

NON-ENZYMATIC AMPEROMETRIC GLUCOSE BIOSENSOR BASED ON COPPER HEXACYANOFERRATE-FILM MODIFIED-GNP-GRAPHITE COMPOSITE ELECTRODE

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ABSTRACT

A non-enzymatic amperometric glucose biosensor based on a modified electrode was fabricated by electrochemical derivatization of copper hexacyanoferrate (CuHCF) film at gold nanoparticles (GNP) adsorbed graphite wax composite electrode. The modified electrode was characterized by cyclic voltammetry and electrochemical impedance spectroscopy (EIS). The electrode was also characterized by scanning electron microscopy (SEM) which indicated that the CuHCF particles sized at 100-120 nm were dispersed uniformly on electrode surface. The modified electrode is exemplified towards the non-enzymatic sensing of glucose using cyclic voltammetry and amperometry. A linear calibration curve for glucose determination is obtained in the concentration range from 8.6×10^{-6} to 1.2×10^{-3} M with a detection limit of 1.3×10^{-7} M. The interference of ascorbic acid has been eliminated by coating the electrode surface with nafion.

KEYWORDS: GNP; Copper hexacyanoferrate; Non enzymatic; Glucose Biosensor.

INTRODUCTION

Diabetes mellitus is one of the principal causes of death and disability in the World, and is highly responsible for heart disease, kidney failure, and blindness. About 200 million people in the world are afflicted with diabetes mellitus. This figure is expected to rise up to more than three hundred million by 2030¹. Frequent testing of physiological blood glucose levels to avoid diabetic emergencies, is crucial for the confirmation of effective treatment²⁻⁵. Therefore, the development of highly sensitive, low-cost, reliable glucose sensors having an excellent selectivity has been the subject of concern for decades, not only in medical science but also in the food industries^{6,7}. Glucose oxidase (GOx)-based glucose biosensors have prevalently had a hold on the glucose sensor research and development over the last four decades and the market place as well. This is due to the high demand of sensitive and reliable blood glucose monitoring in biological and clinical aspects⁸⁻¹¹. There are still some disadvantages of enzyme-based glucose determination. Examples include complicated enzyme immobilization, critical operating

conditions such as optimum temperature and pH, chemical instability, and high cost^{12,13}. The activity of enzyme is obviously affected by the temperature, pH, humidity, and toxic chemicals¹⁴. To solve these problems, many enzyme-free sensors have been investigated to improve the selectivity towards the oxidation of glucose. Numerous processes and methodologies have been developed for creating new glucose sensors using electrochemical methods¹⁵, colorimetry¹⁶, conductometry¹⁷, optical methods¹⁸, and fluorescent spectroscopy¹⁹. Among them, the electrochemical glucose sensors have attracted the greater attention over the last 40 years because of their higher sensitivity and selectivity. Additionally, electrochemical techniques show lower detection limit, faster response time, better long term stability and they are more economical. The goal of the present paper is to study the electrocatalytic property of CuHCF-GNP graphite wax composite electrode towards glucose oxidation. Cysteamine-GNP graphite wax composite electrode has been already reported for CuHCF and NiHCF film modified electrodes from this laboratory²⁰⁻²¹. In the present work, L-

Cysteine-GNP graphite wax composite electrode is used for the preparation of CuHCF film on the electrode surface. This method is simple and copper hexacyanoferrate film is readily formed on a L-Cysteine-GNP adsorbed graphite wax composite electrode surface. The non-enzymatic glucose biosensor based on copper hexacyanoferrate (CuHCF) modified electrodes provides a prominent augmentation of response current toward glucose with a good stability and reproducibility.

EXPERIMENTAL

Chemicals and reagents

Glucose was obtained from Hi-media. Graphite powder was purchased from Aldrich (1-2 μm) Copper chloride, Potassium ferrocyanide were obtained from Merck (Mumbai, India). All reagents were of analytical grade. Doubly distilled water was used for all electrochemical experiments. KOH solution was prepared by double distilled water. L-cysteine solution (20mM) was prepared by double distilled water and copper chloride solution (0.01M) was prepared using ethanol. Potassium ferrocyanide (0.02M) with 0.1M KNO_3 as the background electrolyte was used to derivatize the coordinated copper ion. Glucose solution (0.01M) was prepared using doubly distilled water.

Apparatus

Scanning electron microscopy (SEM) images of the nanoparticles were recorded on a Hitachi Instrument (S-3400N). All the electrochemical measurements were carried out using a CHI electrochemical workstation (660 B) controlled by an IBM personal computer with standard three electrode configuration. The surface-modified CuHCF graphite wax composite electrode was used as working electrode, a platinum electrode as counter electrode and standard calomel electrode as the reference electrode.

Fabrication of Modified Electrode

The citrate capped GNPs were prepared using the reported procedure²². The maximum absorption peak for the synthesized GNP was observed at 530 nm. Graphite powder (1 g) was added to 125 ml of 0.59mM synthesized gold nanoparticles solution and the mixture was stirred for two hours at 300 rpm in room temperature. The stirred mixture was centrifuged at 1450 rpm for 30

minutes and the residue was kept overnight for drying. A GNP graphite wax composite electrode was prepared by mixing 900mg of gold nanoparticles adsorbed graphite powder with paraffin wax in the ratio of 4:1. Gold nanoparticle graphite wax mixture was tightly packed in a glass tube of 3mm diameter. The electrode was removed gently from the tube after it turns hard. The electrode prepared in this ratio showed an excellent hardness and conductivity. The surface of the composite electrode was first dipped in 20 mM of L- cysteine solution for 2 hours. Then, this L-cysteine modified GNP composite electrode surface was dipped in 0.01 M ethanolic solution of CuCl_2 solution for 6 minutes. The cysteine attached to the gold nanoparticles has a greater affinity towards and coordinates with transition metal ion like Cu^{2+} . The Cu^{2+} ion coordinated to amine group of cysteine was then derivatized using 0.02 M potassium ferrocyanide solution dissolved in 0.1 M KNO_3 by cycling the potential between - 0.2 to 1.0 V at the scan rate of 50 mV s^{-1} ²³.

RESULTS AND DISCUSSION

SEM characterization of the surface

Fig.1 shows the SEM images of bare graphite wax composite electrode (a), GNP absorbed graphite wax composite electrode (b) which shows that GNPs are in the range from 6-9nm and CuHCF – GNP- graphite wax composite electrode(c). Figure (c) clearly suggests the formation of CuHCF particles on the electrode surface and that the particle size ranges from 100-120nm.

Cyclic Voltammetric Characterization of Modified Electrode

The GNP-L-Cys-CuHCF modified electrode showed a single redox peak with anodic and cathodic peak potentials at 0.58 V and 0.49 V respectively. Continuous cycling of the GNP-L-Cys-CuHCF electrode in 0.1M KOH solution for more than hundred cycles showed no appreciable change either in the peak current or in peak potential indicating that the modified electrode is highly stable. **Fig. 2** shows the current response of the GNP-L-Cys-CuHCF electrode at various scan rates. It is observed from the results (Inset A) that the current depends linearly with the square root of the scan rate in the range of 10 to 200 mV/s. A plot of $\log i_{pa}$ versus $\log v$, is shown (Inset B)

where, i_{pa} is the anodic peak current in A and v is the scan rate in V/s which has yielded the following results; slope = 0.53 ± 0.02 , intercept =

2.1 ± 0.02 and correlation coefficient 0.999. These results indicate that the redox reaction involves one electron diffusion-controlled process.

Fig. 1. SEM image of (a) Bare graphite electrode (b) GNP adsorbed composite electrode (c) CuHCF-GNP Modified electrode.

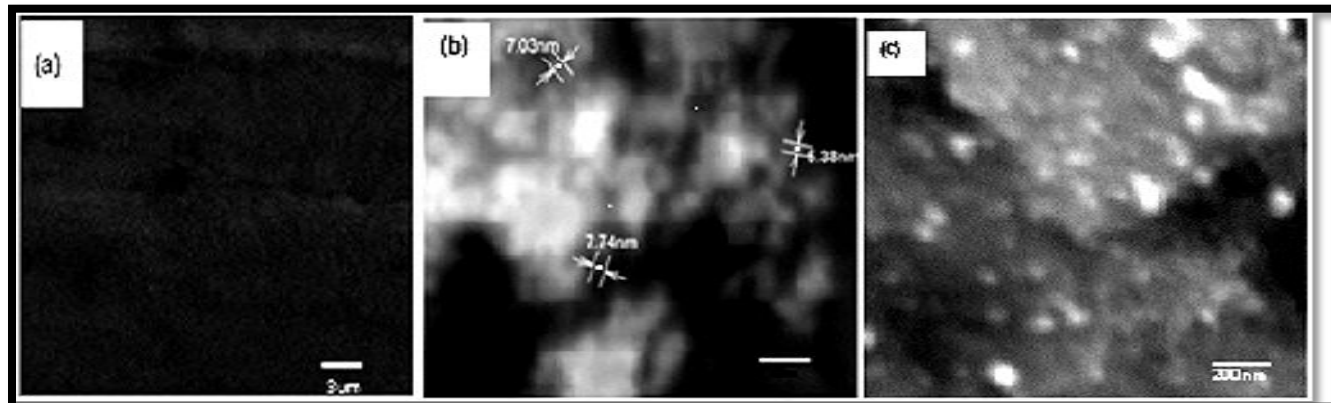
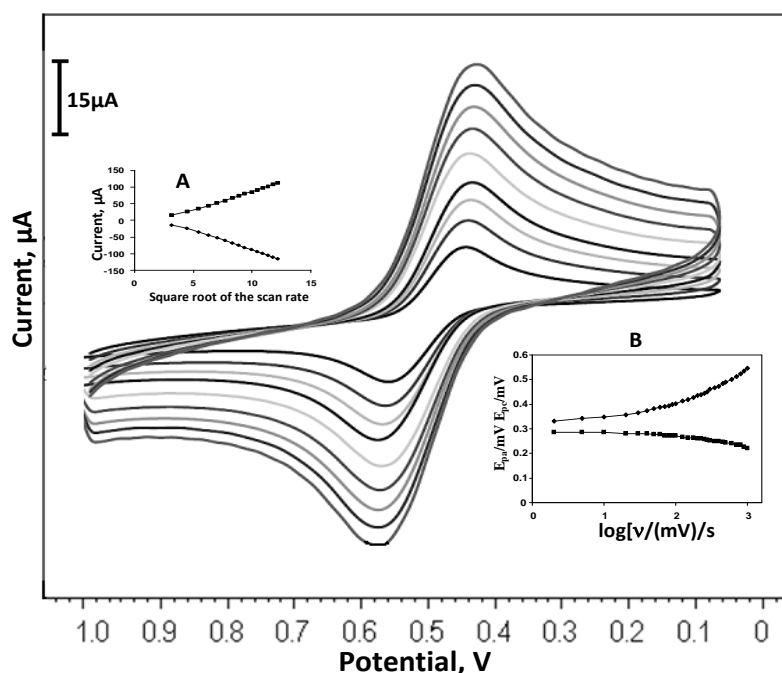


Fig-2 Fig.2.Cyclic voltammogram of the CuHCF modified electrode at different scan rates in 0.1M KOH. The scan rates from inside to outer are $10-150 \text{ mVs}^{-1}$ with increments of 10 from 10 mVs^{-1} ; Inset A: Dependence of peak currents (i_{pa} and i_{pc}) on square root of scan rate (v); Inset B: variation of peak potential vs. logarithm of scan rates.



Electrochemical Impedance Spectroscopy

Electrochemical Impedance Spectroscopy (EIS) was used to investigate the surface bound features of the CuHCF film modified electrode at the electrode-electrolyte interface. **Fig.3.** shows the Nyquist plots of the EIS for the bare electrode and CuHCF film modified electrode at varied potentials (0.54–0.62 V) in the frequency range of 0.1 Hz to 1.0 MHz. The complex plane spectrum was found

to fit well with the Randels circuit. EIS parameters such as charge transfer resistance (R_{ct}), double layer capacitance (C_{dl}), solution resistance (R_s) and Warburg impedance (Z_w) for the electron transfer characteristics of the CuHCF film modified electrode at the formal potential of 0.58 V was found to be $31.32 \text{ k } \Omega \text{ cm}^{-2}$, 15.20 nF cm^{-2} , $298.6 \text{ k } \Omega \text{ cm}^{-2}$ and $8.22 \times 10^{-4} \text{ } \Omega \text{ cm}^{-2} \text{ s}^{1/2}$ respectively and

that of bare electrode are respectively $22.56 \text{ k } \Omega \text{ cm}^{-2}$, 43 nF cm^{-2} , $382.5 \text{ k } \Omega \text{ cm}^{-2}$ and $6.211 \times 10^{-4} \text{ } \Omega \text{ cm}^{-2} \text{ s}^{1/2}$. The schematic representation of the modified Randle's equivalent circuit for this CuHCF film modified electrode is shown in the inset. The impedance data obtained for CuHCF film modified electrode at 0.58 V shows a lower resistance to the charge transfer kinetics which confirms that this modified electrode is a very attractive one for the electron transfer reactions

Electrochemical performances of the modified electrode as glucose biosensors

The electrochemical oxidation of glucose was investigated in 0.1 M KOH using the CuHCF film electrode by cyclic voltammetry. For comparison the voltammetric response in the absence of glucose was also recorded. The corresponding voltammograms are shown in **Fig. 4**. It is clear from the figure that there is a definite enhancement in the oxidation peak current in the presence of glucose indicating that glucose is oxidized by CuHCF via a cyclic mediation redox process.

Fig. 3. Complex impedance plots of the CuHCF film modified electrode in 0.1 M KOH , showing the Nyquist plot obtained for the electrode at the three different potentials (\circ - 540 mV), (\bullet - 580 mV), (\square - 620 mV) and bare composite electrode (bare- $\#$) Inset: Randels equivalent circuit diagram.

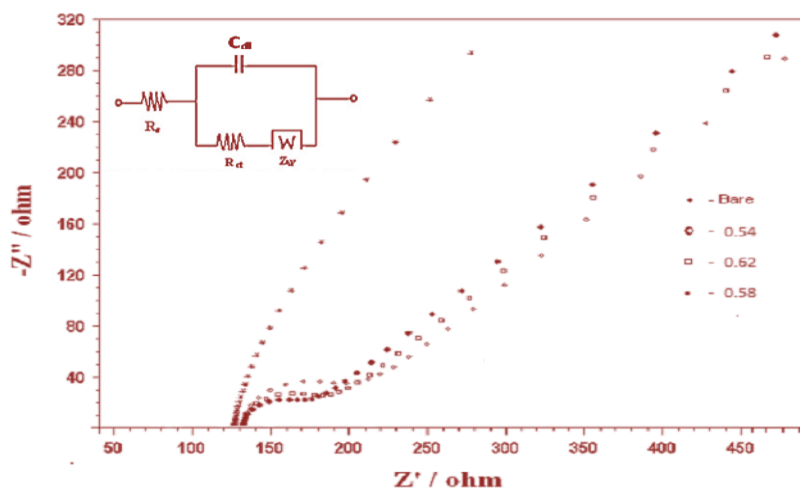
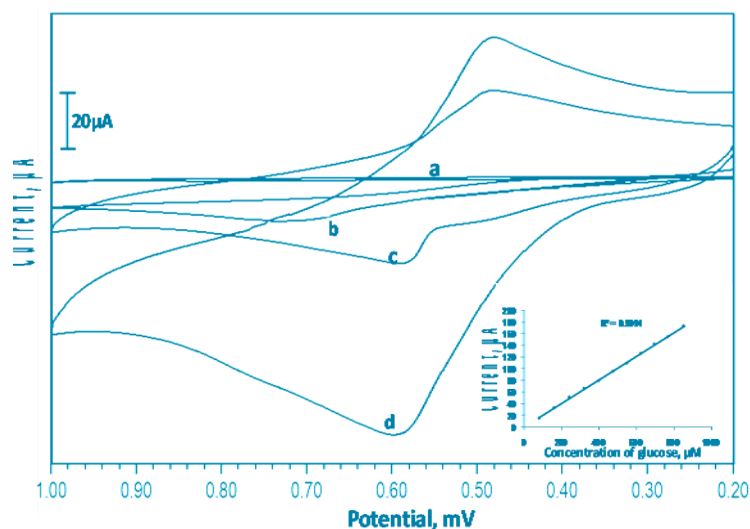


Fig-4: Fig.4. Cyclic voltammograms in 0.1 M KOH at a scan rate of 20 mVs^{-1} (a) bare composite electrode in the absence of glucose (b) bare composite electrode in the presence of $6.5 \times 10^{-5} \text{ M}$ glucose (c) modified electrode in the absence of glucose (d) modified electrode in the presence of $6.5 \times 10^{-5} \text{ M}$ glucose. Inset: calibration graph for glucose measurement.

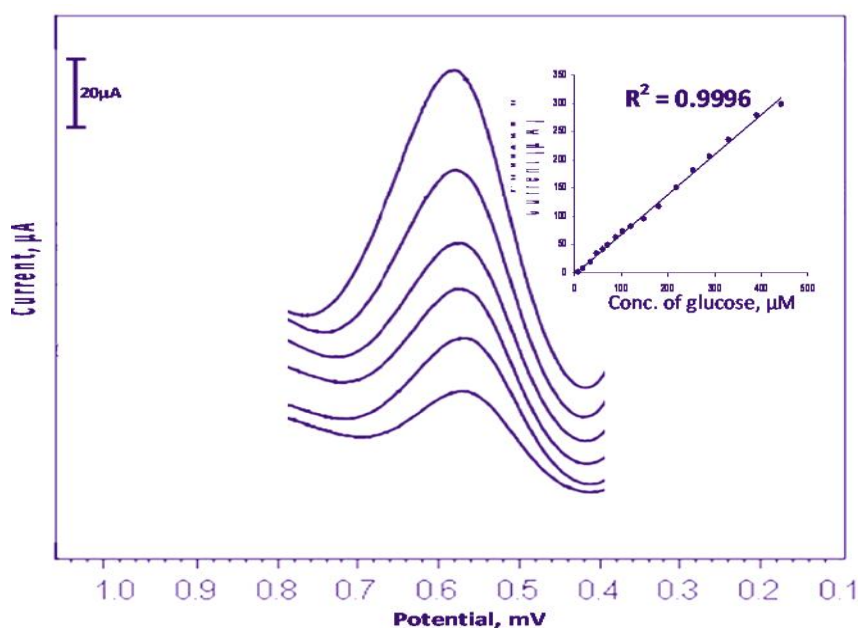


Electrocatalytic oxidation of glucose by Differential Pulse Voltammetry (DPV)

To study the concentration range of glucose that can be determined with the modified electrode, DPVs were recorded for different amounts of glucose (Fig.5). The catalytic currents were then plotted against the amount of glucose taken and the calibration graph is shown in the inset of Fig.5. A linear response for glucose oxidation was

observed in the concentration range from 8.6×10^{-6} to 1.2×10^{-3} M with a correlation coefficient of 0.9996. To study the precision of the method, 15 successive measurements were carried out for 7.2×10^{-5} M glucose and a relative standard deviation of 1.86% was found. A detection limit of 1.3×10^{-7} M glucose was observed for the determination of glucose.

Fig. 5. DPV responses of glucose obtained at the modified electrode at different concentrations; where a–f corresponds to 0.10, 0.15, 0.20, 0.25, 0.30, 0.35 $\times 10^{-4}$ M of glucose in 0.1 M KOH of electrolyte solution. Inset: The calibration plot for glucose measurement.



To study the applicability of the modified electrode as an amperometric sensor in flow systems, hydrodynamic voltammetric (HDV) experiments have been carried out for the electrocatalytic oxidation of glucose. The HDVs for glucose at the bare electrode (curve a) and at the modified electrode (curve b) are shown in Fig.6. From the figure it is seen that the catalytic current for the oxidation of glucose above 0.59 V is maximum and reaches a constant value. The potential can be fixed at any value in the constant current region and the determination of glucose in flow systems can be conveniently carried out. As expected, the electrocatalytic activity of the CuHCF permits convenient detection of glucose at lower potential with high sensitivity. In contrast, the bare electrode offers detection of the glucose only at higher potential with lesser sensitivity.

In order to evaluate the modified electrode as an amperometric sensor for the determination of glucose, amperograms have been recorded with different amounts of glucose and the amperogram obtained is shown in Fig.7. Similar amperograms were obtained for the lower concentration range as well. From the amperograms, a calibration plot was made with the catalytic current against the amount of glucose taken and the results are shown in the inset of Fig.7. A linear relation between the catalytic current and the amount of glucose in the range from 8.6×10^{-6} to 1.2×10^{-3} M is observed with a correlation coefficient of 0.999. The detection limit was found to be 1.3×10^{-7} M. It can be concluded that the CuHCF composite electrode can be used as an amperometric sensor for the determination of glucose with good

sensitivity. The proposed method was compared with the other literature reports (Table. 1.).

Fig.6 Hydrodynamic voltammograms obtained in the presence of 8.6×10^{-5} M glucose (a) bare composite electrode (b) modified electrode

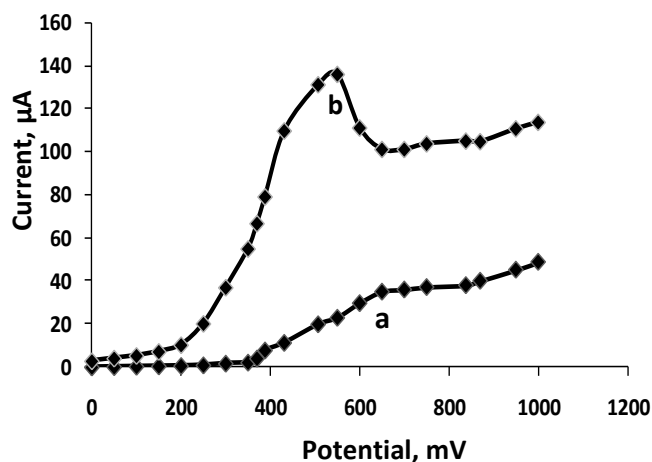
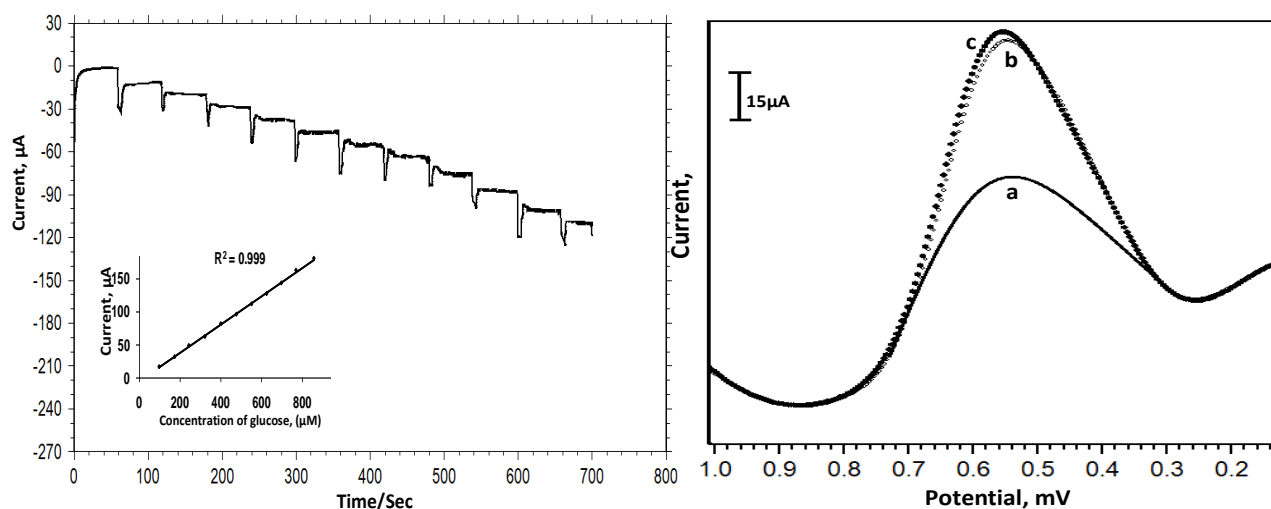


Fig.7. Chronoamperometric response at CuHCF modified electrode for each addition of 6.26×10^{-5} M glucose in 0.1 M KOH stirring rate: 300 rpm; potential: 0.59 V. Fig.7b. Calibration graph for glucose measurement

Fig. 8. (a) CuHCF modified electrode in the absence of glucose (b) CuHCF modified electrode in the presence of 9.9×10^{-5} M glucose. (c) Nafion coated CuHCF modified electrode in the presence of 1mM ascorbic acid with the same glucose concentration (9.9×10^{-5} M).



Interference Study

The CuHCF modified electrode exhibits also a good catalytic activity towards the oxidation of ascorbic acid. The interference of ascorbic can be eliminated using nafion coated CuHCF modified electrode as nafion coating bears negative charges and prevent ascorbate ion from reaching the electrode due to repulsion. The electrocatalytic response of 9.9×10^{-5} M glucose at the nafion coated CuHCF modified electrode in the presence

of 1mM ascorbic acid did not show the interference from ascorbic acid and this observation is similar to earlier reports²⁴.

Mechanism of oxidation

When metal hexachanoferrates such as NiHCF and CuHCF are used as mediators for electrocatalytic oxidation of substrates, the redox system responsible for catalytic oxidation is $\text{Fe(CN)}_6^{4-}/\text{Fe(CN)}_6^{3-}$. But when the modified electrodes with MHCF are used in alkaline medium, the complex

may undergo hydrolysis leading to the formation of Metal hydroxides²⁵. It has been reported that when NiHCF modified electrode is used for the oxidation of glucose, the redox system responsible for oxidation is $\text{Ni}^{2+}/\text{Ni}^{3+}$ system and not $\text{Fe}(\text{CN})_6^{4-}/\text{Fe}(\text{CN})_6^{3-}$ ²⁶. Thus in this work also we propose

Table 1

Electrode Composition	Sensitivity mA/mM	Linear Range mM	LOD (μM)	Reference
Cu nanoporous	220	0.01 - 0.5	40.0	27
Cu implanted BDD	6.4×10^{-3}	0.001 - 0.05	1.50	28
Proposed Method	355	0.0086 – 1.2	0.17	----

CONCLUSION

We have developed a simple method to prepare a CuHCF-GNP sensor. The resulting functional CuHCF film was characterized by SEM and cyclic voltammetry. The electrode modified with the CuHCF film showed a good stability and higher electro-catalytic activity for the oxidation of glucose in alkaline medium. It was found that this modified electrode could strongly enhance the electron transfer and possess excellent electro-catalytic property toward oxidation of glucose. A glucose sensor based on modified electrode was successfully demonstrated for sensitive and selective detection of glucose with excellent performance towards glucose sensing in the absence of enzyme.

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that the redox system responsible for catalytic oxidation of glucose may be $\text{Cu}^{2+}/\text{Cu}^{3+}$ rather than $\text{Fe}(\text{CN})_6^{4-}/\text{Fe}(\text{CN})_6^{3-}$ system which has to be confirmed by XPS studies. Nevertheless, this electrode can be used as non enzymatic glucose sensor for the determination of glucose.

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