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Enzyme-free selective determination of hydrogen peroxide and glucose using functionalized copper nanoparticles modified graphite electrode in room temperature ionic liquid medium

Rajendran Suresh Babu, Pandurangan Prabhu and Sangilimuthu Sriman Narayanan *

Department of Analytical Chemistry, School of Chemical Sciences,

University of Madras, Guindy Campus, Chennai- 600 025, Tamil Nadu, India

Abstract

A novel enzyme-free modified electrode constructed by electropolmerization of L-cysteine on a graphite electrode followed by electrodeposition of copper nanoparticles using ionic liquid as a green electrolyte. The modified electrode exhibits an excellent electrocatalytic activity towards the oxidation of hydrogen peroxide (H_2O_2) and glucose at a reduced overpotential of 0 V and +0.35 V respectively. The immobilization of copper nanoparticle encapsulated by polycysteine formed on the electrode was confirmed by Fourier transform infrared spectroscopy (FT-IR), Raman spectra, X-ray photoelectron spectroscopy (XPS), transmission electron microscope (TEM) and atomic force microscope (AFM) studies. The determination of H_2O_2 and glucose with the modified electrode shows the advantages of ease of preparation, high sensitivity, good selectivity and stability. To demonstrate the practical application of the modified electrode for selective detection of H_2O_2 and glucose has been evaluated by analyzing the real samples of stain remover solutions and human urine samples to determine H_2O_2 and glucose respectively.

Keywords: Copper nanoparticles, glucose, green electrolyte, hydrogen peroxide, room temperature ionic liquid

*Corresponding author:

E-mail: sriman55@yahoo.com (S. Sriman Narayanan)

Phone: +91-44-22202717, Fax: +91-44-22352494

1. Introduction

The pursuit of enzyme-free H_2O_2 and glucose sensing is an enthusiastic and competitive area of research and technology. The development and fabrication of cost effective, simple, accurate, portable and rapid sensors for H_2O_2 and glucose are biologically important. Hydrogen peroxide plays a significant role in the chemical and pharmaceutical industries as an oxidizing, bleaching and sterilizing agent. It also acts as an oxidant in heterogeneous processes that create sulphuric acid and nitric acid in rain and the atmosphere. At high concentrations, H_2O_2 causes irritation to the eyes and skin, and thereby affects human health [1]. Further, the detection of H_2O_2 is an important task in many biological, medical and clinical studies [2, 3]. Since, H_2O_2 is consumed during the reactions of many oxidases, which in turn provides the basis for the construction of several peroxide biosensors. Among the various methods reported for the determination of H_2O_2, electroanalytical methods are generally preferred [4-9] due to their simplicity, low detection limits, rapid response and relatively low costs [9]. The detection of H_2O_2 at most kinds of bare electrodes requires a high overpotential, which in turn causes interferences from many other electroactive and co-existing species. Biosensors that use peroxidase-modified electrodes have also been reported to provide an attractive method for H_2O_2 determination in recent years [10]. Although biosensors provide sensitive determinations of H_2O_2, drawbacks associated with their fabrication and long-term stability limit their application.

Glucose is one among the biologically significant substrates which plays an essential role in biomedical, industrial and clinical applications. The importance of glucose in human metabolism is well known that the glucose levels determine the extent of complications such as diabetes, heart diseases, kidney, vision and nervous damages. Thus, there is always an increasing demand for the development of new methodologies for simple, rapid and reliable quantification of glucose. Despite the immense advances in glucose sensing, there are still many challenges related to the achievement of simple and sensitive sensors with improved and highly desired analytical characteristics for selective detections. Conventional techniques such as spectrophotometry were limited in identifying and quantifying glucose due to the lack of chromophoric and fluorophoric ligands of glucose [11]. Amperometric glucose sensors have two major types, i.e., enzymatic and nonenzymatic sensors, which receives keen interests and are developed rapidly due to the advantages of high sensitivity and quick response [12]. Since Clark reported the first enzyme electrode in 1962 [13] and Updike constructed the first enzymatic biosensor to amperometrically detect glucose in 1967 [14], numbers of studies has been focused on developing electrochemical enzymatic glucose sensors over four decades [15]. As a result of the drawbacks of enzymatic sensor in application to lab-on a-chip and in vitro glucose assay, such as the difficulties in miniaturization, instability of the enzyme, poor reproducibility and interference of oxygen [16], the electrochemical determination of glucose without using enzyme, i.e., enzyme-free glucose sensor, has been one of the ideas of many researchers [16, 17].

Several enzyme-free glucose and H_2O_2 sensors have been reported the electrode modified such Pt, Pd, Au, Ag, Bi, Hg, Ni, Cu and some alloys such as Pt-Pd, Pt-Au, Au-Ru, etc

have been investigated [18-20]. They have some drawbacks such as low sensitivity, poor selectivity, and poisoning by adsorbed intermediates and chloride ions [21]. Out of all these electrode materials Cu and CuO based electrode materials are shown to facilitate the inherent tendency for the mediated electro oxidation of enzyme-free glucose and H_2O_2 determination. Moreover, CuO are very low cost, more stable in air, good sensitivity, excellent selectivity and show no observable self poisoning effect in solutions [22], owing CuO based electrodes is very difficult to form complexation with Cl^- ion since the electronegativity of O is higher than that of Cl^- ion. Thus, the significance of the electrodes modified with the functionalized CuNP based materials for enzyme-free biosensors.

Numerous enzyme and enzyme free sensors based on electrochemical techniques for the determination of H_2O_2 and glucose has been explored in our laboratory [23-25]. In this report, we propose to fabricate the electropolymerization of L-cysteine and electrodeposition of Cu nanoparticles on paraffin wax impregnated graphite electrode (PIGE) using ionic liquid as green electrolyte. The surface morphology of polycysteine functionalized copper nanoparticle (PCFCuNP) modified electrode has been examined by using FTIR-ATR, Raman spectrum, XPS, TEM and AFM. The modified electrode successfully employed for the detection of H_2O_2 and glucose in the alkaline medium using cyclic voltammetry (CV) and linear sweep voltammetric (LSV) techniques. The proposed modified electrode was employed for the amperometric detection of H_2O_2 and glucose successfully. To demonstrate the biological and industrial significance of the modified electrode, we examined the determination of glucose and H_2O_2 in real samples using stain remover solutions and urine samples respectively.

2. Experimental

2.1. Reagents and equipments

L-Cysteine (L-Cys), epinephrine (EP), D-glucose, hydrogen peroxide, ascorbic acid (AA), dopamine (DA), uric acid (UA) was purchased from HiMedia laboratories Pvt. Ltd. 1-ethyl-3-methylimidazoilium ethyl sulphate (purity 98.5%) was obtained from Alfa Aesar. Spectroscopic grade graphite rod (3 mm diameter) was used as received from Aldrich. Two different commercial stain remover solutions were purchased from local market. All other chemicals were of analytical grade and double distilled water was used in experiments.

FTIR spectra were recorded using a Perkin-Elmer RX 1 spectrometer. Raman spectra were recorded with Raman 11i system (Nanophoton Corp., Japan). XPS measurement for surface analysis was performed on a monochromatic 300 W Al Kα X-ray radiation as the X-ray source for excitation (Model XM 1000, Omicron Nanotechnology, Germany). For the study of morphology and size of the PCFCuNP modified electrode were obtained on AFM (XE 70 parks system) and TEM images were obtained using Hitachi, H7650 Microscope. Electrochemical experiments were performed with a CHI 660 B electrochemical analyzer (CH instruments, USA). All experiments were done in a conventional three-electrode cell under room temperature. A graphite electrode modified with PCFCuNP was used as working electrode. A platinum electrode was used as the counter electrode and a saturated calomel electrode (SCE) as the reference electrode. For AFM characterization purpose the PC and CuNPs were electrodeposited on ITO modified electrode instead of graphite electrode were fabricated. All electrochemical experiments were performed in 0.1M NaOH solutions.

2.2. Fabrication of the PCFCuNP modified electrode

The paraffin impregnated graphite electrode (PIGE) was prepared as reported [26] by heating the spectroscopic grade graphite rods (0.3 cm circular diameter, 4.0 cm length) in molten wax with the application of suction for half an hour, until air bubbles ceased to evolve from the rods. After re-establishing the atmospheric pressure, the rods were removed before the paraffin solidified. The lower end of the electrode was polished over a finest quality emery paper and the final mirror polish was obtained with velvette cloth for modification. The mirror polish helps to minimize the residual current. The polished surface was then washed with methanol. The functionalized nickel nanoparticle has been prepared as described earlier [27]. The electrochemical deposition of PC was carried out from a room temperature ionic liquid as a green electrolyte containing 10 mM L-Cys monomer and 0.1 M 1-ethyl-3-methylimidazoilium ethyl sulphate by cycling the potential between -0.6 to 2.0 V at 0.05 V/s. A thin film made with 15 deposition cycles was used in all experiments. When removed from the solution, the PC modified electrode was rinsed with distilled water to remove unbound materials from the electrode surface and then dried by atmospheric condition.

CuNP were deposited potentiostatically on the PC modified electrode in a solution of 0.1 M 1-ethyl-3-methylimidazoilium ethyl sulphate and 1 mM $CuSO_4$ by using amperometric technique at preselected potentials. A constant potential of -1.2 V for 100 s was applied (with respect to SCE reference electrode) for a CuNP deposition. The PCFCuNP modified electrode was rinsed thoroughly with water and dried in room temperature. HRTEM and AFM were used to examine the surface morphology of the modified electrode and to characterize the shape, size, and density of the CuNP.

3. Results and Discussion

3.1 FTIR, Raman and XPS of the PCFCuNP modified electrode

The FTIR study of the PCFCuNP modified electrode was investigated in the reflection mode. The FTIR spectra of PCFCuNP modified electrode (see supporting information Fig.S1) are shown. In the spectra, with the most important band at 2553 cm^{-1} corresponding to the thiol (-SH) group in the L-cysteine [27]. In the case of PCFCuNP modified electrode the peak at 2553 cm^{-1} has disappeared (Fig.S1). The disappearance of peak is attributed to the cleavage of S-H bond and the formation of S-Cu bond which is in confirmation with the XPS study [28]. The peak at 475 and 432 cm^{-1} confirms the formation of Cu-S bond [29]. According to FTIR spectroscopic studies [29] PC can bind onto the MNP through the strong sulfur–metal interaction. Based on the above results it seems that only the thiol group is involved in the bonding with the Cu surface.

The Raman spectrum of L-cysteine and PC modified electrode show absorption at 2565 cm^{-1} which indicates the presence of free –SH group in L-Cys and PC modified electrode [27] (curves not shown). The absence of a peak at this position for the PCFCuNP modified electrode confirms the absence of free –SH group in the modified electrode. The absorption at 1579 cm^{-1} corresponds to NH_3^+ symmetrical deformation and is present in PC modified electrode as well as PCFCuNP modified electrode indicating the presence of PC in both electrodes. The peak at 471 cm^{-1} confirms the formation of Cu-S bond (see supporting information Fig.S2). These observations are in consistent with earlier report [30].

To prove that PC and CuNPs have been immobilized onto the surface of electrode, XPS experiments were performed (see Fig 1a). Two XPS bands of Cu appear at 932.3 and 952.1 eV,

corresponding to the Cu ($2p^{3/2}$) and Cu ($2p^{1/2}$) signals respectively (Fig.1b), which demonstrates the immobilization of CuNP on the PC modified electrode surface. Another two peaks are observed due to existence of O impurity in the sample, which originates from the surface contamination of the CuNP. The S (2p3/2) signal appeared at 162.5 eV indicates the formation of S–Cu bond on the modified electrode (Fig.1c) [31,32]. The above results demonstrated that CuNP were chemically bound to the surface of the PC modified electrode and confirms the deposition of CuNP on the electrode surface. The above results were consistent with the previous reports [33,34].

3.2 AFM and TEM studies of the PCFCuNP modified electrode

The size and morphology of the modified electrode were investigated by AFM and HRTEM. The AFM topographic images of bare electrode, which showed the bare substrate consisting of smooth surface morphology (see supporting information Fig. S3), whereas the PCFCuNP modified electrode shows rough surface morphology was obtained with homogenous deposition of the CuNP nanoparticles of the modified electrode surface (Fig.2). The roughness was increased from bare electrode to modified electrode surface after electrodeposition of CuNP, which clearly showed the CuNP uniformly electrodeposited on the electrode surface. The benefit of using 1-ethyl-3-methylimidazoilium ethyl sulphate as an electrolyte is due to the more rapid nucleation rate than in conventional solvents due to the higher conductivity and viscosity. The size and shape of the nanoparticles is also characterized and confirmed by HRTEM. The image represents the uniform deposition of CuNPs was mainly on PC surface. It also indicates the formation of the CuNP is spherical-like structure. The size of each nanoparticles was found to be ~20 - 30 nm as shown in Fig.3.

3.3. Electrocatalytic oxidation of H_2O_2 at the PCFCuNP modified electrode

The electrocatalytic activity of the PCFCuNP modified electrode for H_2O_2 oxidation was explored in this study. The CV of the PCFCuNP modified electrode in 0.1 M NaOH containing 2 mM H_2O_2 compared with that of a bare electrode CV as shown in Fig. 4A (curve a and b). The electro-oxidation of H_2O_2 at the bare electrode requires a large overpotential, and a poor anodic current was observed at +0.50 V as shown in Fig.4A (curve b). The catalytic oxidation of H_2O_2 at the modified electrode can be clearly seen in Fig. 4A (curve d) where the peak current was markedly enhanced and the peak potential was shifted negatively at 0 V. The oxidation current of CuNP was greatly increased due to the electrocatalytic oxidation of H_2O_2 at 0 V. The concentration dependence of the catalytic current for successive additions of H_2O_2 at the modified electrode resulted in a good linear relationship between the catalytic current and H_2O_2 concentration over the range of 8.3×10^{-6} M to 1.5×10^{-3} M with a correlation coefficient of 0.9962. The detection limit was found to be 2.7×10^{-6} M (S/N=3). Further the increasing peak currents of H_2O_2 oxidation confirm that the CuNPs have the higher catalytic ability together with polycysteine in the electrode surface. The results indicated that the modified electrode can catalyze the mediated electro oxidation of H_2O_2 to H_2O and O_2 due to the existence of Cu(II) ions to the following mechanism (Scheme 1). The higher oxidation state of Cu(III) (e.g., CuO(OH)) is believed to participate the electrooxidation process of in alkaline solutions.

To obtain optimum conditions for amperometric determination of H_2O_2 in flow systems, the hydrodynamic behaviour of H_2O_2 was investigated at the modified electrode over the potential range of -0.2 to 0.2 V. Fig. 4B shows the LSVs of the modified electrode in presence of various concentrations of H_2O_2. This behaviour illustrates that the oxidation of H_2O_2

is greatly enhanced at the modified electrode under dynamic conditions also due to electrocatalysis. Hence a potential of 0 V was selected as the working potential for amperometric determination of H_2O_2 using modified electrode under hydrodynamic conditions.

3.4 Electrocatalytic oxidation of glucose at the PFCuNP modified electrode

The electrocatalytic activity of the modified electrode towards the oxidation of glucose in an alkaline solution was also demonstrated. Fig. 5A displays the CVs of 2.0 mM glucose in 0.10 M NaOH at the modified electrode and bare electrode respectively. A minor signal corresponding to low oxidation with a peak potential of about +0.35 V was observed. Upon addition of 2.0 mM glucose, a single forward oxidative wave, corresponding to the irreversible glucose oxidation, was observed for modified electrode. The oxidation process starts at approximately +0.25 V and +0.35 V vs. SCE was observed at modified electrode, but the peak current of modified electrode was about 2.2 times higher than that of the bare electrode. The negative shift of the peak potential and the increase in peak current for oxidation of glucose may be due to a kinetic effect by an increase in the electroactive surface area and the electron transfer ability of the modified electrode. A good linear response was obtained over the range from 6.6×10^{-6} to 1.3×10^{-3} M glucose with a correlation coefficient of 0.9983. A detection limit of 2.2×10^{-6} (S/N = 3) was observed for the determination of glucose at the modified electrode. The results indicated that the modified electrode can catalyze the electro oxidation of glucose to gluconolactone due to the existence of Cu(II) ions to the following mechanism (Scheme 2).

LSVs were conducted with PCFCuNP modified electrode to investigate its electrocatalytic response under dynamic conditions. In order to obtain maximum catalytic sensitivity, the applied potential is optimized in the potential range of 0 to +0.6 V. Fig.5B shows

the LSVs of various concentration of glucose. As it can be seen, the peak current reaches maximum at +0.35 V for glucose oxidation on modified electrode. The results are similar to that of CV curves described in Fig.5A In order to obtain constant, high sensitivity with a reduced over-potential for glucose oxidation, a potential of +0.35 V is applied during the amperometric determination of glucose.

3.5. Amperometric determination of H_2O_2 and glucose

Figs. 6 and 7 depict the amperometric current–time response of the PCFCuNP modified electrode for the determination of H_2O_2 and glucose. The response current was measured at a fixed potential in a stirring 0.1M NaOH solution. Fig. 6A shows the amperometric response obtained at an applied potential of 0 V for successive addition of 380 mM H_2O_2 to the stirring 0.1M NaOH solution. The modified electrode shows a stepwise increase in current for every successive addition of H_2O_2. The modified electrode exhibited rapid response to the changes in the concentration of H_2O_2 (less than 4 s). The modified electrode showed a linear response to H_2O_2 in the concentration range from 1.6×10^{-5} to 5.0×10^{-3} M with a correlation coefficient of 0.9987. The corresponding calibration graph for the determination of H_2O_2 was shown in Fig.6B. Similarly, the amperometric response for the successive addition of 200 mM glucose at a fixed potential of +0.35 V was shown in Fig. 7A. The modified electrode also shows an increase in current for every successive addition of glucose. The linear range was between 7.3×10^{-6} and 2.2×10^{-3} M and the correlation coefficient was 0.9979. The corresponding calibration graph for the determination of glucose was shown in Fig.7B. The detection limit (S/N = 3) for H_2O_2 was 5.3×10^{-6} and 2.4×10^{-6} M for glucose. The relative standard deviation (RSD) for eight successive determinations of 1.0×10^{-3} M H_2O_2 and 5.0×10^{-4} M glucose were 2.8 and 3.6%,

respectively, indicating the good reproducibility of the proposed modified electrode. The nobility of this modified electrode is reduced the overpotential for the detection of both H_2O_2 and glucose compared to the previously reported so far. The working potential, detection limit and linear concentration range of other related modified electrodes for H_2O_2 and glucose detection have been reported in Table 1.

3.6 Interference study

The selectivity and anti-interference ability of PCFCuNP modified electrode was also evaluated by amperometric technique. Fig.8 shows the amperometric response of the modified electrode to the injection of 82 µM H_2O_2 and 1 mM interfering species including AA, DA, UA, EP, glucose and L-Cys (at the fixed potential of 0 V). It was evident that the influence of interfering species in H_2O_2 response was negligible, because the oxidative potential of H_2O_2 is very less, due to the very high surface area of the CuNP indicating high selectivity of the proposed modified electrode. Obviously glucose electro oxidation occurs only at +0.35 V in the modified electrode. Hence the PCFCuNP modified electrode determined selectively towards H_2O_2 and glucose. The study of interfering species such as chloride ions was also tested at the PCFCuNP modified electrode towards the determination of H_2O_2 and glucose. The influence of the chloride ions was evaluated in the concentration ratio of 1:100. The addition of 1.7×10^{-2} M (1.0 M NaCl) to 1.7×10^{-4} M H_2O_2 or glucose in the background electrolyte solution. The experimental result showed that the peak current did not alter the response of the sensor indicating that the PCFCuNP modified electrode is not poisoned by chloride ion.

3.7 Real Sample analysis

To study the real feasibility of the PCFCuNP modified electrode, the modified electrode was employed to determine H_2O_2 content in commercially available stain remover samples and glucose determination from urine samples obtained from normal human being. The analyses were performed without special treatment for H_2O_2 determination and the human urine samples by diluting 100 times by 0.1 M NaOH. The standard addition method was used for the analysis of the prepared samples. The data given in the Table 2 and 3 shows the satisfactory results for the recovery. These results indicate the suitability of the proposed modified electrode for practical application towards online monitoring of H_2O_2 and glucose analysis.

3.8 Stability and reproducibility

The stability and reproducibility of the PCFCuNP modified electrode was investigated by comparing the response currents of eight similar modified electrodes. The relative standard deviation (RSD) was 3.5% at a H_2O_2 concentration of 0.10 mM and 3.8% at a glucose concentration of 0.10 mM respectively. The modified electrodes were not poisoned by the oxidation product and can be used repeatedly for the determination of glucose and H_2O_2. The sensor was stored in air tight container when not in use and its sensitivity was tested every 5 days. The result demonstrated that the sensitivity was 96% of its initial sensitivity after being stored for 60 days. The good reproducibility and long-term stability of the sensor are desirable for most routine analysis for glucose and H_2O_2 determination. The improved stability of CuNP in electrocatalysis resulted from the protective polymer chain on the copper surface, which prevented the passive layer formation and the possible dissolution of the CuNP and thus efficiently reduced the electrode fouling.

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4. Conclusion

A novel hybrid PCFCuNP modified electrode was fabricated by a simple electrodeposition method using room temperature ionic liquid as green electrolyte. The modified electrode resulted in excellent electrocatalytic activity for the oxidation of H_2O_2 and glucose selectively at the very low potential. The hybrid enzyme free electrochemical sensor showed a wide linear range and good analytical performance with short time response, good repeatability and acceptable stability. The proposed method was successfully applied to the determination of H_2O_2 in stain remover solutions and glucose from human urine samples with good recovery.

Acknowledgments

The authors gratefully acknowledge the funding provided by the University Grants Commission (UGC), New Delhi and the Department of Science and Technology, New Delhi for financial assistance through 'PURSE' program.

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Table captions

Table 1 Comparison of various nanomaterials based sensors for H_2O_2 and glucose detection with previous reports.

Table 2 Determination of H_2O_2 in an antiseptic solution by CV technique using PCFCuNP modified electrode

Table 3 Determination of glucose in human urine samples by CV technique using PCFCuNP modified electrode

Figure captions

Fig. 1. XPS spectra of (a) Survey spectra (b) $2P_{3/2}$ and $2P_{1/2}$ peaks of Cu (c) $2P_{3/2}$ peak of S of the PCFCuNP modified electrode.

Fig. 2 AFM of the PCFCuNP modified electrode.

Fig.3 TEM of the PCFCuNP modified electrode.

Fig. 4(A) CVs in 0.1 M NaOH at a scan rate of 50 mV/s (a) bare electrode in the absence of H_2O_2 (b) bare electrode in the presence of 625 µM H_2O_2 (c) PCFCuNP modified electrode in the absence of H_2O_2 (d) PCFCuNP modified electrode in the presence of 625 µM H_2O_2. (B) LSVs obtained with PCFCuNP modified electrode in various concentrations from (a) 0 µM to (f) 769 µM of H_2O_2. Electrolyte: 0.1M NaOH; stirring rate: 300 rpm.

Fig. 5(A) CVs in 0.1 M NaOH at a scan rate of 50 mV/s (a) bare electrode in the absence of glucose (b) bare electrode in the presence of 625 µM glucose (c) PCFCuNP modified electrode in the absence of glucose (d) PCFCuNP modified electrode in the presence of 625 µM glucose. (B) LSVs obtained with PCFCuNP modified electrode in various concentrations from (a) 0 µM to (f) 769 µM of glucose. Electrolyte: 0.1M NaOH; stirring rate: 300 rpm.

Fig. 6(A) Amperometric responses of the PCFCuNP modified electrode to successive injections of 0.5 mL of 0.01 M H_2O_2 in steps in the supporting electrolyte (0.1 M NaOH) at the applied potential of 0 V. (B) Calibration graph for H_2O_2 determination.

Fig. 7(A) Amperometric responses of the PCFCuNP modified electrode to successive injections of 0.5 mL of 0.01 M glucose in steps in the supporting electrolyte (0.1 M NaOH) at the applied potential of +0.35 V. (B) Calibration graph for glucose determination.

Fig. 8 Amperometric response to the injection of 82 µM H_2O_2, 1 mM AA, 1mM DA, 1 mM UA, 1mM EP, 1 mM glucose and 1 mM L-Cys at the PCFCuNP modified electrode in 0.1 M NaOH. Stirring rate: 300 rpm. Applied potential 0 V.

Fig. S1 FTIR spectrum of PCFCuNP modified electrode.

Fig. S2 Raman spectrum of PCFCuNP modified electrode.

Fig. S3 AFM image of the bare electrode.

Scheme captions

Scheme 1. Electrocatalytic oxidation mechanism of H_2O_2 at the PCFCuNP modified electrode.

Scheme 2. Electrocatalytic oxidation mechanism of glucose at the PCFCuNP modified electrode.

Table 1 Comparison of various nanomaterials based sensors for H_2O_2 and glucose detection with previous reports.

Electrode based on	Applied potential (V)	Linear range (μM)	Detection limit (μM)	Ref.
H_2O_2				
MWCNT/AgNP-Au[a]	-0.20	50-1700	0.5	[35]
CuO nanorod bundles	+0.25	100-800	0.2	[36]
CuNP/PoPD/GCE[b]	-0.30	1-1000	0.1	[37]
AgNP/ITO[c]	-0.40	---	---	[38]
rGO/Tyr[d]	-0.55	100-2100	80	[39]
GO/PB[e]	+0.10	5-1200	0.12	[40]
PCFNiNP[f]	+0.50	3.3-1700	1 .1	[27]
PCFCuNP	0	8.3-1500	2.7	[this work]
Glucose				
CuO/GO/GCE[g]	+0.70	2.79-2030	0.69	[41]
CuO-G- GCE[h]	+0.55	2-400	0.70	[42]
CuO nanorods	+0.60	4-8000	4.0	[43]
Nafion/CuO/GCE	+0.60	50-2550	1.0	[44]
NA/NiONF-rGO/ GCE[i]	+0.60	2-600	0.77	[45]
CuO-MWCNT[j]	+0.40	0.4-1200	0.2	[46]
PCFNiNP	+0.55	1.6-1400	0.5	[27]
PCFCuNP	+0.35	6.6-1300	2.2	[this work]

[a]MWCNT/AgNP-Au - multi-wall carbon nanotube/silver nanoparticle nanohybrids modified Au electrode

[b]Copper nanoparticle/poly(o-phenylenediammine) modified glassy carbon electrode

[c]Silver nanoparticle modified indium tin oxide modified substrate

[d]Reduced Graphene oxide/Tyrosine modified electrode

[e]Graphene oxide/Prussian Blue modified electrode

[f]Polycysteine functionalized nickel nanoparticle modified electrode

[g]CuO based graphene oxide composite modified glassy carbon electrode

[h]CuO nanocubes-graphene nanocomposite modified glassy carbon electrode

[i]Nafion/nickel oxide nanofibers-reduced graphene oxide modified glassy carbon electrode

[j]CuO modified multiwalled carbon nanotube modified electrode

Table 2 Determination of H_2O_2 in an antiseptic solution by CV technique using PCFCuNP modified electrode

Diluted antiseptic solution	Spiked (μM)	Found (μM)[a]	Recovery (%)
Sample 1	----	28.5 ± 0.2	----
	50	78.3 ± 0.04	99.6
	100	127 ± 0.01	98.5
Sample 2	----	29.8 ± 0.1	----
	50	80.1 ± 0.2	100.6
	100	129.4± 0.1	99.6

[a] Average of three measurements

Table 3 Determination of glucose in human urine samples by CV technique using PCFCuNP modified electrode

Human urine	Spiked (μM)	Found (μM)[a]	Recovery (%)
Sample 1	50	51.8 ± 0.3	103.6
	100	104.2 ± 0.2	104.2
Sample 2	50	50.4 ± 0.4	100.8
	100	103.7± 0.2	103.7

[a] Average of three measurements

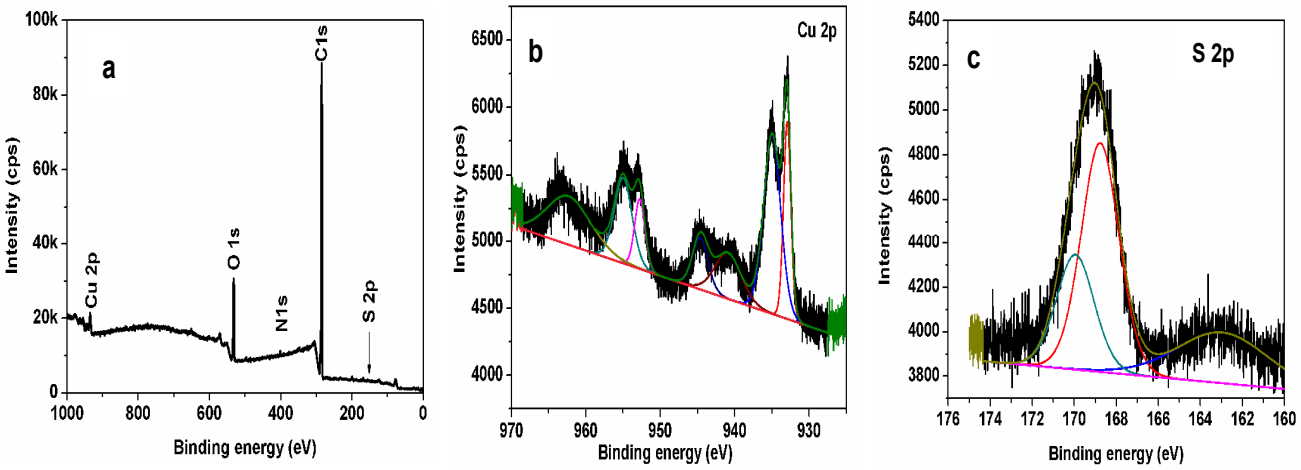


Fig.1

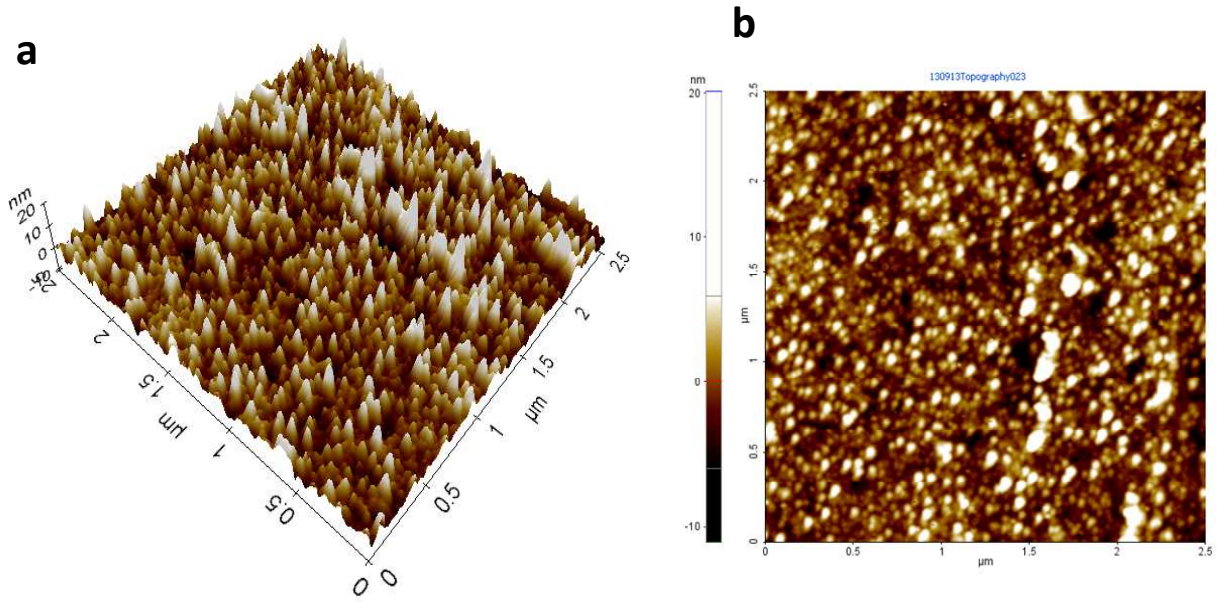


Fig. 2

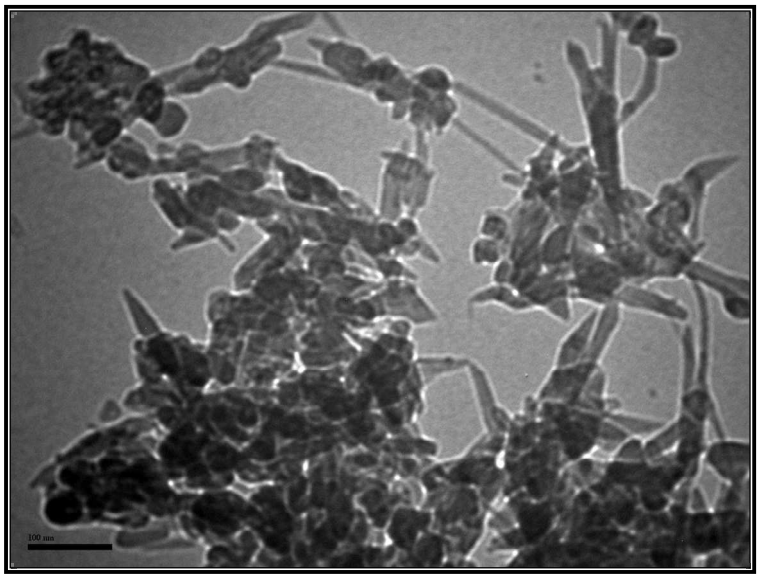


Fig. 3

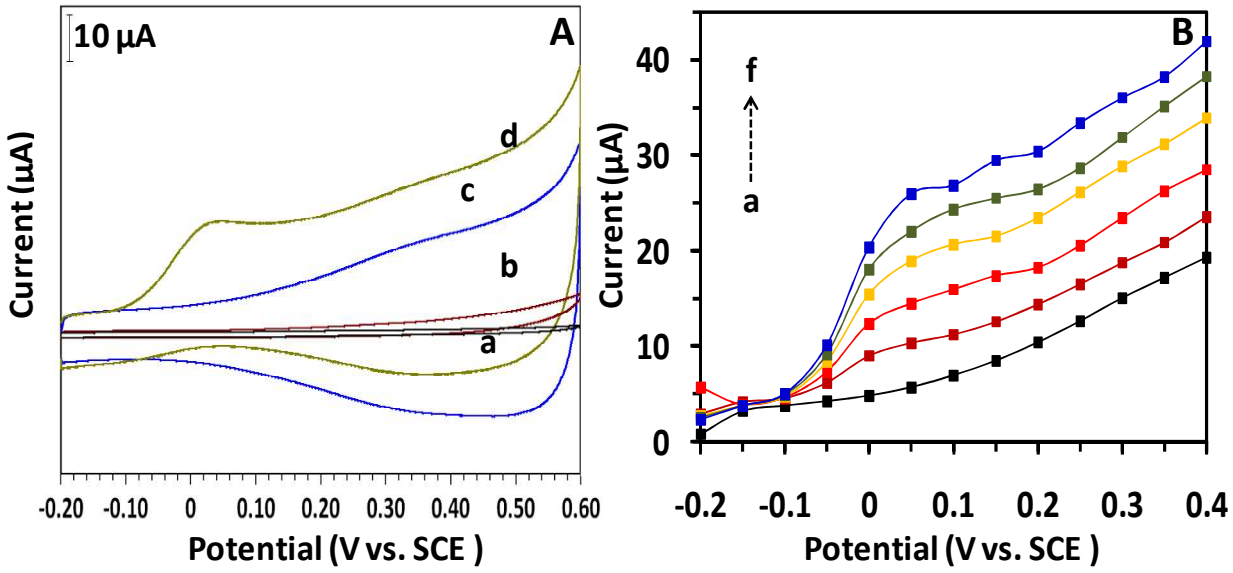


Fig. 4

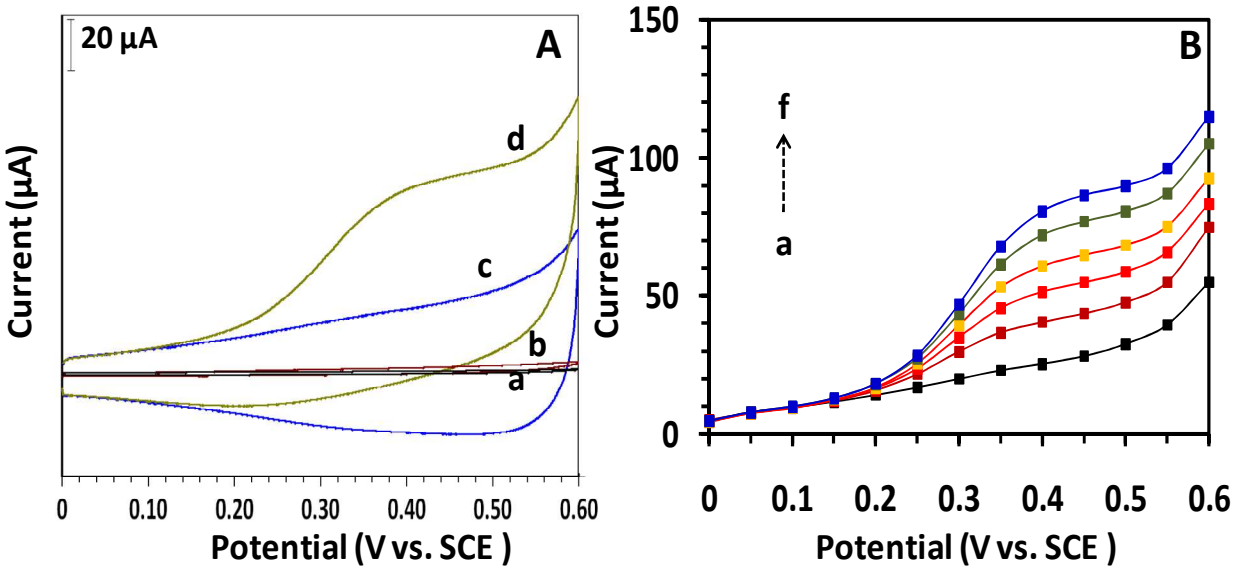
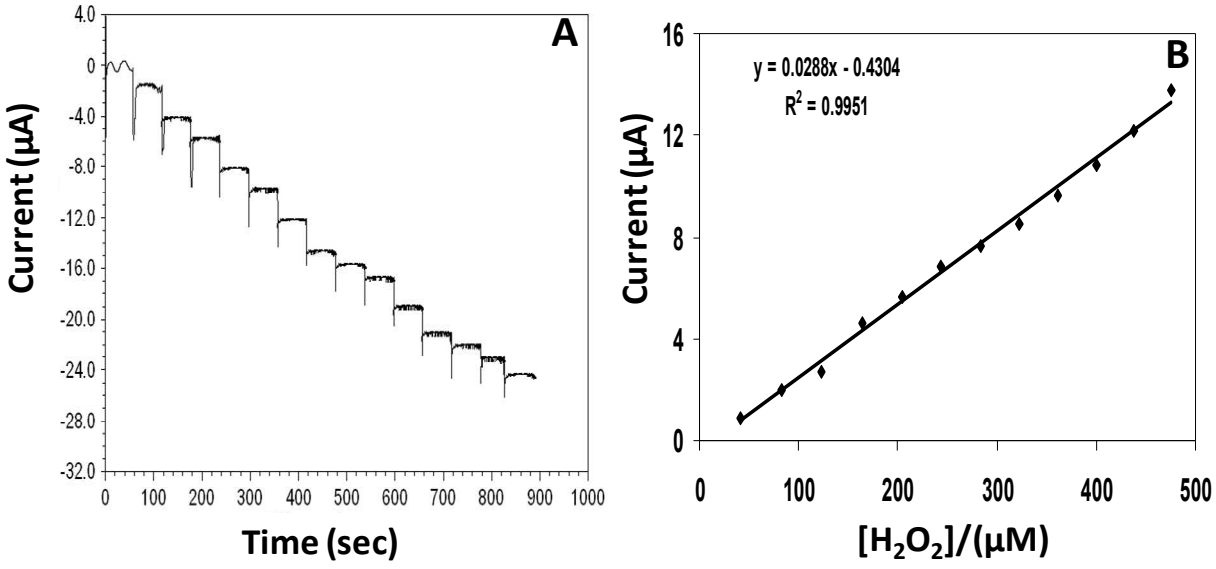


Fig. 5



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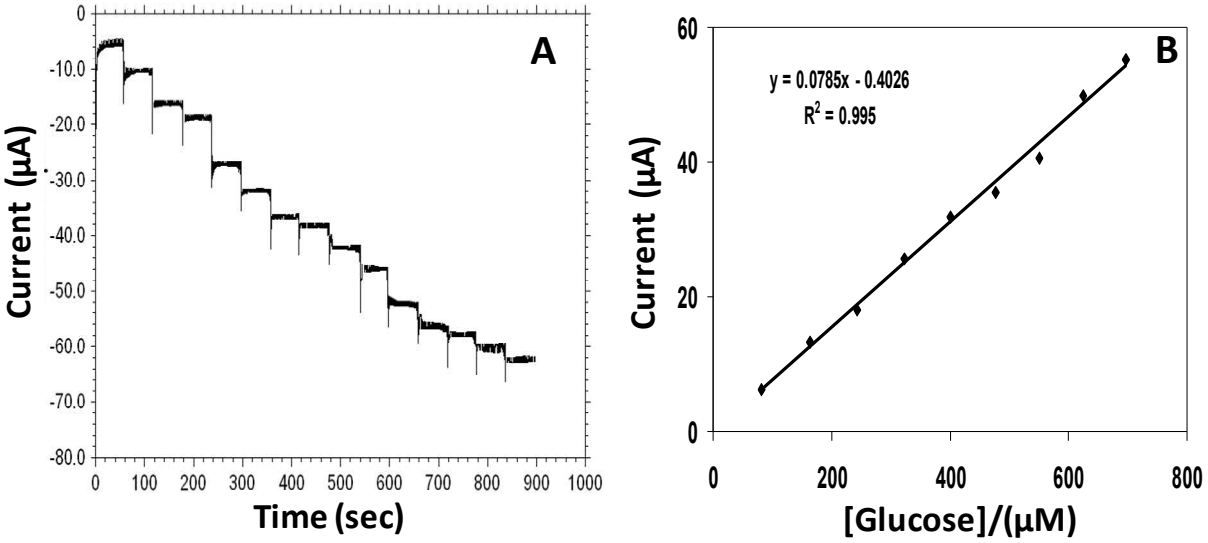

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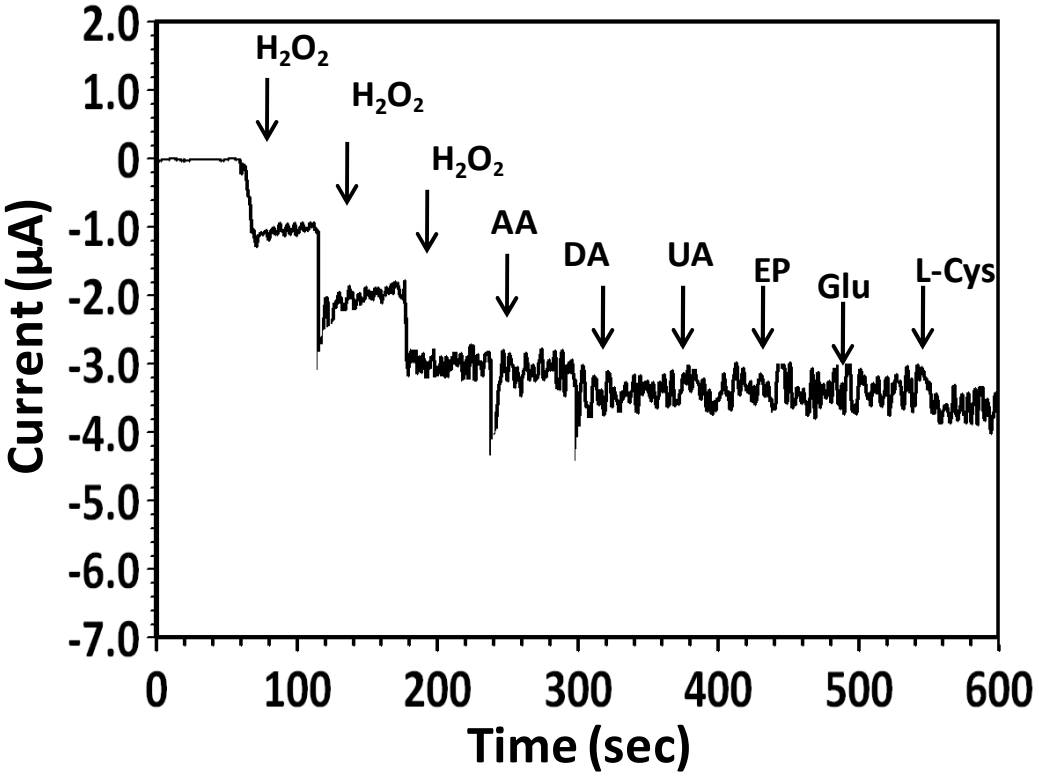
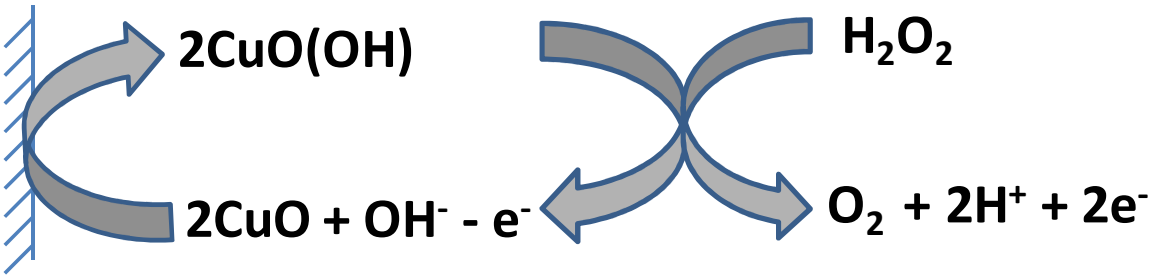
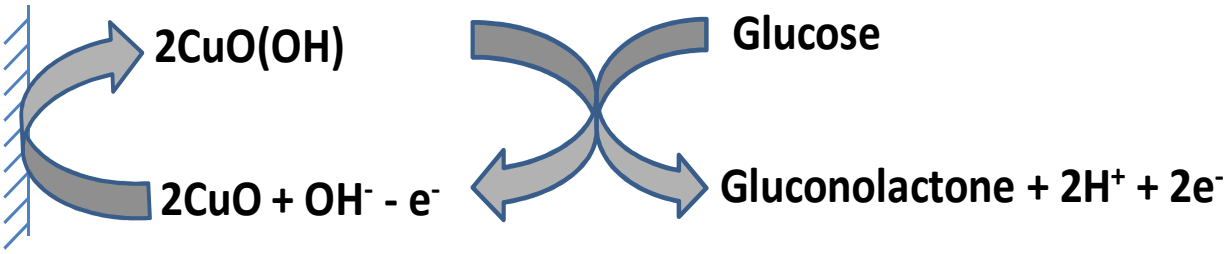


Fig. 8



Scheme 1



Scheme 2

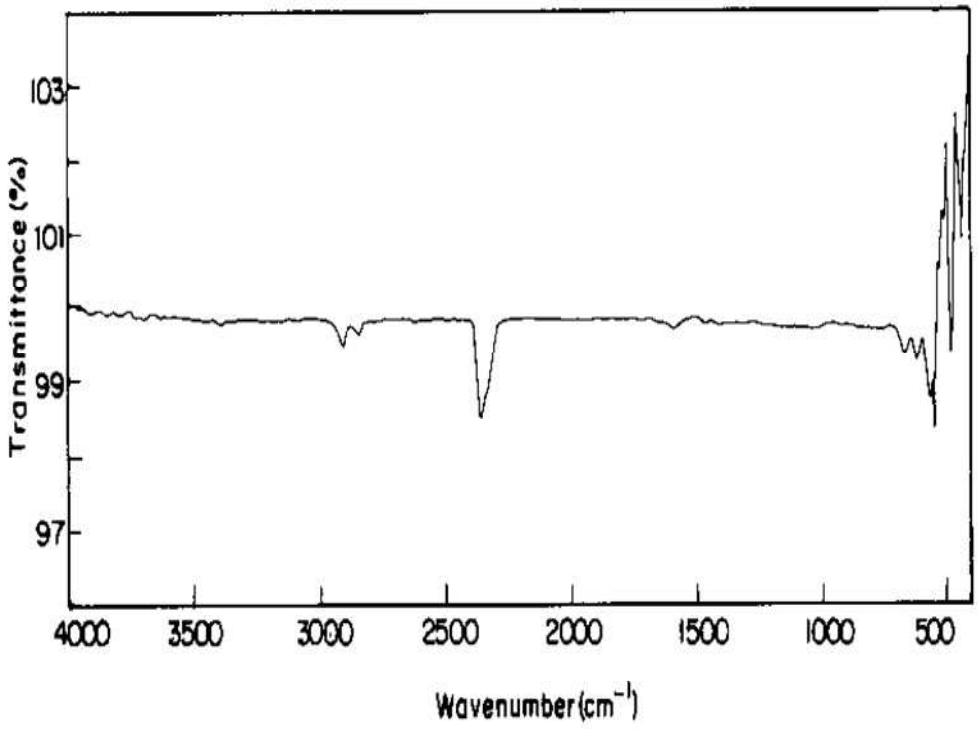


Fig. S1

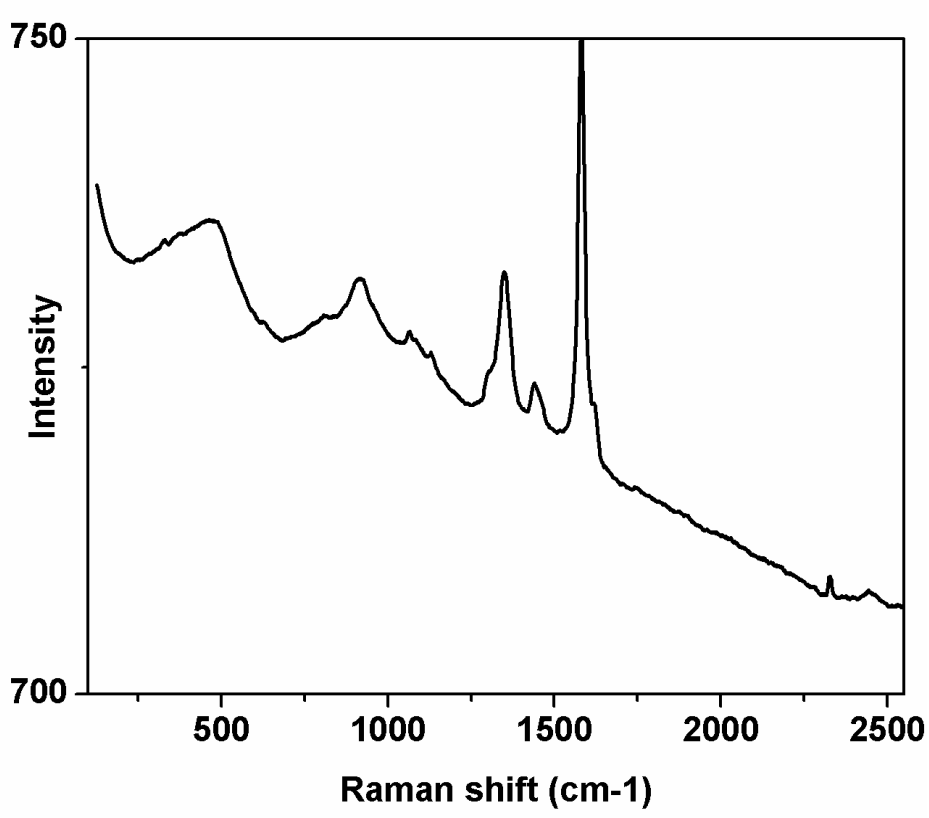


Fig. S2

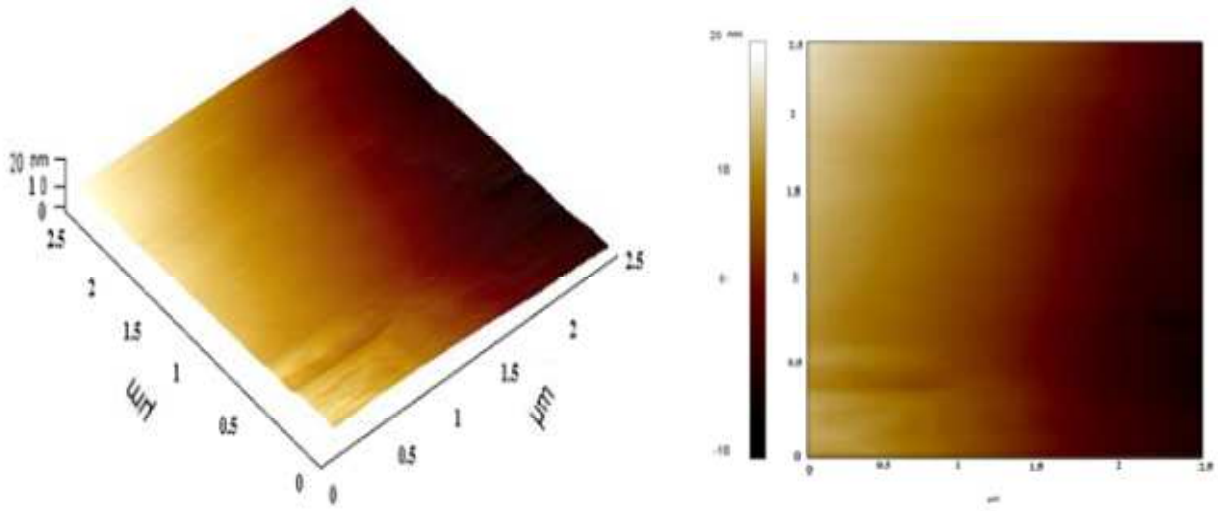
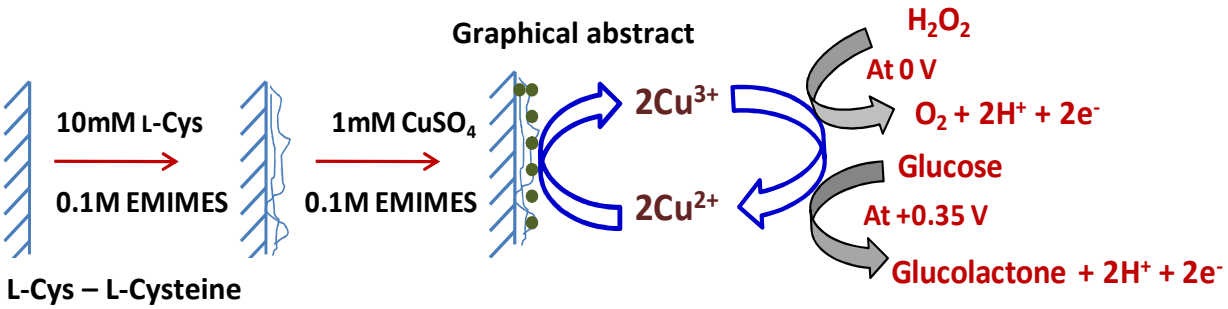


Fig. S3

Graphical abstract

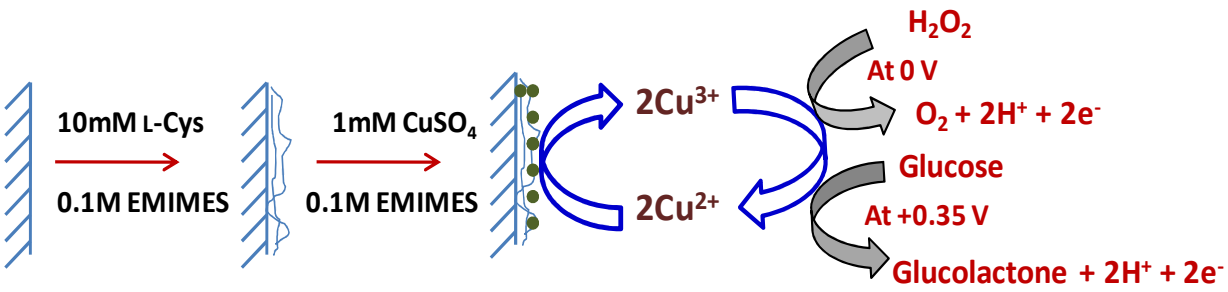


L-Cys – L-Cysteine

EMIMES = 1-ethyl-3-methylmidazoilium ethyl sulphate

Graphical abstract

The proposed method demonstrates a new kind of polycysteine functionalized copper nanoparticle (PCFCuNP) modified electrode using ionic liquid, 1-ethyl-3-methylimidazolium ethyl sulfate (EMIMES) as a green electrolyte. This method also achieved a highly sensitive and selective determination towards H_2O_2 and glucose. Further this report will encourage the researchers to employ the present method for biosensor applications also.



L-Cys – L-Cysteine

EMIMES = 1-ethyl-3-methylmidazoilium ethyl sulphate