



Fabricating NiCoFe₂O₄ decorated PANI nanostructures for high-performance electrochemical detection of 3-Nitro-L-tyrosine biomarkers for rapid diagnosis

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ABSTRACT

A recent development in novel catalytic nanostructures offers numerous opportunities to perform the electrochemical operations towards sensing applications. The organic material PANI has been endorsed as a conducting polymer as it has controlled electrical properties and low density which will be beneficial over the metallic nanostructures. Herein, Nickel Cobalt Ferrite (NiCoFe₂O₄ (NCF)) nanostructures were anchored on the surface of PANI and engineered for electrochemical sensing applications. 3-nitro-L-tyrosine (3-NT) is an important compound for diagnosing oxidizing biomarkers that plays a vital role in therapy and diagnosing pathogenesis. Due to NCF/PANI's conductivity, and superior surface area. It was probed for electrochemical sensing of 3-NT. The NCF/PANI displayed an excellent LOD, broad linear range, better sensitivity, selectivity, and stability. Furthermore, the proposed sensor exhibits sensitive 3-NT detection in biological samples with satisfactory recovery rates.

1. Introduction

3-Nitro-L-tyrosine (3-NT) is an important biomarker for wide range of clinical conditions, including rheumatoid arthritis, diabetes, Alzheimer's disease, atherosclerosis, and cancer. Reactive nitrogen species (RNS) production is uncontrolled and antioxidant defenses in human tissues and cells are the contributing factors of the imbalance condition. It causes human cell damage, Parkinson's disease, cancer, inflammatory, cardiovascular, neuropsychiatric, and neurodegenerative diseases (Shishehbor, Aviles, Brennan, Fu, Goormastic, Pearce, Gokce, Keaney Jr, Penn and Sprecher, 2003, [2–5]). Whenever the human body is under stress from oxidative damage, it is unable to generate enough defenses against antioxidants to eliminate RNS species (nitric oxide-derived compounds). Then the RNS species directly damage the tyrosine residues in the proteins and generate soluble 3-NT [6]. The extensive level

of 3-NT in the body fluids acts as an indicator or a diagnosis for oxidative stress by RNS. The expensive and time-consuming technologies of enzyme-linked immunosorbent assay (ELISA), chromatography, and fluorescence approaches have been existing in the detection of 3-NT [10, 7–9]. The precise platform has been developed for the assessment of 3-NT using an electrochemical sensor due to its numerous benefits. The advantages of the electrochemical sensing technique involve being more sensitive, cost-effective, highly selective, strong, high-speed, non-toxic, and easy to handle [11]. However, electrochemical sensors with bare electrodes have a limitation in selectivity, sensitivity, and reproducibility. To detect the electroactive compounds accurately, the electroactive nanocomposite-modified electrodes have been preferred to get strong electrochemical signals with stability or the electroactive material-coated electrodes have been ideal to enhance the sensing performance towards the target chemical detection [12]. The 3-NT

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compounds have been detected by the electrochemical technique [13, 14].

A wide range of applications for electrochemical sensing technology exists, including environmental monitoring, medical diagnostics, and food safety management systems. Massive efforts have been made over the last few decades to create electrochemical devices that meet important characteristics such as downsizing and simplicity, affordability, stability, speed of response, and selectivity [1]. Due to their programmable electrochemical characteristics, conducting polymers including polyaniline (PANI), polythiophene, and polypyrrole have been used to build electrochemical sensors. Among various materials, PANI is one of the promising polymers because of its benefits of being simple to prepare, having strong conductivity, and being environmentally stable [15,16]. The electrical behaviors of this polymer can be tunable by varying their doping concentration. Moreover, through composites, incorporating any number of compounds to this polymer (PANI), which include transition metals oxide, nanoparticles of metal, silica, and carbon, can effortlessly boost its mechanical strength. Numerous sensor applications, including electrochemical supercapacitors, have been developed using PANI composite materials [17]. The PANI-based nanocomposites will behave as an excellent sensing material owing to their, conjugated structure, high surface area, amine group presence and superior redox characteristics. PANI is a conductive polymers material that have undergone through investigation since it has its peculiar electrochemical features. It can be synthesized in different forms, including nanostructures, films, and composites, making it suitable for various applications. Yet their inability to dissolve in conventional solvents made of organic matter, volatility at higher temperatures, and weak electrochemical equilibrium, consistency, and selectivity limit their sensing application.

Contrarily, transition metal oxide nanoparticles have drawn a lot of interest in electrochemical and biosensing applications due to their electrocatalytic activities and excellent adsorption capacities [17,18]. The nickel cobalt ferrite ($\text{NiCoFe}_2\text{O}_4$) nanocomposite has beneficial properties such as high permittivity and permeability, optimal resistivity ($\sim 10^5\text{--}10^4\text{ ohm cm}^{-1}$), low magneto crystalline anisotropy, high Curie temperature ($\sim 800\text{ K}$), and strong chemical stability [17]. Due to their inherent advantages of being inexpensive, wide availability on earth, and easy to manufacture, 3D transition-metal-based nanomaterials (TMMs) with a variety of structures have recently attracted a lot of attention. [2,3]. One of the promising applications of PANI is in the development of composite materials with metal oxides or transition metals, such as Ni, Co, and Fe. These composites have shown improved electrochemical properties, making them ideal for use in batteries, supercapacitors, and sensors [19]. In particular, the Ni-Co- Fe_2O_4 /PANI (NCF/PANI) composite has attracted significant attention because of its excellent rate capability, good cycling stability and high specific capacitance. Several studies have reported the synthesis process and characterization of NCF/PANI composites and their electrochemical performance. Co-precipitation is a simple and cost-effective technological advance that may be implemented on many different levels without the requirement for expensive equipment. The concluded product's composition may be easily changed by altering precursor concentrations of substances, which allows for specific material character traits. Co-precipitation produces nanoparticles with a limited size distribution and, therefore can be utilized for applications that involve catalysis or drug administration. Co-precipitation processes frequently produce a large volume of the required product, which contributes to affordability and effectiveness. D. Navadeepthy et al. (2019) synthesized a NCF/PANI composite and demonstrated its superior electrochemical properties as an electrode component for supercapacitors [20]. Li et al. (2021) described the procedure for the synthesis of an NCF/PANI composites and its function as an anode layer for batteries powered by lithium-ion, showing the significant capacity, and excellent process stability [21]. Hence, the NCF/PANI composite has shown promising results as an electrode compound for a range of energy storage applications. Further

research is needed to optimize the synthesis and performance of these composites, as well as to explore their potential in other areas such as sensors and catalysis. Improved electrocatalytic behavior is provided by the combination of nanostructured materials through hyper heterojunction of counter components through a synergistic impact. The produced nanocomposite limits the working efficacy of the electrocatalyst by preventing undesired growth or flaking during the reduction or oxidation process, which could cause instabilities. This makes it an effective electrode modifier [22].

Here, we designed a sensor based on the synthesized magnetic nanoparticles with conductive polymer (NCF/PANI) by co-precipitation method to sense the 3-NT biomarkers in human urine and saliva samples.

2. Experimental methods

2.1. Synthesis of NCF-PANI nanocomposite

The synthesis procedure of NCF and PANI was explained detailed in supplementary section (SI). The combination of components of nanocomposite material was developed through the mixture of NCF with PANI, which is with aniline monomer throughout the process of polymerization, as explained in the poly aniline (PA) production part. Scheme1, the visual representation of the synthesis technique.

2.2. Fabrication of modified GCE

Firstly, the bare glassy carbon electrode (GCE) was cleaned with $0.05\text{ }\mu\text{m}$ alumina powder and then washed thoroughly with DI water. Subsequently, the bare GCE was dried at 50° C . The NCF/PANI stock solution was prepared in DI water 1 mL and 2 mg of NCF/PANI and sonicated for 30 mins . Then, the $6\text{ }\mu\text{L}$ NCF/PANI suspension was drop casted on to the mirror surface of GCE and dried for 15 mins . For comparison NCF and PANI were prepared by similar method. In this work, we used 0.1 M phosphate buffer (PB) as an electrolyte. The CV and DPV experiment was carried out in 0.1 M PBS in potential of $+0.6\text{ V}$ to -0.9 V at a scan rate 0.05 Vs^{-1} . The EIS experiment was carried out in a 0.1 M KCl solution containing 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ (FC system).

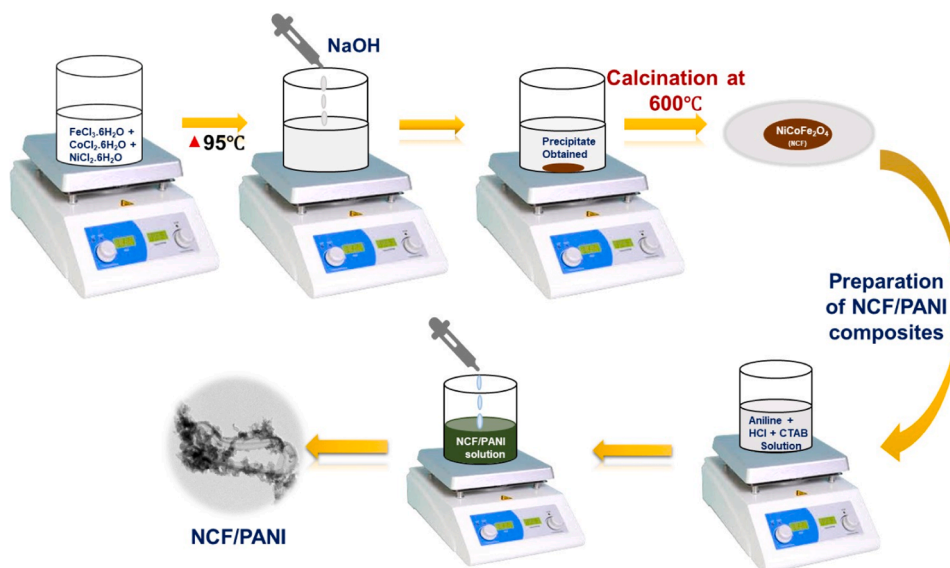
3. Results and discussions

3.1. XRD analysis

The technique of diffraction of X-Ray Diffraction (XRD) in powder is frequently employed for defining substances at the level of the nano-scale. Powdered XRD investigation of a given specimen, along with many microscopy and spectroscopy techniques, can offer significant details regarding the specimen, which includes phases proof of identity, sample purity, the crystallite size, and shape. The XRD patterns of NCF, PANI, and NCF/ PANI were shown in Fig. 1A(a-c). NCF's XRD pattern depicts the strong peaks were observed for $2\theta = 30.3, 35.6, 39.5, 43.3, 53.8, 57.4,$ and 62.9 corresponding to the Ni-Co-Fe crystal planes of (220), (311), (222), (400), (422), (511), and (440) respective planes of the spinel ferrite with cubic symmetry [23]. By using the Debye-Scherrer formula, the average Ni-Co-Fe crystallographic size was estimated toward being 13.46 nm using the following formula[24],

$$D = K \lambda / \beta \cos \theta \quad (1)$$

Where ' λ ' = $1.54\text{ \AA}$ (wavelength of X-rays), ' K ' is a geometrical factor (constant), ' β ' is full width at half maximum of the desired peak at the diffraction angle ' θ '. A diffuse broad-range range peak extending from 20° to 30° verified PANI's amorphous composition. The huge peak at around $2\theta = 24.3^\circ$ depicts the amorphous character of something like the intermittently parallel PANI chains [25]. In the XRD of PANI, they were observed in the region of $2\theta = 20\text{--}25^\circ$. Whereas the NCF/PANI's



Scheme 1. Graphical representation of NCF/PANI preparation.

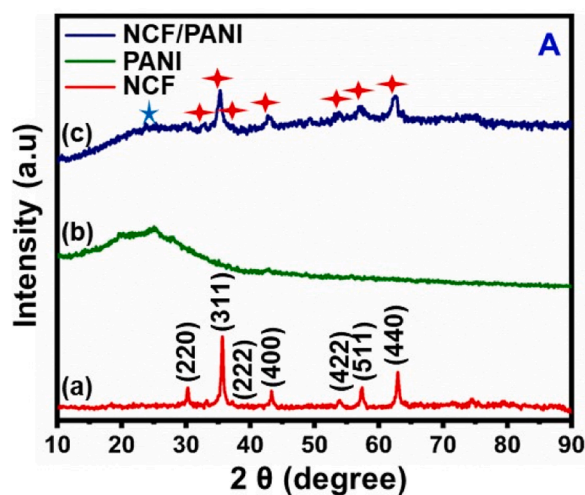


Fig. 1. XRD patterns of synthesized materials: (a) NCF (b) PANI and (c) NCF/ PANI.

XRD (c) exhibit characteristic distinguishing peaks of PANI and polycrystalline NCF, in decreasing order ferrite percentage. All the major peaks have appeared concerning NCF/ PANI nanocomposite.

3.2. SEM analysis

The Scanning Electron Microscopy (SEM) images of the pure NCF and PANI are indicated in Fig. 2. Fig. 2(A,B) shows that the NCF material had a porous structure, with interconnected pores of varying sizes throughout the material. Fig. 2(C) depicts the PANI having a porous structure with a smooth surface, which was assembled by a aggregated rod. The PANI chains have been generated in environments that are acidic and exist as polycations featuring powerful bonds with each other charged PANI, which creates a rod shape. The reason for that PANI influence builds up the surface area of the NCF, which results in boosted surface contact between it and the electrolytes. Fig. 2(D,E) The NCF/ PANI nanocomposite material is synthesized in the plate-like nanoparticles at a thickness of approximately 50 nm. The physical structure of the end product stays unchanged when polyaniline is incorporated among NCF sheets. In addition, the implementation of NCF

nanoparticles will effectively eliminate PANI chain entanglement due to the repulsion between charged NCF nanoscale in an acidic atmosphere. The SEM image of the NCF/ PANI nanocomposite demonstrates that is generated of several different nanoparticles that connect in order to form a structure that is porous.

The Transmission Electron Microscopy (TEM) micrograph of the NCF/PANI composite shown in Fig. 2 (F-H) reveals an idea of the spherical shape distribution of NCF nanoparticles embedded in the polyaniline matrix at different magnifications. Fig. 2 (H) reveals the existence of non-crystal PANI which is depicted in the amorphous layer and crystal layer of NCF. The TEM images of the sample were consistent with the structure of SEM images. The SAED pattern of the NCF/PANI sample in Fig. 2(I) presents clearly that the composite is of polycrystalline nature which is confirmed by the appearance of bright spots. It is promised that the NCF nanoparticles are well bound in the PANI matrix. The purity of the synthesized NCF/PANI nanocomposite has been confirmed by the elemental mapping studies in Fig. 3 subsequently depicts a consistent dispersion of each of the elements – Ni, Co, Fe, C, N, O. This results confirms the successful formation of NCF/PANI composite.

3.3. Electrochemical investigation of NCF/PANI

Electrochemical impedance spectroscopy (EIS) is used to analyze the interfacial properties of the modified electrodes. The EIS Nyquist plot was observed in 0.1 M KCl and FC system as a redox probe. The EIS plot exhibit a semicircle along with a straight line, the semicircle indicating the electron charge transfer process, while the straight line indicating the diffusion process. From the Fig. 4 A it was clear that the NCF/PANI/ GCE exhibit significantly less electron transfer resistance (R_{ct}) than the other modified and bare GCE. The R_{ct} value is equal to the diameter of the semicircle, Fig. 4 A displays the Nyquist plot of bare GCE has higher resistance (554.7 Ω) is due to the poor active sites. NCF/GCE reveals a R_{ct} of 402.8 Ω less than the bare GCE as because of having more active sites of NCF nanoparticle. The PANI/GCE has a R_{ct} value of 170.2 Ω due to high active sites on the conducting polymer. Whereas, the R_{ct} value of NCF/PANI/GCE is 132.5 Ω , the insertion of conducting polymer provides more surface area enabling more electron transfer rate.

The electron transfer ability of the modified GCE was investigated in Cyclic Voltammetry (CV) analysis in FC system. Fig. 4B displays the CV response of modified and unmodified electrode. The bare depicts the redox peak with a high peak-to-peak separation ($\Delta E_p = 290$ mV). Then,

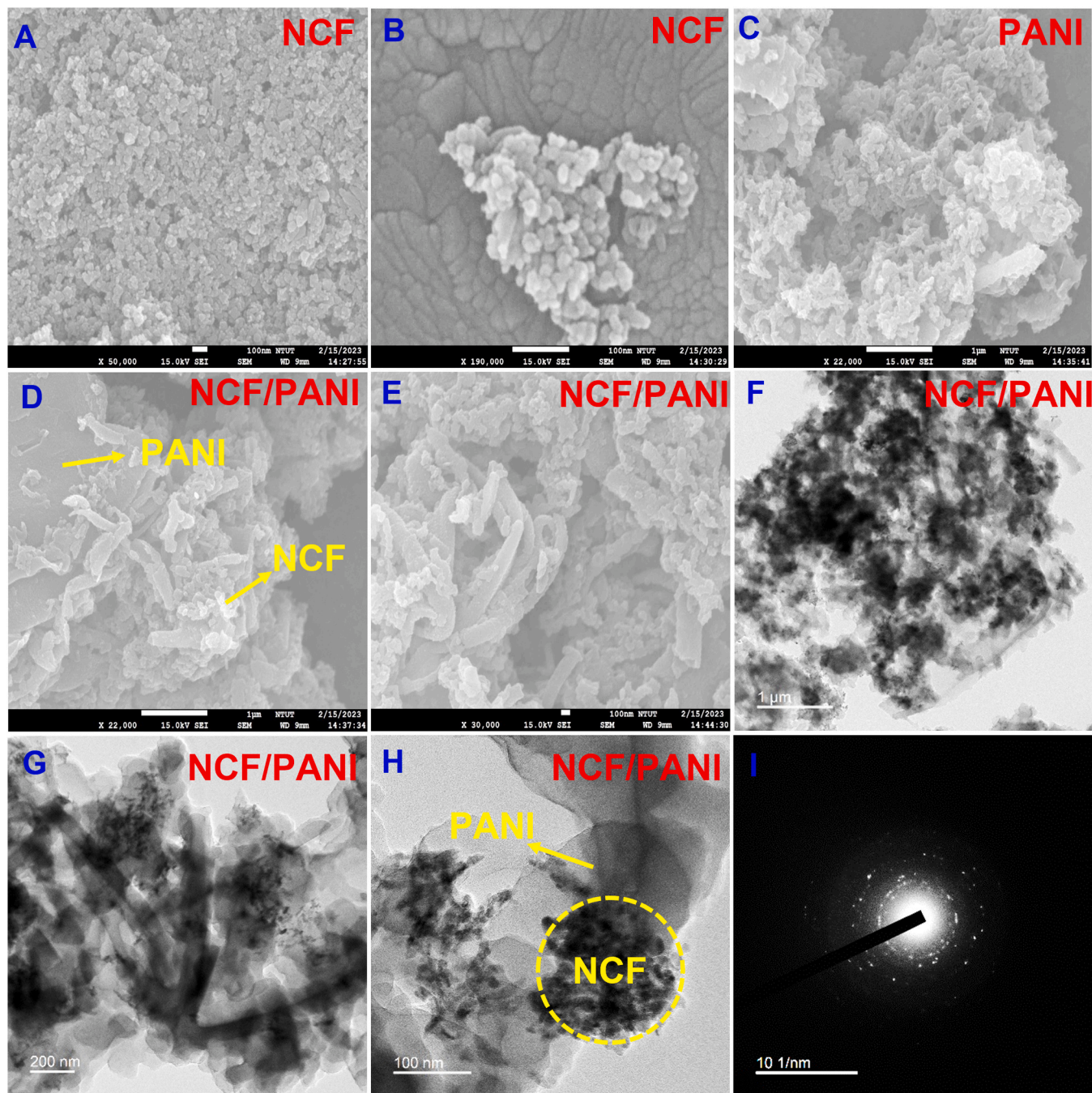


Fig. 2. SEM and TEM morphology analysis of (A,B) NCF (C) PANI (D-H) NCF/PANI at different magnifications, and (I) SAED pattern.

NCF/GCE shows an increased peak than the bare GCE with a ($\Delta E_p = 173$ mV). The PANI/GCE displays a redox current higher than the bare GCE and NCF/GCE with a $\Delta E_p = 129$ mV. The NCF/PANI/GCE displays a distinct redox peak current with a low peak-to-peak separation ($\Delta E_p = 123$ mV) value than the other modified electrodes as because these films allows easier permeation of $[\text{Fe}(\text{CN})_6]^{3-/4-}$ ions, a higher surface area, and the higher active sites is due to the effect between the NCF and PANI composite. Among, the low peak to peak separation, higher current indicates the best conductivity and electron transferring ability.

To check the electrocatalytic activity and the surface of the NCF/PANI/GCE, CV study was investigated in FC system. Fig. 4C displays a CV response of NCF/PANI/GCE at increasing scan rate from 20 mVs^{-1} – 300 mVs^{-1} . The corresponding linear plot was displayed in Fig. 4D. The electrochemical active surface area (EASA) was calculated using

Randle's Sevcik Eq. (1),

$$I_p = (2.69 \times 10^5) n^{3/2} D^{1/2} A \nu^{1/2} C \quad (1)$$

Where D is the diffusion coefficient, I_p is the peak current value, ν is the scan rate, C is the bulk concentration of $[\text{Fe}(\text{CN})_6]^{3-/4-}$ solution, and n represents the number of electrons involved during the electrochemical reaction. The calculate EASA values of bare GCE, NCF/GCE, PANI/GCE, and NCF/PANI/GCE were 0.058, 0.091, 0.098, and 0.117 cm^2 . The results depict that the NCF/PANI/GCE has a higher EASA which demonstrates that the NCF/PANI/GCE provides benefits for the electrochemical sensing detection.

In order to investigate the effect of pH on the electrochemical behaviour of $100 \mu\text{M}$ of 3-NT in 0.1 M PBS, different pH was studied. In Fig. 5A the peak current gets increased from pH 3–7 and gets decreased

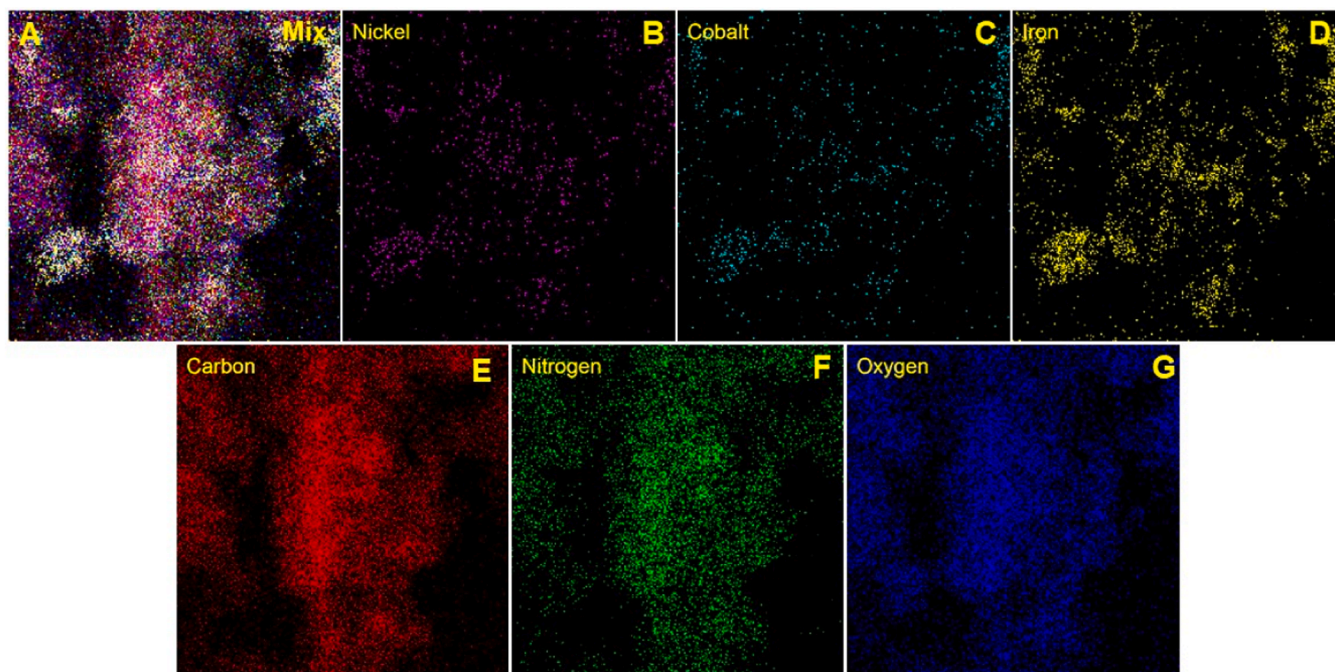


Fig. 3. TEM mapping of a hybrid composite of NCF/PANI sample (A) Mix, (B) Nickel, (C) Cobalt, (D) Iron, (E) Carbon, (F) Nitrogen, and (G) Oxygen.

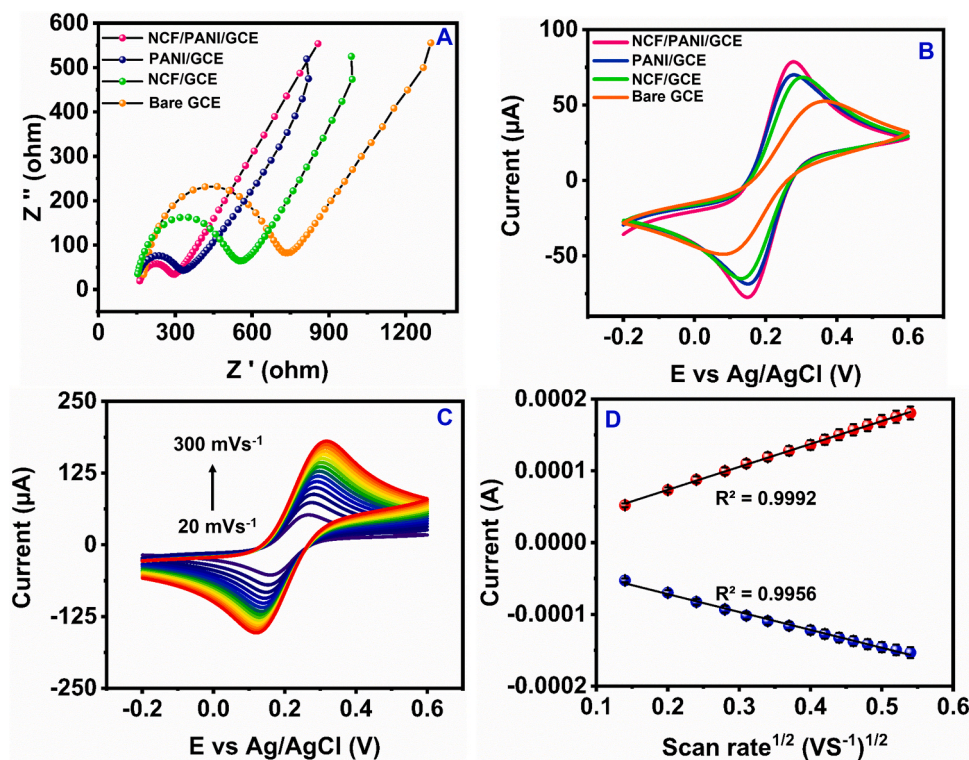


Fig. 4. (A) Nyquist plots of bare GCE, NCF/GCE, PANI/GCE, NCF/PANI/GCE, (B) CV response of different modified electrodes, (B) CV curves of NCF/PANI/GCE at increasing scan rate 20 mVs^{-1} – 300 mVs^{-1} , (C) respective linear plot of current response against square root of scan rate. The EIS experiment was carried out in a 0.1 M KCl solution containing $5 \text{ mM } [\text{Fe}(\text{CN})_6]^{3-/4-}$ (FC system).

from 9 – 11 which was due to the slow charge transfer kinetics and significant potential changes towards the more negative values. As in highly acidic or basic pH the protons mobility was greatly affected. Thus, pH 7 was chosen as an ideal pH for the further electrochemical sensing performance. Fig. 5B depicts the corresponding linear plot of different pH's current response and potential.

Fig. 5C displays the CV response of different modified and unmodified electrodes in 0.1 M PBS holds $100 \mu\text{M}$ of 3-NT. Among all the modified and unmodified electrodes, the NCF/PANI/GCE exhibits a high current response. Fig. 5D depicts the corresponding bar diagram of current response from which also it was clear that higher current is observed for NCF/PANI/GCE. The result was owing to the fact that the

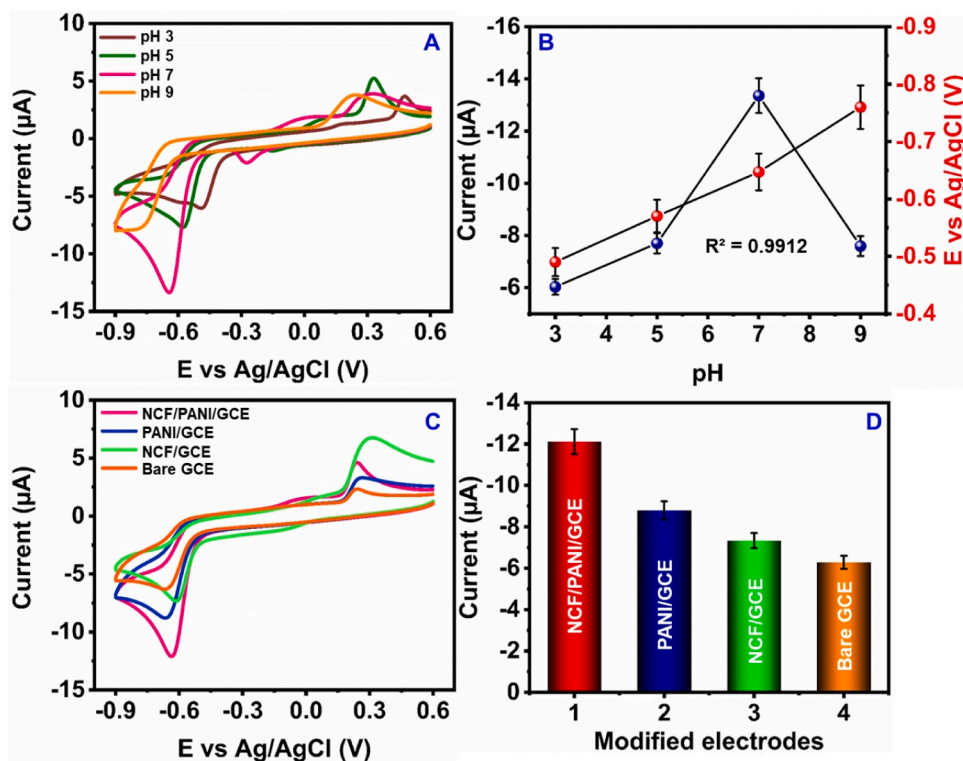


Fig. 5. A) CV curves of NCF/PANI/GCE in 0.1 M PBS at different pH contains 100 μM of 3-NT in 0.1 M PBS, B) corresponding plot of different pH against current and potential, C) CV response of different modified electrodes in presence of 100 μM of 3-NT at a scan rate of 50 mVs^{-1} in 0.1 M PBS, and D) corresponding bar diagram of current response of different modified electrodes.

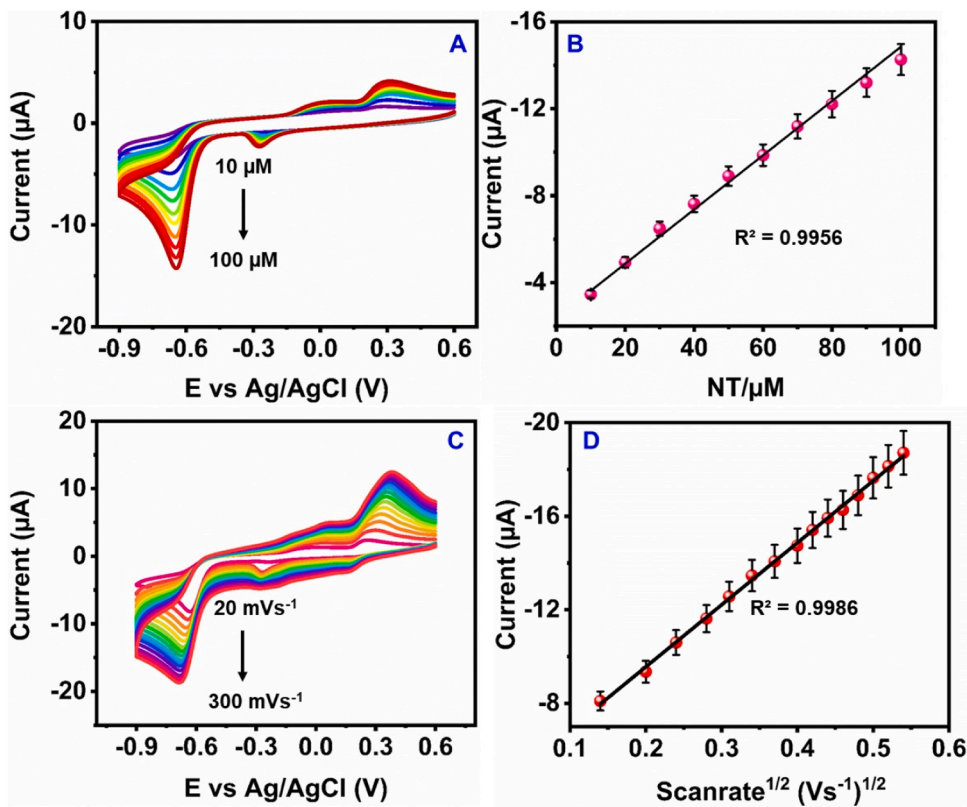


Fig. 6. A) CV response of NCF/PANI composite in increasing concentration of 3-NT from 10 – 100 μM in 0.1 M PBS at a scan rate of 50 mVs^{-1} , B) respective linear plot of current response, C) CV response of NCF/PANI composite at scan rate of 20 mVs^{-1} – 300 mVs^{-1} in 0.1 M PBS, D) corresponding linear plot of current response against square root of scan rate.

interaction between the NCF and PANI results in enhanced electron transfer ability between electrode and electrolyte interface. Further, best performing electrode NCF/PANI/GCE was subjected for 5 consecutive runs ($n=5$) in the presence of $100\ \mu\text{M}$ 3-NT in $0.1\ \text{M}$ PBS to investigate the electrodes effect towards analyte adsorption. The result are presented in Figure S1. It is evident that even after 5 CV runs, the electrodes displayed a stable current response for the detection of 3-NT indicating the superior electrocatalytic property of NCF/PANI/GCE. No significant decline in peak current response for 3-NT was witnessed showing the negligible adsorption effect in the surface of electrode.

The CV response of NCF/PANI/GCE for different increasing concentration from $10 - 100\ \mu\text{M}$ in $0.1\ \text{M}$ PBS at a scan rate of $50\ \text{mVs}^{-1}$ containing $100\ \mu\text{M}$ of 3-NT (Fig. 6A). The reduction peak current gets linearly increased on increasing the concentration of 3-NT. Corresponding linear plot was depicted in Fig. 6B. From the calibration result good linearity $R^2 = 0.9956$ value was obtained which proves the catalytic activity of the NCF/PANI/GCE. Thus, NCF/PANI/GCE is an excellent electrochemical catalyst for the sensing of 3-NT. The electrochemical process undertaken by NCF/PANI/GCE was investigated by CV method in $0.1\ \text{M}$ PBS contains $100\ \mu\text{M}$ of 3-NT. As shown in the Fig. 6C the peak current gets raises as the scan rate gets gradually increased implying a linear relationship between scan rate and current. Fig. 6D depicts the corresponding linear plot of current against square root of scan rate. Thus, the reduction of 3-NT on NCF/PANI/GCE to be a diffusion-controlled process[13]. The possible electrochemical detection mechanism of 3-NT was displayed in Scheme 2.

3.4. DPV analysis of 3-NT at NCF/PANI

The qualitative analysis of the prepared sensor was investigated in Differential Pulse Voltammetry (DPV) analysis. The analysis was accomplished in $0.1\ \text{M}$ PBS solution by increasing concentration of 3-NT from $0.01 - 1268\ \mu\text{M}$. Fig. 7A displays the DPV curve response of increasing concentration of 3-NT depicting that the current increases

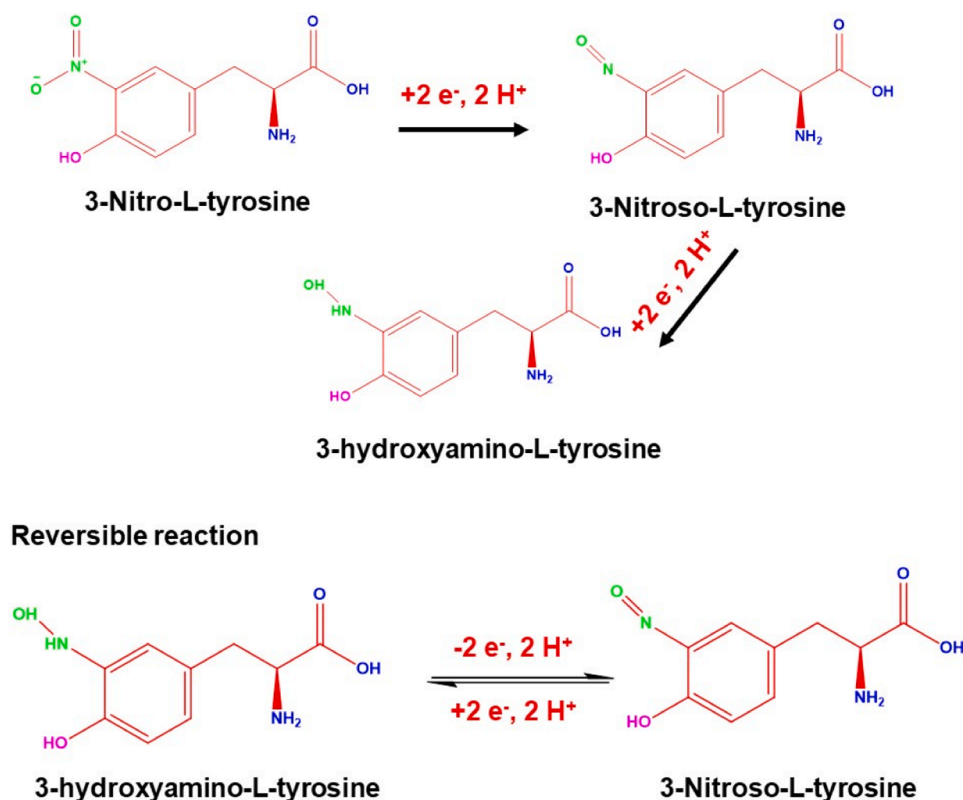
linearly as the concentration of 3-NT gets increased. Fig. 7B displays the corresponding plot of the current response against the concentration of 3-NT. The corresponding linear plot with a coefficient correlation of $R^2 = 0.9634$. The LOD was calculated as $0.02\ \mu\text{M}$ using the formula $\text{LOD} = 3\ s/q$ [26]. The proposed sensor was compared with the other previously reported results in Table S1. The anti-interference ability of this NCF/PANI/GCE had been evaluated in the presence of a few interfering compounds such as ascorbic acid (AA), hydrogen peroxide (H_2O_2), sodium nitrite (NaNO_2), enrofloxacin, 2-nitrofluorene, and uric acid (UA). Fig. 7C displays this selectivity of the proposed sensor. Negligible change in the current response was observed which proves the anti-interfering ability.

3.5. Reproducibility and stability of the NCF/PANI

The reproducibility and stability of NCF/PANI/GCE in $0.1\ \text{M}$ PBS containing $100\ \mu\text{M}$ of 3-NT. The storage stability of the NCF/PANI/GCE was investigated by DPV in Fig. 8A, the electrode was stored in a refrigerator at $4\ ^\circ\text{C}$ for 10 days and investigated on the first, fifth, and tenth days. A negligible change in the current response was obtained and final current maintains about 96.5% of the initial current, which verifies the improved stability of the nanocomposite. In addition, the modified electrodes were tested by three different GCE contains $100\ \mu\text{M}$ of 3-NT depicted in Fig. 8B. Finally, the RSD value of the proposed sensor was calculated to be 2.96%, which was acceptable. The results show that NCF/PANI/GCE exhibits good stability and reproducibility.

3.6. Real sample analysis

Investigating the practicability of the proposed sensor NCF/PANI/GCE via DPV in $0.1\ \text{M}$ PBS using human urine and saliva. The sample was first centrifuged at $6000\ \text{rpm}$ for 20 min. Then the sample was diluted with PBS solution and a known concentration of 3-NT in human urine and saliva. A good recovery results were obtained which was



Scheme2. Electro redox mechanism of 3-Nitro-L-tyrosine.

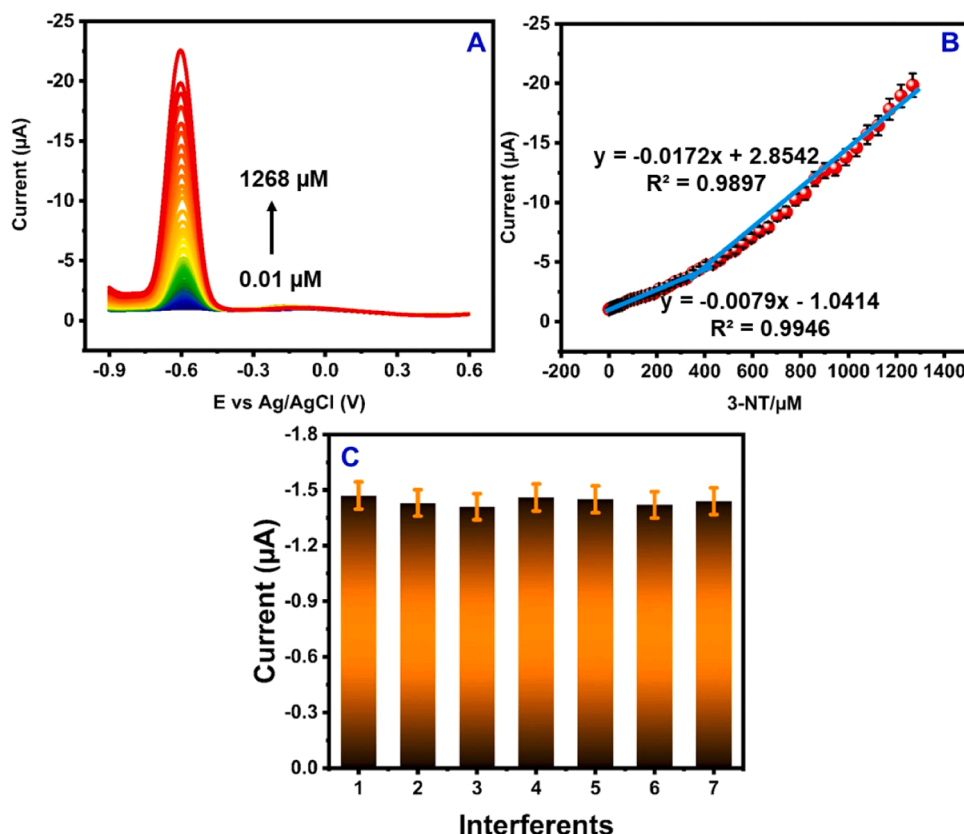


Fig. 7. A) DPV response of 3-NT at NCF/PANI in 0.1 M PBS with increasing concentration of 3-NT from 0.01 – 1268 μM, B) corresponding calibration plot of DPV response, C) interference analysis of drugs and ions over 3-NT on NCF/PANI/GCE in 0.1 M PBS.

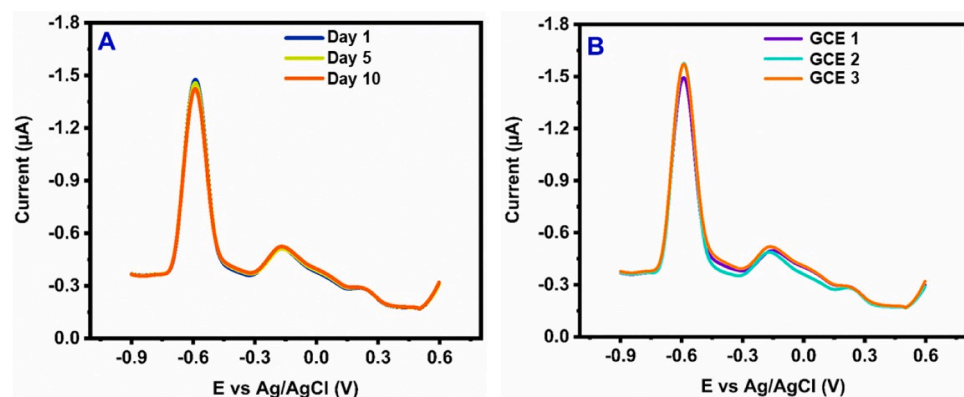


Fig. 8. A) DPV response of NCF/PANI/GCE stability test for about 10 days in 0.1 M PBS contains 100 μM of 3-NT, B) the DPV reduction current response for three independent NCF/PANI/GCE modified GCE's in 0.1 M PBS containing 100 μM of 3-NT.

displayed in Table S2. For all the real samples, the newly fabricated electrode demonstrates good retrievals suggesting it as a reliable and proficient platform for the determination of 3-NT in human urine and saliva samples.

4. Conclusion

Here, the transition metal oxides with a conducting polymer (NCF/PANI) were successfully synthesized and characterized to confirm the formation of composite. Compared to the other sensors, our NCF/PANI exhibiting better conductivity owing to the incorporation of metal ferrite with a conducting polymer. The voltammetry studies evidently proved that the NCF/PANI modified GCE has a good electrochemical

stability, large surface area, better conductivity, that are beneficial for electrochemical sensing performance. Furthermore, wide linear range (0.01 – 1268 μM), the LOD, selectivity, reproducibility, and stability has also been analysed. The real sample analysis using human urine and saliva exhibits good recoveries.

CRediT authorship contribution statement

Subalakshmi Pragalathan: Conceptualization, Software, Writing – original draft, Writing – review & editing. **Sundaresan Ruspika:** Conceptualization, Data curation, Methodology, Software, Writing – original draft, Writing – review & editing. **Shen-Ming Chen:** Conceptualization, Project administration, Supervision, Validation,

Visualization. **Aravinth Dhanasekaran:** Methodology, Writing – original draft. **V Velmurugan:** Investigation, Supervision, Visualization, Writing – review & editing. **Balaji Ramachandran:** Conceptualization, Writing – original draft, Writing – review & editing. **Jagan Mohan:** Software, Writing – original draft. **Narendhar Chandrasekar:** Writing – original draft, Writing – review & editing.

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.

Data availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.jece.2024.112455](https://doi.org/10.1016/j.jece.2024.112455).

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