- ➢ 32% of the teenagers feel playing PUBG has made them lazier where as 48% of them feel it has not made them lazier.

Conclusion

From this research we can conclude that, the effect of PUBG depends on the use of PUBG. When the game is played in limit it is not harmful in any way. It can increase the reactive skills of the teenagers. Most of the parents also do not consider PUBG as an issue if played in limit. Hence, the game should have certain time restrictions which will help the teenagers to play in a controlled time limit. The teenagers like the game because of the features of making new friends around the world and interacting with them. In this way, it helps in improving the social skills of the teenagers.

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A Study on Technological Innovations in Indian Banking Sector

K.Pranathi   **R. Meena Shanthi**
Lecturer in Commerce   Lecturer in Commerce
St. Ann's Degree College For women   St. Ann's Degree College For women
Mehdipatnam, Hyderabad.   Mehdipatnam, Hyderabad.
E-mail: pranathi.goud@gmail.com   E-mail: ramdasmeena.1584@gmail.com

Abstract: The banking sector in India has seen a number of changes. Over the years many regulatory reforms and technology advancements have sent in to meet the challenges of changing needs and perceptions of customers. Most of the banks have begun to take an innovative approach towards banking with the objective of creating more value for customers in the banks. Today we have electronic payment system along with currency notes. India's financial sector is moving towards a scenario, where it can have new instruments along with liquidity and safety. Arrival of plastic money, introduction of Electronic Clearing Service (ECS) in late 1990's, introduction of Electronic Funds Transfer, Real Time Gross Settlement (RTGS), introduction of NEFT (National Electronic Funds Transfer), mobile banking, online banking are the various innovations in banking. Banks are investing heavily in adoption of these innovations. This paper highlights the benefits of technological innovations in Indian banking sector.

Keywords: Indian Banking Sector, Innovative Banking, Technological innovations, Recent Trends, Customer Satisfaction.

1. Introduction:

The Banking sector has been immensely benefited from the implementation of superior technology during the recent past, almost in every nation in the world. Productivity enhancement, innovative products, speedy transactions seamless transfer of funds, real time information system, and efficient risk management are some of the advantage derived through the technology. Information technology has also improved the efficiency and robustness of business processes across banking sector. India's banking environment. Indian banking industry is the midst of an IT revolution. Technological infrastructure has become an indispensable part of the reforms process in the banking system, with the gradual development of sophisticated instruments and innovations in market practices.

1.1 Financial innovation:

In 1969, a landmark was registered in the Indian Financial Sector, when 14 Banks were nationalized by the center. It is a matter of great pride to mention that during the economic meltdown in 2008-09, our Indian Bank stood firm and remained unaffected. This shocked the whole world as few Banks of the developed nations crumbled down but Indian Bank stood strong and also witnessed many positive developments in this industry. To improve the regulation in this sector, several notable efforts were made by the Policy Makers i.e. The Reserve Bank of India, Ministry of Finance and other Government and financial sector agencies. It must be appreciated that the Banks are in a position

To meet the demands of the customers and are also adding the economic development of the nation at large. India's Banking sector has made rapid strides in reforming and aligning itself to the new competitive business environment. Technology has swiftly turned as a boon to the customers. With Technology the Bank branches becomes only one of the many channels that are now accessible to customers for performing routine banking transactions. The Information Technology has transformed the functioning of the businesses worldwide. It has bridged the gaps in the terms of reach and the coverage of system. It also enabled better decision making based on the latest and the accurate information. It has not only tried to reduce the cost but also tried its level best to improve the efficiency in the many new processes, products and services offered by the Banks and the IT centers. Banks that employ IT solutions are perceived to be innovative and should be capable of satisfying the customers with their services.

The Banking sector has been immensely benefited from the implementation of superior technology during the recent past, almost in every nation in the world. Productivity enhancement, innovative products, speedy transactions seamless transfer of funds, real time information system, and efficient risk management are some of the advantage derived through the technology. Information technology has also improved the efficiency and robustness of business processes across banking sector. India's banking environment. Indian banking industry is the midst of an IT revolution. Technological infrastructure has become an indispensable part of the reforms process in the banking system, with the gradual development of sophisticated instruments and innovations in market practices.

2. Objective of the Study

- To study how innovations have contributed to the development of Indian banking.
- To study the challenges faced by Indian Banks.

Research Methodology:

The research is mainly based on secondary data. Data has been collected from different sources like scholarly articles, annual reports of the selected banks, newsletters, and various web sites.

3. Literature review

The banking industry is undergoing a rapid and radical transformation due to the all-pervasive influence of Information Technology (IT) and breath taking developments in the technology of telecommunications and electronic data processing. This process has radically altered the traditional ways of doing banking business and al- lowed banks to wipe out the difference in time, distance and speed. Many banks use state-of-the-art technology to provide their customers with better value-added services tailored to their needs. In this innovative error, there is no way a bank can remain lukewarm to technology and yet hope to grow. The choice is between survival and extinction but to survive in these times there is a need for the banks to adopt an innovative culture. Proponents and theorists in this area still emphasize on a firms performance

An innovation is an idea, practice, or object that is perceived to be new by a person or adopting entity. When an innovation emerges, diffusion unfolds which entails communicating or spreading of the news of the innovation to the group for which it is intended (Rogers, 1995). Adoption however is the commitment to and continued use of the innovation. The diffusion of innovations theory provide explanations for when and how a new idea, practice or newly introduced information and communication medium is adopted or rejected over time in a given society.

Berger (2003), the usage of information technology (IT) broadly referring to computers and peripheral equipment, has seen tremendous growth in service industries in the recent past. The most obvious example is perhaps the banking industry, where through the introduction of IT related products in internet banking, electronic payments, security investments, information exchanges, banks now can provide more diverse services to customers with less manpower.

Dr. Rangarajan committee had drawn up in 1983-84 the first blue print for computerization and mechanization in banking industry and looked into modalities of drawing up a phased plan for mechanization for the banking industry covering period 1985-89. The committee in its report in 1984 recommended introduction of computerization and mechanization at branch, regional office / zonal office and head office levels of banks. In 1988 another committee was constituted under the chairmanship of Dr. Rangarajan for making plans for computerization for the next five years from 1990-94 for the banking industry. It identified the purpose of computerization as improvement in customer service, decision making, housekeeping and profitability.

The Second wave of development was in Total Branch Automation (TBA) which came in late 1980s. This automated both the front-end and back-end operations within the same branch. TBA comprised of total automation of a particular branch with its own database. In the third wave, the new private sector banks entered into the field of automation. These banks opted for different models of having a single centralized database instead of having multiple databases for all their branches. This was possible due to the availability of good network infrastructure.

Narasimhan Committee (1991): The banking industry has introduced various new customer services and products using IT. The banking industry has gone through many changes as a result of the introduction of IT. In fact, the structure of the industry is continuously changing because of rapid development of IT . Banks are the backbone of the economy of the country. Implementation of information technology and communication networking has brought revolution in the functioning of the banks and the financial institutions. The status of automation in the banks in India is not uniform. There are banks functioning for decades, having a sizable number of branch networks in the rural and semi-urban centers. Compared to this, there are banks which are generally regional in character and not having a large number of branches in the country. In the recent past a few private sector banks have been established with the latest technology. Foreign banks located at major commercial centers of the country also transact their business in a computerized environment. The level and extent of automation in the banks are generally vary because of their history, work culture and policies/strategies adopted by their management in branch expansion and investment in technology.

According to the World Bank (2003) report on ICT and the Millennium Development Goals, information technology reduces transaction costs per customer and enables banks to provide small loans and services to a larger number of rural customers.

Ananthakrishnan G. described customer's services in the banks. The discriminating customer's expectations have begun to change in terms of quality and service. With the advent of computers and ATMs, the gap between the customers and the banking personnel is widening. Unless a change of heart occurs, even the largest banks will find it hard to survive on their assumed false glory. Banks which take care to see the 66 reality and react early will survive and prosper, while those who continue the traditional path will find their market share eaten away.

3.1 Innovation in banking sector

Over the years, the banking sector in India has seen a number of changes. Most of the banks have begun to take an innovative approach towards banking with the objective of creating more value for customers. Information technology has given rise to new innovations in the product designing and their delivery in the banking and finance industries. Technology offers a chance for banks to build new systems that address a wide range of customer needs including many that may not be imaginable today. Financial innovation associated with technological change totally changed the banking philosophy and that is further tuned by the competition in the banking industry. Challenging business environment within the banking system create more innovation in the fields of product, process and market. Today, we have electronic payment system along with currency notes. Financial sector is moving towards a scenario, where it can have new instruments along with liquidity and safety.

ATM

ATMs are widely used electronic channels in banking. It is operated by plastic card with its special features. It is a computer controlled device at which the customers can make withdrawals, check balance without involving any individuals. ATM can be interior (i.e., located in the branch premises) or exterior (located anywhere outside the branch premises).

An "automated teller machine (ATM)" is a computerized telecommunication device that provides customers of financial institutions with access to financial transactions in public place without the need of human clerk or bank teller

The banks increased their penetration further with the total number of ATMs reaching 0.18 million in 2015. However, there was a decline in growth of ATMs of both Public Sector Banks as well as Private Banks. Public Sector Banks recorded a growth of 16.7 per cent during 2014-15 maintaining a share of around 70 per cent in total number of ATMs.

Table1: ATMs of Scheduled Commercial Banks (As at end-March 2013)

S.No.	Bank Group	On-site ATMs	Off-site ATMS	Total numbers of ATMs
1	2	3	4	5
I	Public sector banks	40241	29411	69625
II	Nationalized banks SBI Group	20658 18708	14701 13883	35359 32591
III	Private sector banks 1. Old private sector 2. New private sector banks	15,236 4054 11182	27,865 3512 24353	43101 7566 35535
III	Foreign banks	283	978	1261
IV	All SCBs (I+II+III)	55760	58254	114014

Debit Card

Debit card (also known as a bank card plastic card or check card) is a plastic payment card that can be used instead of cash when making purchases. It is similar to a credit card, but unlike a credit card, the money comes directly from the user's bank account when performing a transaction.

Some cards may carry a stored value with which a payment is made, while most relay a message to the cardholder's bank to withdraw funds from a payer's designated bank account. In some cases,

the primary account number is assigned exclusively for use on the Internet and there is no physical card.

In many countries, the use of debit cards has become so widespread that their volume has overtaken or entirely replaced cheques and, in some instances, cash transactions. The development of debit cards, unlike credit cards and charge cards, has generally been country specific resulting in a number of different systems around the world, which were often incompatible. Since the mid-2000s, a number of initiatives have allowed debit cards issued in one country to be used in other countries and allowed their use for internet and phone purchases.

Unlike credit and charge cards, payments using a debit card are immediately transferred from the cardholder's designated bank account, instead of them paying the money back at a later date.

Debit cards usually also allow for instant withdrawal of cash, acting as an ATM card for withdrawing cash. Merchants may also offer cashback facilities to customers, where a customer can withdraw cash along with their purchase.

Credit Card

A credit card is a payment card issued to users (cardholders) to enable the cardholder to pay a merchant for goods and services based on the cardholder's promise to the card issuer to pay them for the amounts so paid plus the other agreed charges.The card issuer (usually a bank) creates a revolving account and grants a line of credit to the cardholder, from which the cardholder can borrow money for payment to a merchant or as a cash advance. In other words, credit cards combine payment services with extensions of credit. Complex fee structures in the credit card industry may limit customers' ability to comparison shop, help ensure that the industry is not price-competitive and help maximize industry profits. Because of this, legislatures have regulated credit card fees.

A credit card also differs from a cash card, which can be used like currency by the owner of the card. A credit card differs from a charge card also in that a credit card typically involves a third-party entity that pays the seller and is reimbursed by the buyer, whereas a charge card simply defers payment by the buyer until a later date.

Table 2: Credit and Debit Card Issued by Scheduled Commercial Banks

(In millions)

S.No.	Bank Group	Outstanding number of credit cards		Outstanding number of debit card	
		2012	**2013**	**2012**	**2013**
1	2	3	4	5	6
I	Public sector banks	3.1	3.5	214.6	260.6
II	Nationalized banks SBI Group	0.8 2.2	0.9 2.6	97.7 112.0	118.6 136.4
III	Private sector banks 1. Old private sector 2. New private sector banks	9.6 0.04 9.6	11.1 0.04 11.1	60.6 13.9 46.0	67.3 15.4 51.9
III	Foreign banks	4.9	5.0	3.8	3.3
IV	All SCBs (I+II+III)	17.7	19.5	278.4	331.2

RTGS

Real Time Gross Settlement (RTGS) is an electronic form of fund transfer where the transmission takes place on a real time basis. 'Real Time' refers to the process of instructions that are executed at the time they are received, rather than at some later time. On the other hand "Gross Settlement" means the settlement of funds transfer instructions occurs individually (on an instruction by instruction basis). The settlement of funds actually takes place in the books of RBI and thus the payments are considered as final and irrevocable. The attraction of RTGS is that the payee banks and their customers receive funds with certainty and finality during the same day enabling them to use the funds immediately without exposing themselves to risk. RTGS system, do not create credit risk for the receiving participant because they settle the each payment individually, as soon as it is

accepted, liquidity risks remains, as well as the possibility of the risks being shifted outside the system.

NEFT

National Electronic Funds Transfer (NEFT) is an Indian system of electronic transfer of money from one bank or bank branch to another. Under NEFT, individuals, firms and corporates can electronically transfer funds from any bank branch to any individual, firm or corporate having an account with any other bank branch in the country participating in the Scheme. The funds under NEFT can be transferred by individuals, firms or corporates maintaining accounts with a bank branch. Even individuals not having a bank account can deposit cash at the NEFT-enabled branches with instructions to transfer funds using NEFT. However, such cash remittances will be restricted to a maximum of Rs.50, 000/- per transaction. Such walk-in-customers have to furnish full details including complete address, telephone number, etc. NEFT, thus, also help in transfer of funds even without having a bank account. This is a simple, secure, safe, fastest and cost effective way to transfer funds especially for Retail remittances.

What are the minimum and maximum amount of remittance under RTGS and NEFT?

	RTGS	**NEFT**
Minimum amount	Rs. 2 lakhs	No maximum limit
Maximum amount	No upper ceiling	No upper ceiling

(However, maximum amount per transaction is limited to Rs.50,000/- for cash-based remittances and remittances to Nepal.)

Electronic Clearing Service (ECS)

Electronic Clearing Service is a retail payment system that can be used to make bulk payments/receipts of a similar nature especially where each individual payment is of a repetitive nature and of relatively smaller amount. This facility is meant for companies and government departments to make/receive large volumes of payments rather than for funds transfers by individuals.

Internet Banking

It is a service provided by banks so that people can find out information about their bank account, pay bills etc using the Internet. Internet Banking allows you to conduct bank transactions online, instead of finding a bank and interacting with a teller. In a broad sense, it is the use of electronic means to transfer funds directly from one account to another, rather than by cheque or cash. Real Time Gross Settlement (RTGS) is an electronic form of fund transfer where the transmission takes place on a real time basis.

4. Challenges ahead in Banking Sector

Developing countries like India, has a huge number of people who don't have access to banking services due to scattered and fragmented locations. But if we talk about those people who are availing banking services, their expectations are raising as the level of services are increasing due to the emergence of Information Technology and immense competition between the services and products provided by different banks. Since, foreign banks are playing in Indian market, the number of services offered has increased and banks have laid emphasis on meeting the customer expectations. India's banking sector has made rapid strides in reforming and aligning itself to the new competitive business environment. The major challenges faced by banks today are as to how to cope with competitive forces and strengthen their balance sheet. Today, banks are groaning with burden of NPA"s. It is rightly felt that these contaminated debts, if not recovered, will eat into the very vitals of the banks.

High transaction costs -A major concern before the banking industry is the high transaction cost of carrying non- performing assets in their books. The growth led to strains in the operational efficiency of banks and the accumulation of nonperforming assets (NPA"s) in their loan portfolios.

Regulatory pressure -Regulatory requirements continue to increase, and banks need to spend a large part of their discretionary budget on being compliant, and on building systems and processes to keep up with the escalating requirements.

Timely technological up gradation- Already electronic transfers, clearings, settlements have reduced translation times. To face competition it is necessary for banks to absorb the technology and upgrade their services.

IT Revolution- The Indian banks are subject to tremendous pressures to perform as otherwise their very survival would be at stake. The application of IT and e-banking is becoming the order of the day with the banking system heading towards virtual banking.

Intense Competition- the RBI and Government of India kept banking industry open for the participants of private sector banks and foreign banks. The Indian banking sector was introduced to competition when, in accordance with the suggestions of the first Narasimham Committee, entry was deregulated and both domestic and foreign banks were allowed to expand their branch networks. Due to this lowered entry barriers many new players have entered the market such private banks, foreign banks, non-banking finance companies, etc. The foreign banks and new private sector banks have spearhead the hi-tech revolution.

Privacy and Safety- Among the most important aspects of savings, i.e., safety, liquidity and profitability, safety is at the top most priority. The areas which might endanger security in e-banking can be:

Credit risk- Liquidity, interest rate risk, market risks Legal risk

Global banking -The impact of globalization becomes challenges for the domestic enterprises as they are bound to compete with global players. The numbers of Foreign Banks have become a major challenge for Nationalized and private sector banks.

Financial inclusion has become a necessity in today's business environment. Whatever is produced by business houses, that has to be under the check from various perspectives like environmental concerns, corporate governance, social and ethical issues. In India, RBI has initiated several measures to achieve greater financial inclusion, such as facilitating „Basic savings bank deposit accounts‟ and GCCs for small deposits and credit.

5. Conclusion:

The Banking sector in India has become stronger in terms of capital and the number of customers. It has become globally competitive and diverse aiming, at higher productivity and efficiency. Exposure to worldwide competition and deregulation in Indian financial sector has led to the emergence of better quality products and services. Reforms have changed the face of Indian banking and finance. The banking sector has improved manifolds in terms of Technology, Deregulation, Product & Services, Information Systems, Etc.

The pre and post liberalization era has witnessed various environmental changes which directly affects the aforesaid phenomena. It is evident that post liberalization era has spread new colors of growth in India, but simultaneously it has also posed some challenges. Banks have to adopt a holistic approach to fulfill the ever changing needs of customers and to grab a better market share. Development of sophisticated products with low cost technology is the key. This calls for in- depth analysis of customer needs the market and competitor trends. This analysis plays a very important role in devising new strategies, products and services. The better the banks understands their customers, the more successful they will be meeting their needs.

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STUDY ON INFLATION ACCOUNTING

D. Sujatha[1], P. Sireesha[2]
[1]Lecturer, St Anns College for women, Mehdipatnam
sujathagadamalla@gmail.com
[2]Lecturer, St Anns College for Women,
sirisha.ravi8@gmail.com

ABSTRACT

Inflation accounting is a term that describes a set of accounting systems framed to correct the problems caused by the use of historical cost in terms of inflation. It is the term used in countries with high inflation. The primary goals of an information accounting system is timely and accurate for users of that information. The traditional basis of accounting for the use of historical data is the use of this information. Accounting thinkers and economics scholars have been thinking about a solution. Accountants have been working on solving these problems by providing solutions in the form of accounting standards.

Inflation accounting consists a high range of accounting models developed to correct problems arising from historical cost accounting in the presence of high inflation.

This article explains the main issues in inflation accounting debate highlights and summarises the main proposals for altering present accounting practice, as well as it brings out the challenges and oppurtunities in application of methods used in this type of accounting.

Keywords: accounting, inflation, management, economics, hyperinflation, historical data

HISTORY OF INFLATION ACCOUNTING

Accountants in the United Kingdom and the United States have discussed the effect of inflation on financial statements since the early 1900s, beginning with index number theory and purchasing. Inflation accounting is a term that describes a set of accounting systems framed to correct the problems caused by the use of historical cost in terms of inflation. It is the term used in countries with high inflation. Themajor goals of an information accounting system is timely and exactness for users of that information. The traditional basis of accounting for the use of historical data is the use of this information. Accounting thinkers and economics scholars have been thinking about a solution. Accountants have been working on solving these problems by providing solutions in the form of accounting standards.

1. INTRODUCTION

Irving Fisher's 1911 book The Purchasing Power of Money was used as a source by Henry W. Sweeney in his 1936 book Stabilized Accounting, which was about Constant Purchasing Power

Accounting. This model by Sweeney was used by The American Institute of Certified Public Accountants for their 1963 research study (ARS6) Reporting the Financial Effects of Price-Level Changes, and later used by the Accounting Principles Board (USA), the Financial Standards Board (USA).

During the Great Depression, some corporations restated their financial statements to reflect inflation. At times during the past 50 years standard-setting organizations have encouraged companies to supplement cost-based financial statements with price-level adjusted statements. During a period of high inflation in the 1970s, the FASB was reviewing a draft proposal for price-level adjusted statements when the Securities and Exchange Commission (SEC) issued ASR 190, which required approximately 1,000 of the largest US corporations to provide supplemental information based on replacement cost. The FASB withdrew the draft proposal.

It is the inflation accounting model required in International Financial Reporting Standards implemented in 174 countries.

WHAT IS A HISTORICAL COST?

A historical cost is a estimation of value used in accounting in which the value of an asset on the balance sheet is recorded at its standard price when attained by the company. The historical cost method is used for fixed assets in the United States. It is also known as cost principle of accounting.
While the **cost** in the traditional **accounting** refers to **historical cost**, in **inflation accounting** it represents the **cost** that prevails at the time of reporting. The **inflation accounting** has an inbuilt and automatic mechanism to match the **cost** and revenue at current values. States under generally accepted accounting principles (GAAP).

Process of Inflation Accounting Inflation Accounting refers to the process of tweak the financial statements of a company to reveal the real financial position of the company during the inflationary period. Inflation Accounting involves recording of business transactions at present value. When a company operates in a country where there is a eloquent amount of price inflation or deflation. Inflation accounting is a term that describes a set of accounting systems framed to correct the problems caused by the use of historical cost in terms of inflation. It is the term used in countries with high inflation. The primary goals of an information accounting system is timely and accurate for users of that information. The traditional basis of accounting for the use of historical data is the use of this information.. Accounting thinkers and economics scholars have been thinking about a solution. Accountants have been working on solving these problems by providing solutions in the form of accounting standards.

To counter this issues, in certain cases companies are permitted to use inflation adjusted figures, rephrase the numbers to reflect current economic values. IAS 29 of International Financial Reporting Standards (IFRS) is the guide for entities whose functional currency is the currency of a hyper inflationary economy or over extension. The IFRS defines hyperinflation as prices, interest,

and wages linked to a price index rising 100% or more additive over three years. Companies that fall under this category may be need to refurbish their statements periodically in order to make them relevant to current economic and financial conditions. Following two points are important in the process of Inflation Accounting

- ➢ Inflation accounting is the practice of adjusting financial statements according to price indexes.55%unique
- ➢ Numbers are restated to reflect current values in hyper inflationary business environments.

METHODS OF ACCOUNTING FOR CHANGING PRICES

International accounting bodies have suggested a number of methods for measuring the impact of changing prices on the profitability and financial position of the business units. However, no single method has gained universal acceptance.

Noteworthy among the various methods the following are the generally accepted methods of accounting for price level changes:

- ➢ Current Purchasing Power Method [CPP method].
- ➢ Current Cost Accounting Method [CCA method].
- ➢ Current Value Accounting Technique (CVAT)

CURRENT PURCHASING POWER (CPP) METHOD

The CPP method [also termed as Constant rupee method] attempts to restate all items in the financial statements in terms of units of equal purchasing power. It seeks to eliminate the effects of changes in the general price levels or in the value of money itself. Money as a measuring rod is somewhat defective, because its value keeps on changing due to inflation or deflation.

It is important to note that under CPP method only the changes in the general purchasing power of money is relevant and not the value of individual asset. For example a particular asset has become cheaper over the period of time as against the increase in the general price index. In such a case, the value of such an asset will be raised in accordance with the general price index.

STEPS INVOLVED IN CPP METHOD

Conversion Factor: For converting historical rupees into equivalent uniform rupees [current purchasing prices] as at the date of balance sheet an index depicting the changes in the power of rupee is required.

THIS IS DONE THROUGH A CONVERSION FACTOR, WHICH IS CALCULATED AS FOLLOWS

$$\text{Conversion Factor} = \frac{\text{Price Index at the date of conversion}}{\text{Price at the date of transaction}}$$

For this purpose most broad-based retail or consumer price is used. And in case of transactions occurring throughout a period, an average price index of the period is used. Such transactions include items like sales, purchase of goods, payment of expenses, etc. The average price index may be calculated by taking the average of the opening and end of the period price index numbers.

A COMPANY HAS THE FOLLOWING TRANSACTIONS AT THE GIVEN DATES AND PRICE INDICES FOR THE FIRST QUARTER OF 2008

	₹	Price Index
Opening Balance (Jan. 1)	8,000	100
Cash Sales (Feb. 1)	15,000	104
Payment to Creditors (March 1)	10,000	106
Cash Purchases (March 1)	2,000	108

Payment of Expenses (March 31)	4,000	110
Closing Balance	7,000	110

Calculate Monetary Gain or Loss.

Solution :

Statement of Cash		Convertional Accounting ₹	Conversion Factor	Converted Values ₹
Opening Balance		8,000	110/100	8,800
Add: Cash Sales		15,000	110/104	15,865
	(a)	23,000		24,665
Less: Cash Payments				
(i) Creditors		10,000	110/106	10,377
(ii) Purchases		2,000	110/108	2,037
(iii) Expenses		4,000	110/110	4,000
	(b)	16,000		16,414
Closing Balance [(a) – (b)]		7,000		8,251
				₹
Expected balance				8,251
Actual Balance				7,000
Monetary Loss				1,251

Illustration 5. Compute the net monetary result of Apex Ltd. as on 31.12.2011 from the data given below :

	1.1.2011 ₹	31.12.2011 ₹
Cash	20,000	25,000
Debtors	50,000	60,000
Creditors	70,000	80,000
Loan	30,000	30,000
Retail price index numbers :		
January 1, 2011		200
Average for the year		210
December 31, 2011		230

Solution :

	₹
(i) Monetary Assets (Cash + Debtors) as on 1.1.2011	70,000
Monetary Assets (Cash + Debtors) as on 31.12.2011	85,000
∴ Additions of Monetary Assets during the year	15,000
(ii) Monetary Liabilities (Creditors + Loan) as on 1.1.2011	1,00,000
Monetary Liabilities (Creditors + Loan) as on 31.12.2011	1,10,000
∴ Additions of monetary liabilities during the year	10,000

(iii) Calculation of monetary gain on account of holding of monetary liabilities :

Converted value of monetary liabilities (1.1.11) $=1,00,000 \times \dfrac{230}{200}$ — 1,15,000

Add: Converted value of monetary liabilities (Additions in 2002)$=10,000 \times \dfrac{230}{210}$ — 10,952

1,25,952

Less: Historical value of liabilities on 31.12.2011 — 1,10,000

Gain on holding of monetary liabilities — 15,952

(iv) Calculation of monetary loss on account of holding of monetary assets

Converted value of monetary assets (1.1.2007) $=70,000 \times \dfrac{230}{200}$ — 80,500

Add: Converted value of monetary assets (Additions in 2002) $=15,000 \times \dfrac{230}{210}$ — 16,429

96,929

Less: Historical value of assets as on 31.12. 2007 — 85,000

Loss on holding of monetary assets — 11,929

(v) Net Monetary Gain = ₹ 15,952 – 11,929 = ₹ 4,023

2. LIMITATIONS OF CPP METHOD

The index numbers are statistical averages and the CPP method is based on indices. Hence, it would be very difficult to apply with precision to individual firms.

There are various price indices, which characterize different price situations. Hence, it would be a difficult task to select a suitable price index.

The method deals with changes in the general price level and not with the changes in prices of individual firms. However, the only relationship is that the individual prices move with the general price index to some extent.

Hence, a large number of accountants, economists, and Government authorities do not favor this method.

For example, a machine costing Rs. 80,000 has a life of eight years with zero scrap value and it has been used for three years. If the machine can be purchased now for Rs.80,000 then the net replacement cost of the asset would be Rs.70,000 i.e., the current price of the machine less depreciation for three years.

Plant and machinery, Motor vehicles, Office equipment, Fixtures and fittings, Ships and Aircrafts, etc., are normally valued at their net current replacement cost. Land and buildings occupied by the owner himself should be shown in the balance sheet at their value to the business, which will comprise the market value for existing use plus estimated acquisition cost.
If the open market is not available, then the net replacement cost of the buildings and open market value of land for its existing use plus the estimated acquisition cost should be taken as their value to the business. Long-term investments should also be shown in the balance sheet at their value to the business.

Quoted investments should be valued at their unit market value and unquoted investments are valued on the basis of the current cost net worth of the company. Inventories should be valued at power of the current replacement cost as on the date of the balance sheet and the net realizable value.

DEPRECIATION ADJUSTMENT

The profit and loss account should be charged for depreciation with an amount equal to the value of fixed assets consumed during the period. When the fixed assets are valued on the basis of their net current replacement cost, the depreciation charge should be based on such cost. The depreciation charge may be computed either on the basis of total replacement cost of the asset or on average net current cost of assets.

AVERAGE CURRENT COST MAY BE COMPUTED AS FOLLOWS

$$\frac{\text{Current cost at the beginning of the year } + \text{ Current cost at the end of the year}}{2}$$

The current depreciation charge is obtained by apportioning the average net replacement cost over the expected remaining useful life of the fixed assets as at the beginning of the period.

When the fixed assets are revalued every year there will also be shortfall of depreciation representing the effect of price rise during the period. This shortfall is called backlog depreciation, which should be charged either to general reserves or against the related revaluation surplus on the fixed assets. The need for adjustment of backlog depreciation will arise whenever a depreciating asset is revalued

Under the CCA technique, cost of sales are to be calculated on the basis of cost of replacing the goods at the time they are sold. The important principle is that current costs must be matched with current revenues. As for sales are concerned, it is current revenue and out of the costs, all operating expenses are current costs. But in case of inventories, certain adjustments will have to be made, known as cost of sales adjustment.

COST OF SALES ADJUSTMENT CAN BE CALCULATED WITH THE HELP OF THE FOLLOWING FORMULA

$$COSA = (C-O) \cdot I_a \left(\frac{C}{I_c} - \frac{C}{I_c} \right)$$

Where
- C = Historical cost of closing stock
- O = Historical cost of opening stock
- I_a = Average Index number
- I_c = Index number appropriate to closing stock
- I_o = Index number appropriate to opening stock

This has been explained in the following illustration.

ILLUSTRATION

CALCULATE THE 'COST OF SALES ADJUSTMENT' (COSA) FROM THE FOLLOWING

Historical Cost	Index Number	₹
Opening Stock	52,000	100
Purchases	2,20,000	110 (Average)
Total goods	2,72,000	
Less : Closing Stock	72,000	120
Cost of sales	2,00,000	

Solution :

$$COSA = (C-O) - I_a\left(\frac{C}{I_c} - \frac{O}{I_o}\right)$$

$$Or\ COSA = (72,000 - 52,000) - 110\left(\frac{72,000}{120} - \frac{52,000}{100}\right)$$

$$= 20,000 - 110\,(600 - 520)$$

$$= 20,000 - 8,800 = ₹\,11,200.$$

Cost of sale adjustment can also be calculated as under :	₹
Opening stock at average index $= 52,000 \times \dfrac{110}{120}$	57,200
Add : Purchases at average index	2,20,000
Total goods available at average index	2,77,200
Less : Closing stock at average index $= 72,000 \times \dfrac{110}{120}$	66,000
Current Cost of Sales	2,11,200
COSA = Current Cost of Sales – Historical Cost of Sales	

$$= ₹\,2,11,200 - 2,00,000 = ₹\,11,200$$

(ii) **Depreciation Adjustment.** Under the CCA method assets are shown in the balance sheet on current replacement costs after allowing for depreciation. This will require an adjustment in depreciation also. Current year's depreciation under CCA can be calculated with the help of following formula :

Current year's depreciation (CCA method)

$$= \frac{\text{Opening Current Value of Assets} + \text{Closing Current Value of Assets}}{2 \times \text{Life of Asset}}$$

And, Depreciation Adjustment = Current year's depreciation of CCA – Depreciation on historical cost

HOWEVER, THERE ARE MANY DIFFICULTIES IN THE OPERATION OF CCA TECHNIQUE

- ➢ It is very difficult to determine the 'value to the business' of a real asset.
- ➢ There is an element of subjectivity in this technique.
- ➢ It does not hold good during the periods of depression.

SOME IMPORTANT ADJUSTMENTS REQUIRED UNDER THE CCA TECHNIQUE

CURRENT COST OF SALES ADJUSTMENT (COSA)

Under the CCA technique, cost of sales are to be calculated on the basis of cost of replacing the goods at the time they are sold. The important principle is that current costs must be matched with current revenues. As for sales are concerned, it is current revenue and out of the costs, all operating expenses are current costs. But in case of inventories, certain adjustments will have to be made, known as cost of sales adjustment.

COST OF SALES ADJUSTMENT CAN BE CALCULATED WITH THE HELP OF THE FOLLOWING FORMULA

$$COSA = (C-O) \cdot I_a \left(\frac{C}{I_c} - \frac{C}{I_c} \right)$$

Where
- C = Historical cost of closing stock
- O = Historical cost of opening stock
- I_a = Average Index number
- I_c = Index number appropriate to closing stock
- I_o = Index number appropriate to opening stock

This has been explained in the following illustration.

CURRENT VALUE ACCOUNTING TECHNIQUE

In the Current Value Accounting Technique of price level accounting all assets and liabilities are shown in the balance sheet at their current values.

The value of the net assets at the beginning and at the end of the accounting period is ascertained and the difference in the value in the beginning and the end is termed as profit or loss, as the case may be. In this method also, like replacement cost accounting technique, it is very difficult to determine relevant current values and there is an element of subjectivity in this technique.

ILLUSTRATION
THE FOLLOWING ARE THE BALANCE SHEETS OF XYZ CO

Liabilities	2010 ₹	2011 ₹	Assets	2010 ₹	2011 ₹
Equity Share Capital	2,00,000	2,00,000	Land & Buildings at cost (Purchased in 2003)	1,00,000	1,00,000
Profit and Loss Account	15,000	20,000	Plant ad Machinery (Cost ₹ 1,50,000 Purchased in 2003)	75,000	67,500
Sundry Creditors	25,000	30,000	Inventories	30,000	37,500
			Sundry Debtors	20,000	25,000
			Cash	15,000	20,000
	2,40,000	2,50,000		2,40,000	2,50,000

The General price index was 100 in 2003 (base year), 200 in 2010 and 250 in 2011. No dividend was paid in 2011.

You are required to prepare :
(i) Supplementary Income Statement at current values.
(ii) Supplementary Comparative Balance Sheet at current values.

Solution :

	Conversion of Assets at Current Values (2011 Index)				
Name of Asset	Conversion Factor	Historical Costs 2010 (₹)	2011 (₹)	Converted Values 2010 (₹)	2011 (₹)
Land & Buildings	250/100	1,00,000	1,00,000	2,50,000	2,50,000
Plant & Machinery	250/100	75,000	67,500	1,87,500	1,68,750
Inventories	250/200	30,000	37,500	37,500	37,500
Sundry Debtors	250/200	20,000	25,000	25,000	25,000
Cash	250/200	15,000	20,000	18,750	20,000
	Conversion of Liabilities at Current Values (2011 Index)				
Sundry Creditors	250/200	25,000	30,000	31,250	30,000

Calculation of Loss from Holding Current Assets	Historical Values ₹	Current Value ₹
Inventories	30,000	37,500
Sundry Debtors	20,000	25,000
Cash	15,000	18,750
	65,000	81,250

Loss = Current Values − Historical Values of Current Assets = ₹ 81,250 − 65,000 = ₹ 16,250

Calculation of Gain Arising From Current Liabilities

From Sundry Creditors (Current Value − Historical Value) = ₹ 31,250 − 25,000 = ₹ 6,250
Net Loss from Holdig Curret Assets and Curret Liabilities = 16,250 − 6,250 = ₹ 10,000

Supplementary Income Statemet At Current Values

	₹		₹
To Additional Depreciation, current Values on Plant & Machinery	11,250 (1)	By Balance of P/L/A/c (20,000–15,000)	5,000
To Loss on Net Current Assets	10,000	By Loss for the year	16,250
	21,250		21,250

(1) Depreciation Required = 1,87,500 − 1,68,750 = 18,750
Less : Depreciation already provided = 7,500
= 11,250

Supplementary Comparative Balance Sheet at Current Values					
Liabilities	2010	2011	Assets	2010	2011
Equity Share Capital	2,00,000	2,00,000	Land & Buildings	2,50,000	2,50,000
Revalution Reserve	2,87,500	2,87,500	Plant and Machiery	1,87,500	1,68,750
Sundry Creditors	31,250	30,000	Inventories	37,500	37,500
			Sundry Debtors	25,000	25,000
			Cash	18,750	20,000
			Loss	—	16,250
	5,18,750	5,17,500		5,18,750	5,17,500

3. CONCLUSION

The primary **conclusion** of using **inflation accounting** is that **inflation**

➢ Distorts government budget **accounting** by exaggerating interest expense.
➢ Reduces the national debt to its nominal value instead of its real value

➢ Causes recessions, and increases the structural deficit.
➢ Distorts the tax system, and results in slower economic growth.

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TRENDS IN PRESENT & FUTURE IN PERSPECTIVE OF COMMERCE & MANAGEMENT

STRATEGIES IN MANAGEMENT

P.Sireesha ,
Faculty of commerce ,
St.Anns college for women ,Mehdipatnam

D.Sujatha
Faculty of commerce ,
St.Anns college for women ,Mehdipatnam

ABSTRACT:
Anyone who wants to shove an organization safely and successfully into the future needs a "north star" or a strategy. Despite of the type of organization or the state of the wider economy, a clear strategy increases the possibility of achieving success, however that term is defined as "Management strategies are a collection of processes that businesses use to ensure their activities remain aligned to the company's mission, objectives and strategic plan.
 Management strategies exist because, in the long-run, organizations can only achieve top performance if they have a clear strategy in place and the strategy is anchored throughout the company. Otherwise, the ship would be driving forward with no clear direction, potentially toward the iceberg.

In present scenario i.e,pandemic situation management need to focus on management techniques to get maximum output from human as well as financial and technical resources.

The prime focus on this paper is strategies to be implemented by the management in this pandemic situation and the new emerging trends of management.the main aim of this paper is to bring the techniques available to the management to dirct and control to get better output with the strategies implemented in the organization .

Key words :strategy ,pandemic emerging trends ,management

What are Management Strategies?

Management strategies, are a series of techniques for controlling and directing a business to achieve a set of preset goals. They include strategies for goal-setting, leadership, business administration and operational activities.

A strategy lays the foundation for achievement of goals by:

- Determining the goals and objectives of the organization.
- Establishing the timeline for achieving those goals; short, medium or long-term.
- Establishing the resources necessary for carrying out those goals.
- Providing a clear sense of direction for the company and its employees

employees can better see how their daily activities fit into the organizational plan. Providing consistency and discipline in the way that managers manage. So decisions are not made in a vacuum. Allowing managers to orient themselves in uncertain conditions, such as increasing digitization or regulatory change.

What is a Strategy?

Anything that a manager does, or consciously chooses not to do, to achieve the organization's goals is a strategy. It provides the blueprint for what the organization will do, what type of business it wants to be, how it will make decisions, how it will optimize its strengths and reduce the impact of its weaknesses and how it will behave toward its customers, employees and stakeholders, among other things.

Within this broad definition, there are three features that all management strategies have in common.

 First, a strategy will deal with long-term objectives rather than one-time or routine incidents.

Second, a strategy will provide a clear road map for getting the company from where it is now to where it wants to the future – there must be a clear connection between the strategy and its intended objective.

Third, a strategy will take into account the likely behavior of competitors, customers and employees. Organizations cannot predict the future, but by estimating what customers and competitors will do next.

The Five Stages of Strategic Planning

There is no common mold for developing management strategies. The one that is perhaps the most well known proposes a five-stage process, namely:

- Goal-setting.
- Situational analysis.
- Strategy formulation.
- Strategy implementation.
- Evaluation and control.

The benefit of the five-stage model lies in its digilence. The model is envisaged as a un interrupted activity, with lessons learned from the evaluation stage feeding back into future goal-setting.

Goal-Setting: Clarifying the Vision

The idea is to define:

- Core objectives or what the company stands for (mission, vision, core values, strategic focus areas).
- The process of how to accomplish each objective (improving service delivery standards, breaking new markets, cutting costs, improving results, reducing risk, improving team relationships and so on).

The goals fall into four categories:

> ❖ **customer** (improve loyalty, satisfaction or repeat business),
> ❖ **financial** (revenue targets, return on investment),
> ❖ **internal** (efficiency, risk management, innovation and other business capabilities) and
> ❖ **growth/learning** (corporate culture, staff engagement and development).

The idea is to connect the dots between the big-picture objectives and the operational elements, so you can develop specific action plans for your departments, work functions and teams.

Situation Analysis: Where Do You Stand in the Market?

A **situation analysis** evaluates the company's internal environment (resources, strengths and weaknesses), external environment (state of the industry, regulation,

economic conditions, labor market and so on) and competition, to give a picture of the organization's situation in the market.

A good place to start is by doing a **SWOT analysis**, looking at the company's

Strengths (e.g. assets, resources, knowledge, good reputation),

Weaknesses (e.g. high production costs, limited service line, limited marketing budget),

 Opportunities (e.g. government incentives, local business partnerships, strong market growth) and

Threats (e.g. discounting by competitors, increasing supplier costs, technology and systems becoming obsolete).

 When doing a SWOT analysis, you look at both internal and external factors to figure out the company's critical success factors and potential profitability.

Many other tools are useful in this context, including the PEST or PESTLE frameworks, Core Competencies, Porter's 5 Forces, Market Segmentation and Scenarios Forecasting. Articles on how to run these analyses are readily available online.

Strategy Formulation: Choosing the Best Strategy

Strategy formulation is the process of choosing the **best** and most appropriate course of action to achieve an objective.. It's a good idea to fix some numbers or other targets to the objective so you can evaluate performance as you weigh one alternative against another. Benchmarking is a useful tool. This allows you to agree on some quantitative and qualitative criteria for assessing performance.

For example, you might measure things like units sold, revenue, net profit, return on investment, earning per share and cost of production (quantitative criteria) as well as flexibility, response to change, skill acquisition, employee motivation and other intangibles (qualitative criteria)

Strategy Implementation: Time for Action

Strategies are just ideas until you put them into action.

Managers who have dedicate a lot of time and effort to selecting the right strategy may feel they have every reason to be confident about the strategy's success but, in reality, implementation is a complex and demanding process. There may be weak senior-level commitment, budgetary pressure or ambiguity in job functions. There may be a lack of accountability or resistance to the change. Whatever the challenge, you're going to need to align people of all levels to the strategy. Some tips include:

- Communicate widely and openly.
- Ensure clearly defined tasks and responsibilities so everyone knows what is expected of them and what needs to be done.
- Put the focus on the consistent implementation of activities.
- Promote the team spirit ("We're all in this together").
- Closely control the process, so that deviations from targets are detected early and countermeasures are quickly put in place.

Strategy Evaluation: Has it Worked?

The final stage of the strategic management process sheds light on how effective your strategies have been in achieving the desired results. If the strategy is working as expected you should be hitting all or most of your benchmarks. If results are below par then either something has gone wrong in the implementation phase or the strategy itself is poor. In the worst case, you have to go back to the goal-setting stage and re-frame your plans in the light of your results.

Common Sources of Error in Strategies

There must be a **clear and logical connection** between the intended objective and the strategy you're formulating to achieve it. If you run a situational analysis and there are still gaps and weaknesses in the strategy, it's not yet ready for implementation.

Another source of error is **inside-out thinking**. This happens when companies look primarily at the technical side of their product and fail to pay sufficient attention to the market and customers. This is how "golden faucets" are made – products that are innovative but just too expensive for consumers. Although innovation is an important source of competitive advantage, a strategy like this would be out of alignment with the customer's priorities.

 bottom-up method of strategy development. Certainly, a good strategy must be broken down for individual departments and functions so that each functional area

understands what's expected of it. But if you start at the departmental level and try to put the puzzle pieces together, there's a fair chance that the pieces won't fit. The result is not a coherent strategy, but a patchwork of policies that are cut and sewn together without any sense of cohesion across the entire organization.

The Benefits of Strategic Management:

While many organizations understand the importance of strategic planning and spend a great deal of time and money coming up with the strategic plan, it still remains something that is reviewed just once a year or worse – a glossy document that sits on the shelf.

You might be thinking: Is it worth embarking on a strategic planning process? There are many benefits that go along with strategic planning. The key to successful strategic planning is to build in measures and implementation steps that allow you to engage your staff and monitor the results at regular intervals

Here are the top 5 benefits of strategic planning:
1. It allows organizations to be proactive rather than reactive
A strategic plan allows organizations to foresee their future and to prepare accordingly. Through strategic planning, companies can anticipate certain unfavourable scenarios before they happen and take necessary precautions to avoid them. With a strong strategic plan, organizations can be proactive rather than merely reacting to situations as they arise. Being proactive allows organizations to keep up with the ever-changing trends in the market and always stay one step ahead of the competition.
2. It sets up a sense of direction
A strategic plan helps to define the direction in which an organization must travel, and aids in establishing realistic objectives and goals that are in line with the vision and mission charted out for it. A strategic plan offers a much-needed foundation from which an organization can grow, evaluate its success, compensate its employees and establish boundaries for efficient decision-making.
3. It increases operational efficiency
A strategic plan provides management the roadmap to align the organization's functional activities to achieve set goals. It guides management discussions and decision making in determining resource and budget requirements to accomplish set objectives — thus increasing operational efficiency.
4. It helps to increase market share and profitability
Through a dedicated strategic plan, organizations can get valuable insights on market trends, consumer segments, as well as product and service offerings which

may affect their success. An approach that is targeted and well-strategized to turn all sales and marketing efforts into the best possible outcomes can help to increase profitability and market share.

5. It can make a business more durable

Business is a tumultuous concept. A business may be booming one year and in debt the next. With constantly changing industries and world markets, organizations that lack a strong foundation, focus and foresight will have trouble riding the next wave. According to reports, one of every three companies that are leaders in their industry might not be there in the next five years… but the odds are in favour of those that have a strong strategic plan!

If you're considering adopting a strategic plan at your organization, or are looking for a way to align your staff and daily operations on your existing one, then Envisio may just be the solution you've been looking for. Envisio's cloud-based strategic plan management software helps organizations manage their strategic and operating plans, track performance, and report to stakeholders.

Three Management Strategies You Can Use Today For Higher Team Performance

1) A Clear Reason Why- Give employees the big picture of how their work and responsibilities are going to make an impact, large or small. Share the big picture around these concepts:

- Why is this project important, what is the opportunity?

- How will the project create value for customers?

- How will it create value for the company?

- How does my workday connect to the wider world outside of the office?

- How does the work lead to more of what the employees want in their lives?

2) Evoke curiosity - Instead of saying "Do this, this way", ask every employee the question: "How can we best accomplish this? What do you think?" "I wonder what would happen if we did this?" Even if you already have a good idea of what needs to be done, an inclusive conversation will take you and your products to unexpected places.

3) Trust and empower - Allow curiosity and engagement to unfold. As employees demonstrate engagement and capability, allow them to spread their wings and have more and more control of their work.

As you find employees that choose to be co-owners with the business, who decide to make the business' success, their success, do whatever you need to do to keep them for the long-term. These are your superstars, and will form your core team. It is around this core of 'lead by example' team members that you will recruit and grow new team members and take the team to the next level. Ownership Mentality is a choice. It cannot be forced, coaxed, ordered, or 'implemented' by management. Ownership Mentality is a frame of mind that comes from a deep and personal identification with work and its outcomes. This identification is an expression of "I Am!". It is an existential statement, and comes from the unfolding of curiosity and possibility. This is the ultimate 'bottom-up' work paradigm – which can result in outsized results and is a key component for high performance teams.

Strategic management development trends :

Balanced score card :

This system views organization from four perspectives :

1.learning and growth .

2.business process .

3.customers and finance .

4.financial perspective for gathering and analyzing data.

FUTURE TRENDS IN STRATEGIC MANAGEMENT

1.Emerging technology proliferation.

2.Environment controls…

3.Time to market .

4.Workplace social environment .

5.The complexity of business growth leads to collaboration.

6.Digitization to manage information overload.

7.Digital skill enhancement among leaders.

8.Contexual problem solving.

9Hyper automation (machine learning).

10.Human augmentation .

STRATEGY PRESENT AND FUTURE REQUIRES :

Strategy present requires	Strategy future requires
1.Objectives	1.Resetting of objectives
2.Business definition	2.Redefning of business definition
3.Positoning	3.Re-positioning
4.Segmentation and focus	4.Re segmentation and focus
5.Differentiation	5.Re-differentiation

Conclusion

This chapter has explained elements of organizational design that are vital for executing strategy. Leaders of firms, ranging from the smallest sole proprietorship to the largest global corporation, must make decisions about the delegation of authority and responsibility when organizing activities within their firms. Deciding how to best divide labor to increase efficiency and effectiveness is often the starting point for more complex decisions that lead to the creation of formal organizational charts. While small businesses rarely create organization charts, firms that embrace functional, multidivisional, and matrix structures often have reporting relationships with considerable complexity. To execute strategy effectively, managers also depend on the skillful use of organizational control systems that involve output, behavioral, and clan controls. Although introducing more efficient business practices to improve organizational functioning is

desirable, executives should avoid letting their firms become "out of control" by being skeptical of management fads. Finally, the legal form a business takes is an important decision with implications for a firm's organizational structure.

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