



CuO Microspheres Modified Glassy Carbon Electrodes as Sensor Materials and Fuel Cell Catalysts

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This work describes the electrocatalytic oxidation of glucose on CuO microspheres prepared by initially depositing copper galvanostatically from an optimized sulfate bath on glassy carbon (GC) surface and subsequently subjecting the Cu deposits to potential cycling in 0.1 M KOH. The electrochemical behaviour and electrocatalytic activity of the electrode were characterized using cyclic voltammetry (CV) and chronoamperometry. Scanning electron microscopy (SEM) and X-ray Photoelectron Spectroscopy (XPS) were employed to characterize the morphological and chemical oxidation states of CuO in the film. These studies show that CuO modified GC electrodes exhibit good catalytic performance and high stability. Anodic oxidation current density was found to be 0.0496 mA cm⁻² for 1 mM of glucose. Modified electrode was validated as a sensing matrix by carrying out *in vitro* analysis of glucose in human blood serum and in the anolyte of the microbial fuel cell (MFC). The CuO/GC electrode shows very good sensitivity (709 mA M⁻¹ cm⁻²) and excellent linearity ($R = 0.998$). Our results present the scope of using CuO modified GC substrate as non-enzymatic glucose sensor for *in vitro* analysis and as an electrocatalyst for fuel cell application.

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Electro-oxidation of glucose has attracted a lot of interest due to its potential application in several areas, ranging from medical application to wastewater treatment and from biological and chemical fuel cell applications to analytical applications in food industry.¹⁻⁴ The investigation of glucose electrochemical oxidation began in 1960s and has remained a very attractive research area. One way to derive energy from glucose is to feed it into a fuel cell that directly oxidizes fuel to generate electricity. Over the past decades, extensive efforts have been made to study glucose as a fuel for both enzymatic and microbial fuel cells. Typically enzymatic glucose fuel cells employ glucose oxidase for glucose oxidation reaction, the most critical technical hurdle for this type of fuel cell is its extremely low performance.⁵ Furthermore, the limited stability of enzymes results in rather poor fuel cell durability. In this regard, microbial fuel cells are better than enzymatic fuel cells because they can be less susceptible to poisoning and loss of activity under normal operating conditions, and this leads to relatively long-term durability. But the most difficult problem of microbial fuel cells is the difficulty in transferring the electrons from the microbe to the electrode.⁶ For this reason, the performance of microbial glucose fuel cell is also extremely low.⁷ As both enzymatic and microbial fuel cells face above mentioned technical hurdles, direct glucose fuel cells that use a membrane electrolyte and metal catalysts have been receiving attention.

Although the direct oxidation has several advantages, such as easy electrode preparation, long device life time and high thermodynamic efficiency in fuel cell applications, it has not been investigated, and only a few significant activities in this area address this subject.^{8,9} The process of surface modification provides an interesting platform to alter the basic structure of the underlying substrates and thereby physical and chemical properties could be modified to find applications in various fields such as sensors and catalysis. So chemically modified electrode surface provides an attractive way of combining the benefits of heterogeneous catalysis with the advantages arising from three dimensional structural distributions of redox centers. Electrochemical oxidation of carbohydrates at various types of catalytically active electrode materials have been reported on Au,¹⁰⁻¹² Pt,^{13,14} Cu,¹⁵⁻¹⁸ Ni and Ni-alloys.^{19,20} Oxidation of carbohydrates in alkaline medium at Cu and Ni electrodes results in higher analytical currents when compared to Au and Pt electrodes. This has been attributed to a catalytic effect which involves multi-electron oxidation mediated by surface Cu and Ni oxide layers. It has been demonstrated that, among different materials glucose oxidation is most active in alkaline solution.²¹ The

oxidation of glucose in strong alkaline media is particularly interesting in sugar fed fuel cells.²² On the other hand the chemical instability of glucose during oxidation at higher pH and its degradation kinetics were widely investigated in the past years²³ showing the formation of complex mixtures of high and low molecular weight carbohydrates and aldehydes rather than simple gluconate.

CuO nanomaterials are relatively stable and have been used as electrocatalyst for glucose oxidation. CuO is a *p*-type semiconductor with a narrow bandgap of 1.2 eV. It has been studied extensively because of its application in several fields such as catalysis, semiconductors, batteries, gas sensors etc.²⁴⁻³⁰ CuO nanostructures have promising application in the field of non-enzymatic glucose sensors because of their high specific surface area, good electrochemical activity and electron transfer reaction at low over potential. Amperometric glucose sensing is one of the most promising techniques among various glucose testing methodologies, in which the enzyme glucose oxidase (GOx) is used as the crucial constituent to catalyze the oxidation of glucose to gluconolactone.³¹⁻³⁶ Even though GOx is quite stable compared to other enzymes, thermal and chemical instability of GOx prohibits enzymatic glucose sensors from being used for continuous monitoring in fermentation process or in human bodies requiring sterilization.^{37,38} In recent years considerable attention has been given for the development of enzyme-free electrode. Zhang et al. synthesized three different nano structures of CuO (wires, platelets and spindles) and concluded that electrocatalysis of CuO nano spindles was stronger than that of CuO nanowires and platelets.³⁹ Since the electrocatalytic property of chemically modified electrode depends on the physical and chemical state of the modifiers on the electrode surface, developing new preparation methods will be of great significance. Several reports related to the non-enzymatic glucose sensing is being published every year although they face certain tribulations such as lack of sensitivity, selectivity, and stability and being more expensive.

In this work we report modification of GC by CuO by galvanic deposition of Cu from sulfate-citrate based bath containing surfactant SLS (sodium lauryl sulfate) followed by electrochemical potential cycling. To the best of our knowledge no work has been reported for modifying GC using copper electrochemically from plating bath containing surfactant SLS as template. Further, the morphology variation observed for CuO on glassy carbon surface by changing the concentration (higher and lower) of copper in the bath using the same surfactant has been investigated in this work. Electrochemical potential cycling was employed for the chemical conversion to their corresponding metal hydroxide [Cu(OH)₂]. The process of modification by CuO microspheres on glassy carbon surface is simple and does not involve

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the use of any complicated and expensive equipment. Thus developed CuO/GC exhibits excellent catalytic current toward glucose oxidation with lower operation potential. The modified electrode was characterized using scanning electron microscope (SEM), X-ray photoelectron spectroscopy (XPS), and Cyclic voltammetry (CV). In addition we have also studied the effect of concentration on oxidation of glucose, selectivity, stability and amperometric response of the modified GC. Our studies showed that GC modified using $\text{Cu}(\text{OH})_2$ act as good electrocatalyst for glucose oxidation in alkaline condition and at the same time the results indicate the scope of using our electrodes for the non enzymatic sensing of glucose for in-vitro applications.

Experimental

Reagents.— D-(+)-Glucose and Dopamine hydrochloride were purchased from Sigma-Aldrich. $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, Citric acid monohydrate, Saccharin and KOH were obtained from Merck. L-Ascorbic acid was purchased from Sisco research laboratories (Mumbai, India). Sodium lauryl sulfate (SLS) from S-d fine chemicals (Mumbai). Methanol and Ethanol were purchased from Otto Inc (Mumbai). All reagents used were of analytical grade and used as received. Solutions used in this study were prepared with milli pore water.

Apparatus.— Cyclic voltammograms were recorded using PG-STAT 12/30/302 Autolab potentiostat/galvanostat using GPES software of version 4.9.007. A conventional three electrode set up was used with glassy carbon electrode as the working electrode, Pt electrode as counter electrode and calomel electrode (1N KCl) as the reference electrode. X ray photoelectron spectroscopy (XPS) of the modified electrode was recorded using Multilab- 2000 of Thermo-scientific make. The modified electrode was affixed to the sample holder. All spectra were recorded using an X-ray source (Al K α radiation) with a scan range of 0-1200 eV binding energy. The collected high-resolution XPS spectra were analyzed using the XPS peak fitting software program. The energy scale has been adjusted on the carbon peak (C 1s) spectra at 284.5 eV. SEM images of the electrode surface were taken using Hitachi S3000-H, Japan and images were recorded at 25 kV using secondary electron detector. Real samples were analyzed spectrophotometrically by Cary 5000 UV-Vis-NIR spectrophotometer (Varian) at 490 nm.

Modification of glassy carbon electrode by CuO.— The GC (3 mm diameter) electrode was subjected to polishing with different grades of emery paper followed by washing with doubly distilled water and subsequently ultrasonicated successively in ethanol and doubly distilled water for ten minutes. An electrochemical cell with a three electrode configuration was used in our study. A platinum rod and saturated calomel electrode (SCE) were employed as counter and reference electrodes respectively. Copper was deposited galvanostatically on the GC surface from a sulfate based bath containing CuSO_4 ($0.00249 \text{ g mL}^{-1}$), saccharin (0.0005 g mL^{-1}), citric acid monohydrate (0.06 g mL^{-1}), sodium lauryl sulfate (0.0002 g mL^{-1}) by maintaining the current density as -17 mA cm^{-2} using Hg/HgSO_4 as reference electrode.³⁹ Afterwards Cu deposited electrode was immersed in 0.1 M KOH solution and the electrode was subjected to electrochemical cycling for 20 cycles between the potential range -0.85 V and 0.75 V vs. Hg/HgCl_2 at a scan rate of 50 mV/s . Electrochemical cycling leads to the formation of CuO film on the GC surface. Two GC electrodes were modified in order to study the effect of concentration of Cu on glucose oxidation. First electrode was modified from a bath containing 0.2 M CuSO_4 and the second electrode from a bath containing 10 mM concentration of CuSO_4 .

Electrocatalytic oxidation of glucose.— Cyclic voltammetry was carried out in a three compartment cell containing 15 mL 0.1 M KOH at a scan rate of 50 mV/s in the potential window of -0.85 V to 0.75 V vs. $\text{Hg}/\text{Hg}_2\text{Cl}_2$. Oxidation current density for glucose oxidation

was measured from their corresponding CVs. Effect of concentration was studied by varying the glucose concentration from $66 \mu\text{M}$ to 6 mM . Chronoamperometry was carried out in a stirred solution of 0.1 M KOH and glucose is injected at various intervals at a constant applied potential of 0.35 V , and this method was used for the invitro glucose detection in blood serum.

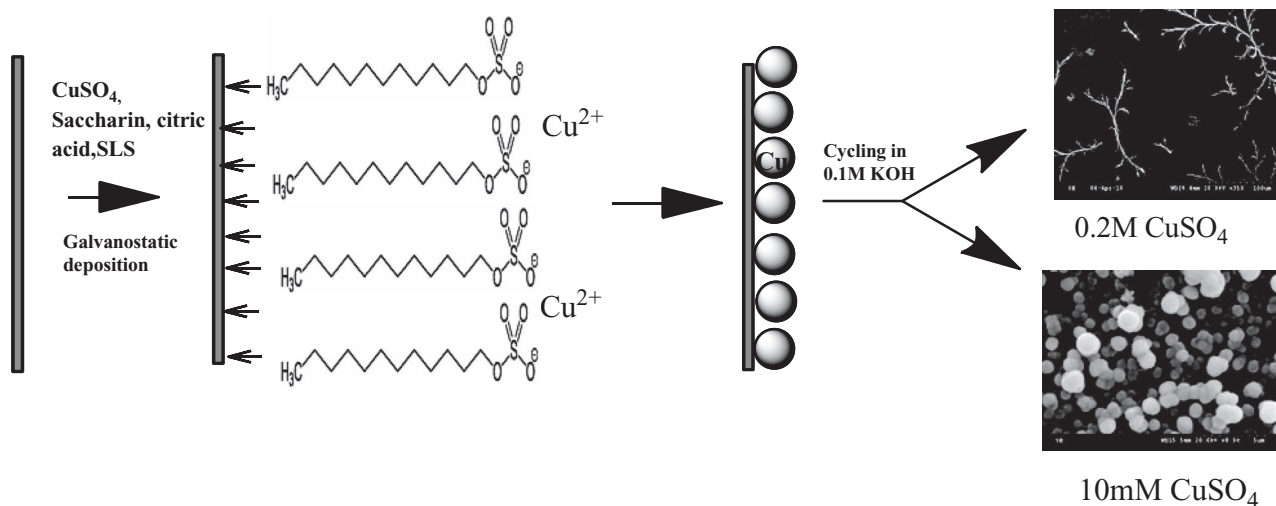
Real sample analysis.—

- Glucose in blood serum - Serum samples were collected from nearby health center and the performance of the modified electrode was measured by adding $50 \mu\text{L}$ of serum to constantly stirred solution of 0.1 M KOH and the amperometric response was measured at 0.35 V . Thus obtained values were compared with the lab reports.
- Glucose levels in the anode compartment of the microbial fuel cell (MFC) - In our laboratory research and development of mediatorless microbial fuel cell (MFC) is being carried out with the microorganisms *Acetobacter aceti* and *Gluconobacter roseus* as biocatalysts and glucose as fuel.^{40,41} The performance of fuel cell is evaluated in terms of fuel consumption. Routine analysis of glucose consumption is being carried out in our laboratory using Phenol-sulphuric acid method with the help of Cary 5000 UV-Vis-NIR spectrophotometer (Varian) at 490 nm. We are making attempts to see that the method described in this work can substitute the spectrophotometric protocol. Aliquots from the anolyte of the MFC are added to stirred solution of 0.1 M KOH and the glucose concentration is detected amperometrically. The values obtained are compared with the values obtained spectrophotometrically.

Results and Discussion

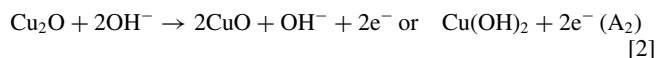
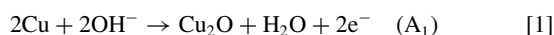
Galvanostatic deposition of copper on GC surface and transformation to CuO.— Glassy carbon surface was modified by galvanostatic deposition of copper from citrate- and sulfate based bath containing sodium lauryl sulfate and saccharin as additives.^{39,42} Galvanostatic method was adopted for getting better control over the deposit thickness. Here saccharin was added to suppress the dendritic growth and citrate act as complexing agent. Deposition was done using Hg/HgSO_4 as reference electrode (to avoid chloride poisoning) and platinum as counter electrode. Optimum time for Cu deposition was 300 seconds according to the catalytic activity of glucose. By lowering the time non uniform film formation is observed, which lowers the active sites. While increasing the time bulk copper deposition rather than thin film formation is observed. This also reduces the active sites. Cu deposited GC electrode is then transferred to 0.1M KOH and subjected to electrochemical cycling at a scan rate of 50 mV/s in the potential range -1.2 V to 0.75 V using $\text{Hg}/\text{Hg}_2\text{Cl}_2$ as reference electrode. While proceeding the potential cycling process, the redox current decreased indicating that CuO get deposited on GC surface. The optimum number of cyclic potential scan was 20 according to the catalytic activity of the analyte. Scheme 1 shows CuO formation on GC surface.

Cyclic voltammograms of CuO/GC in 0.1 M KOH after cycling is shown in Figure 1. A number of well defined peaks are observed in both anodic and cathodic half cycles that relates to the series of redox process. According to the previous reports the oxidation peaks corresponds to $\text{Cu}(0)/\text{Cu}(\text{I})$ - A_1 , $\text{Cu}(\text{I})/\text{Cu}(\text{II})$ - A_2 , large wave at 0.4 V can be associated with the formation of soluble species ($\text{Cu}_2\text{O} + \text{OH}^- \rightarrow \text{HCuO}_2^-$ (soluble) + e^- - A_3) during the oxidation of Cu. The peak expected for $\text{Cu}(\text{II})/\text{Cu}(\text{III})$ at 0.5 V appears to be flat, due to fast electron transfer and oxygen evolution followed by the reaction.⁴³ Examination of the reverse scan revealed, initially a small cathodic peak at 0.5 V which has been attributed to the reduction of $\text{Cu}(\text{III})$ to $\text{Cu}(\text{II})$ and reduction peaks at -0.6 V and -0.85 V corresponds to $\text{Cu}(\text{II})/\text{Cu}(\text{I})$ - C_2 , $\text{Cu}(\text{I})/\text{Cu}(0)$ - C_1 respectively. The I-E characteristics of this voltammogram are in good agreement with those found in the literatures.^{20,31-33} The possible reaction mechanism



Scheme 1. Schematic representation showing the methodology used for Cu deposition on GC and conversion to CuO via potential cycling in 0.1 M KOH. First step shows Cu^{2+} along with surfactant SLS approaching GC surface, second step shows Cu deposition, third step indicates CuO formation via potential cycling.

for the series redox process during conversion of copper to copper hydroxide is as follows.⁴⁴⁻⁴⁸



The oxidation peaks of copper cover the entire potential ranges which makes this electrode unamenable for electrochemical studies. So in order to prepare a stable copper oxide layer the lower limit of the applied potential is restricted to -0.85 V to avoid the reduction of Cu oxide to Cu⁴² and in this case concentration of CuSO_4 was maintained as 0.2 M. Figure 2a shows the cyclic voltammetric response of CuO/GC cycled in the potential range of -0.85 V to 0.75 V after 20 cycles at a scan rate of 50 mV/s. In comparison with Figure 1 there is

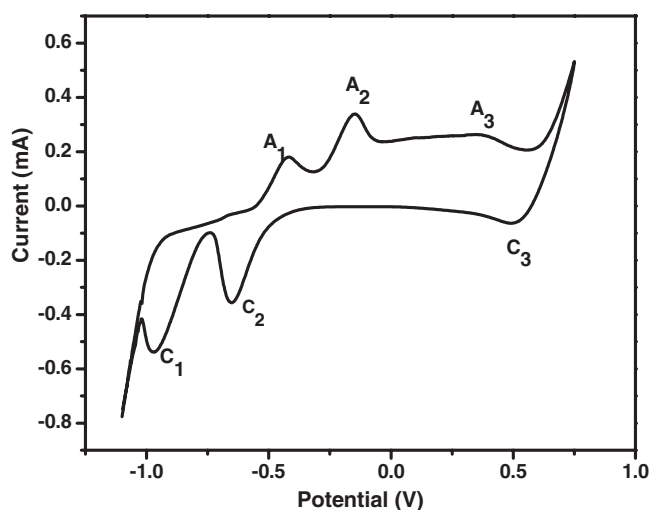
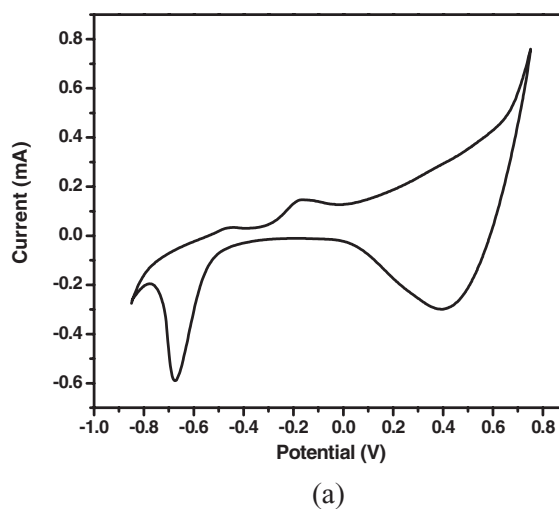


Figure 1. Cyclic voltammetric response of CuO/GC in 0.1M KOH after 20 cycles. The potential range used for cycling is from -1.2 V to 0.75 V vs Hg/HgCl₂ at a scan rate of 50 mV/s.

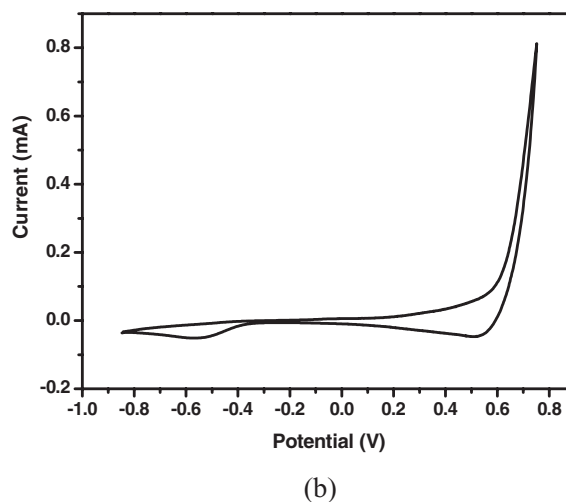


Figure 2. Cyclic voltammetric response of (a) CuO/GC prepared from 0.2 M CuSO_4 (b) CuO/GC prepared from 10 mM CuSO_4 in 0.1 M KOH after 20 cycles. The potential range used for cycling is from -0.85 V to 0.75 V vs Hg/HgCl₂ at a scan rate 50 mV/s.

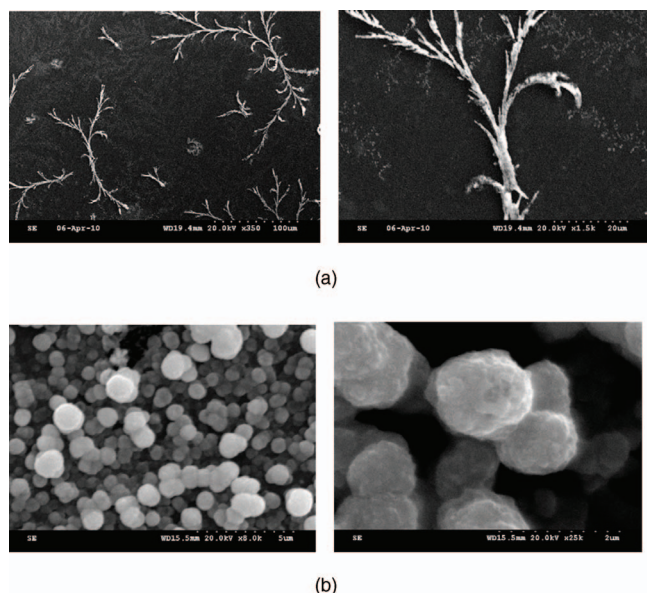


Figure 3. SEM images of CuO modified GC. (a) SEM image of dendritic CuO prepared using 0.2 M CuSO_4 . (b) SEM image of CuO microspheres prepared using 10 mM CuSO_4 .

a decrease in peak height of A_1 . During potential cycling from -0.85 V there arises a broad reduction peak at 0.48 V vs. $\text{Hg}/\text{Hg}_2\text{Cl}_2$ which might correspond to $\text{Cu(II)}/\text{Cu(III)}$ redox couple.²⁰ Since more Cu is converting to Cu(III) state increase in current is observed while restricting the potential to -0.85 V, compared to the previous case where the starting potential is -1.2 V. CuO formation takes place initially if the starting potential is more negative potential which shows low current response. Thus a stable CuO layer is formed on the electrode surface.⁴⁹

A second electrode is modified using a similar bath composition with the concentration of CuSO_4 adjusted to 10 mM. Figure 2b represents the cyclic voltammogram of GC electrode modified using 10 mM CuSO_4 after alkaline cycling in 0.1 M KOH. After potential cycling the anodic peaks A_1 , A_2 were minimized to a great extent and only peak corresponding to $\text{Cu(II)}/\text{Cu(III)}$ exists. This suggests that this electrode can be used for electrochemical studies in the potential range -0.8 to 0.75 V without any interfering effects by other oxidation peaks^{50,30}

Surface characterization.—Scanning electron microscopic analysis.—Structural morphology of CuO modified GC of different concentration were analyzed using SEM studies. Copper deposition from the bath containing 0.2 M CuSO_4 after subjected to cycling in 0.1 M KOH show a dendritic growth. (hereafter referred to as dendritic CuO - Figure 3a) and copper deposition from the bath containing 10 mM CuSO_4 after subjected to cycling in alkali shows that the CuO film consists of spherical structures of nearly $1\ \mu\text{m}$ dimensions (hereafter referred to as CuO microspheres Figure 3b). Surface of CuO microspheres appear to have nodules. Dendritic appearance can be due to excess concentration of copper compared to surfactant and additives. While decreasing the concentration of copper, template effect of SLS causes copper deposit to have microsphere morphology.

X-ray photo electron spectroscopy studies.—Oxidation state of Cu in CuO microspheres and dendritic CuO is obtained by XPS analysis. The results show both CuO belong to same oxidation state. Figure 4 shows XPS spectra of C (1s), O (1s), Cu (2p). Carbon 1s emission peak is observed around 284.5 eV, which is used as reference in the present XPS measurement. XPS is a powerful technique for the study of transition metal compounds having localized d orbitals. From the XPS analysis it is clear that copper exist in divalent state mainly in d^9

character. XPS detected $2p_{3/2}$ and $2p_{1/2}$ main peaks at 934.05 eV and 954.1 eV respectively. The peak fit of Cu $2p_{3/2}$ peak revealed a main peak at 934.05 accompanied by a series of satellite peak on the high binding energy side at 942 and 944.15 . The shake-up satellite peaks are evident and diagnostic of an open $3d^9$ shell, corresponding to Cu^{2+} state.^{48,51} O 1s binding energy peak appeared at 530 eV, originating from CuO.

Electrocatalytic oxidation of glucose and effect of concentration on modified electrode.— Since surface immobilized layer often imparts optimally fast electron transfer characteristics to an underlying substrate, we tried to investigate the possibility of using the Cu(OH)_2 films for the electrocatalytic oxidation of glucose in alkaline medium. Electron transfer mediating ability of metal oxide and semiconductor electrodes can be enhanced by choosing proper molecule for surface anchoring which could later be used for sensing and catalytic application. We have studied the direct electrochemical oxidation of glucose in alkaline medium. For comparison we have also carried out similar experiments in bare electrode and copper modified electrode in alkaline condition. Figure 5 shows CVs of bare GC(a), Cu/GC (b), and CuO microsphere/GC (c) in 0.1 M KOH at a scan rate of $50\ \text{mV/s}$ in the presence of glucose. No catalytic current response was observed for unmodified and Cu modified electrode, while GC modified with CuO microsphere shows good catalytic current response. These results suggest that Cu(OH)_2 modified electrode are capable of performing direct oxidation of glucose in alkaline medium.

By the addition of glucose characteristic oxidation peak was observed at the potential range from 0.2 V to 0.6 V vs. $\text{Hg}/\text{Hg}_2\text{Cl}_2$ at a scan rate of $50\ \text{mV/s}$. This may be attributed to the proposed involvement of Cu (II) and Cu (III) surface species in the oxidation of glucose.⁵⁰ This CuO layer acts as an effective mediator for electron transfer between glucose and GC electrode. Figure 6a shows the cyclic voltammetric response for glucose addition in dendritic CuO modified electrode. With increase in concentration of glucose the oxidation peak current proportionately increases. Oxidation starts from 0.2 V with a shoulder peak at 0.5 V. This shows that CuO prepared by our method acts as an efficient catalyst for glucose oxidation. Inset shows plot of current density (j) vs. concentration, in which current density reaches plateau at higher concentration.

In the case of GC modified with CuO microspheres oxidation over potential was found to be less. The oxidation starts from 0.2 V with peak potential at 0.35 V. Figure 6b corresponds to electrocatalytic effect of CuO microspheres modified GC for glucose addition in 0.1 M KOH. The lowering of the over potential is perhaps due to kinetic effect caused by an increase in electro active surface area (i.e. the dendritic structure changes to microsphere). Current density (j) vs. concentration plot shows a good range of linearity from $0.8\ \text{mM}$ to $6\ \text{mM}$ with $R = 0.9988$. The linear regression equation was found to be $j\ (\text{mAcm}^{-2}) = 0.01115 + 0.05C\ (\text{mM})$. A shift in the peak potential toward anodic side is observed with the increase in concentration of glucose in both cases. It can be noted from the figure that anodic current density increases with concentration of glucose and exhibits a linear relation between current and concentration for CuO microspheres, while CuO dendritic structure shows a deviation from linearity with increase in concentration. CuO microspheres show glucose oxidation without any surface saturation even at high concentration. Current density for glucose oxidation was measured to be $0.0469\ \text{mA cm}^{-2}$ for $1\ \text{mM}$ of glucose. Linear relationship between oxidation current density and glucose concentration makes CuO microspheres modified electrode applicable for glucose sensing.

The exact mechanism of glucose oxidation on the copper modified electrode in alkaline medium is hard to confirm. But the most accepted mechanism suggests the catalytic role of metal oxy-hydroxide in oxidation process as proposed by Fleischmann et al.⁵² We observed that oxidation of glucose occurs at the peak potential of the redox couple $\text{Cu(II)}/\text{Cu(III)}$ and the addition of glucose results in increasing current density for the oxidation reaction and decreasing current density for the reduction process. So the ratio of anodic peak current and cathodic peak current increases in the presence of glucose. The

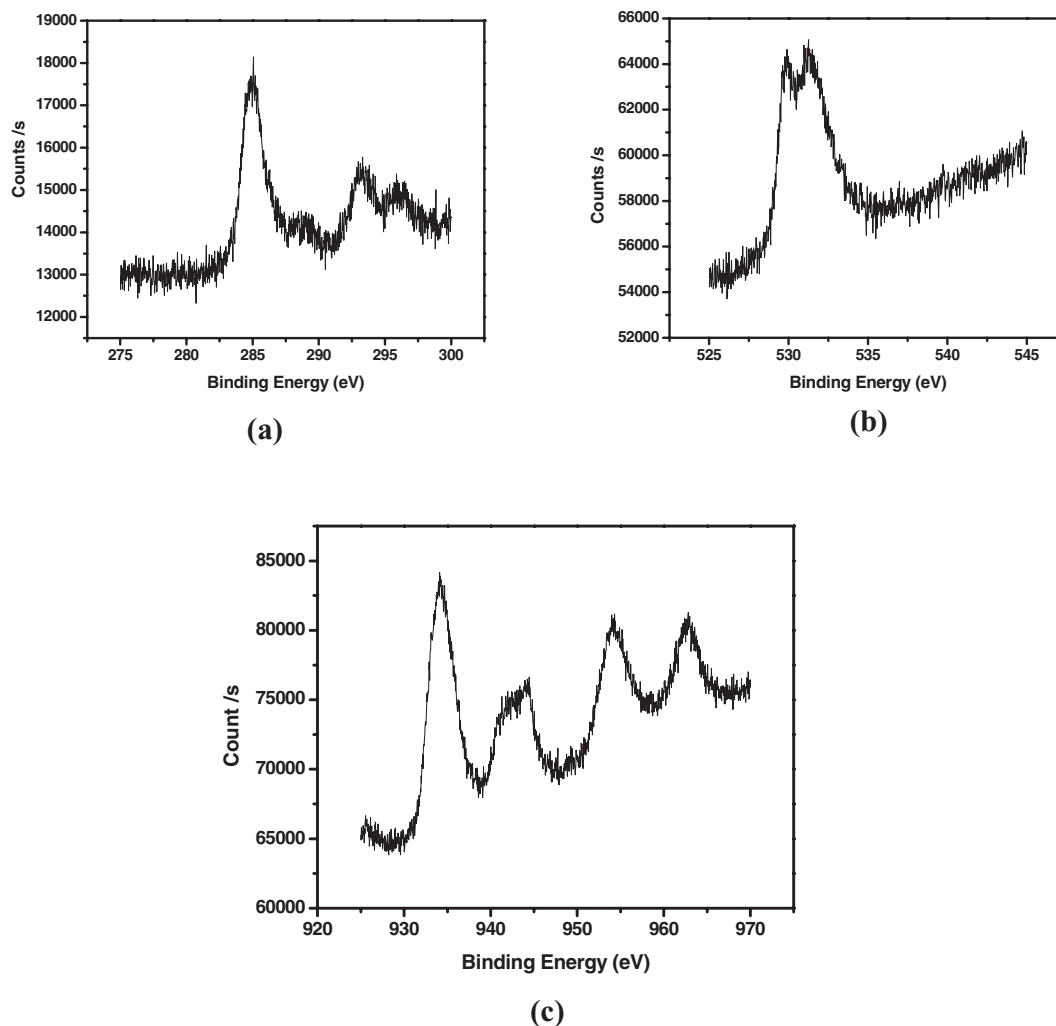


Figure 4. X-ray photo electron spectrum of CuO microspheres (a) C 1s (b) O 1s (c) Cu 2p of the CuO.

proposed mechanism in our case is the direct reaction between glucose and Cu(OOH) formed on the electrode surface. In dilute alkaline conditions oxidation begins with the deprotonation of glucose which isomerises to enediol form followed by the adsorption on electrode

surface for oxidation.⁵¹ Based on these points the proposed mechanism was explained in terms of the following equations.

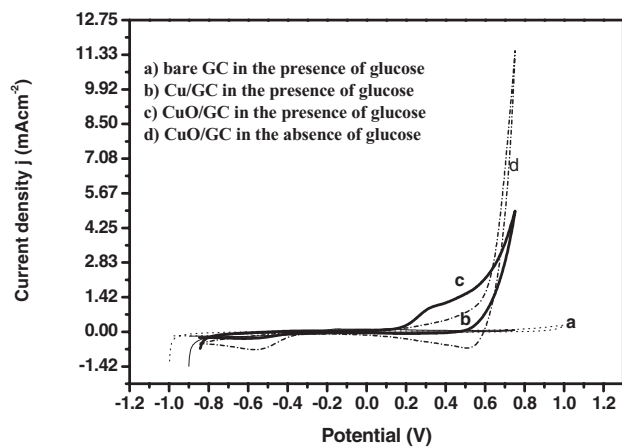
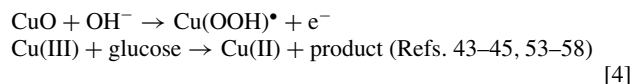
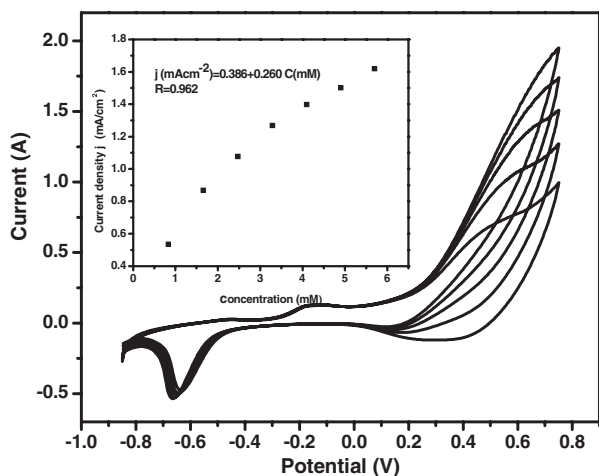


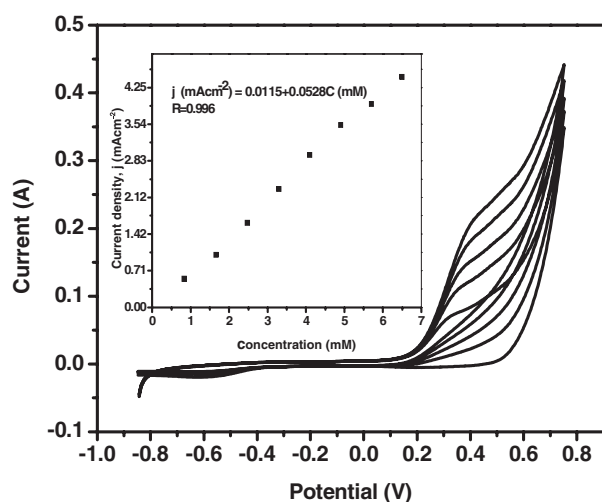
Figure 5. Cyclic voltammograms of (a) bare GC (b) Cu modified GC (c) CuO microsphere modified GC at a potential sweep rate of 50 mVs^{-1} in 0.1 M KOH solution in the presence of 0.83 mM of glucose. (d) CuO microspheres in the absence of glucose.

The effect of oxidation current of glucose with respect to scan rate was examined by cyclic voltammetry in 0.1 M KOH solution containing 5 mM glucose (SI Fig-1).⁵⁹ A good linearity between square root of scan rate and peak current was observed in the range $10\text{--}200 \text{ mVs}^{-1}$. The regression equation was found to be $I (\text{mA}) = 2.14107 + 86.64 v^{1/2} (\text{mV/s})$ with $R = 0.988$ and this confirms that the oxidation of glucose is diffusion controlled.

Amperometric determination of glucose on CuO/GC.— In order to find application of the modified electrode for invitro sensing of glucose amperometric response of the electrode was analyzed in constantly stirred 0.1 M KOH solution. Amperometric response of glucose addition on dendritic CuO was done at a potential of 0.5 V (SI Fig-2).⁵⁹ Two different ranges ($66 \mu\text{M}$ to 0.456 mM and 0.8 mM to 6 mM) of glucose concentration were tested by successive addition of glucose in 0.1 M KOH . Dendritic CuO/GC displays a linear range from $66 \mu\text{M}$ to 3 mM and a deviation at higher concentration is observed, the regression equation was found to be $I (\text{mA}) = 0.4824 + 0.21C (\text{mM})$ and the sensitivity is $2970 \text{ mA M}^{-1} \text{ cm}^{-2}$. Figure 7 shows amperometric response curve of glucose in 0.1 M KOH at CuO



(a)



(b)

Figure 6. Cyclic voltammetric response for glucose addition in 0.1 M KOH at 50 mV/s – (a) dendritic CuO (b) CuO microspheres. Each addition corresponds to 0.83 mM. Inset shows plot of current density vs concentration.

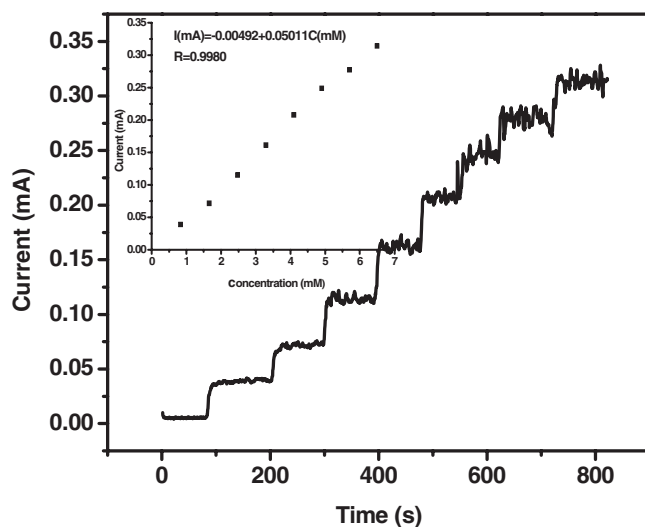


Figure 7. Amperometric response of CuO microsphere/GC for glucose oxidation in 0.1 M KOH (concentration range 0.8 mM to 6 mM glucose). Inset shows the current vs concentration plot.

microspheres/GC at a potential of 0.35 V. Linearity is observed at lower (SI Fig-3)⁵⁹ as well as at higher concentration of glucose. The regression equation was found to be $I \text{ (mA)} = -0.00492 + 0.050C \text{ (mM)}$ with $R = 0.9980$ and sensitivity is $709 \text{ mA M}^{-1} \text{ cm}^{-2}$ for CuO micro spheres. The observed sensitivity of the electrode in this study is far greater than that of similar non-enzymatic sensors already reported. There are reports based on carbon nano tubes (CNT) based non-enzymatic sensors, even though their sensitivity is comparable (comparison is given in the Table I) with our reports they have used expensive CNT also the over potential for glucose oxidation is high. Our method follows simple and cost effective procedure. Response time of the electrode to achieve steady state is less than 7 s. Even though sensitivity of detection obtained in the case of dendritic CuO/GC is high, it shows a deviation from linearity at higher concentration but this is not observed for CuO microspheres.

Real sample analysis.—

- a) Human serum sample analysis - In an attempt to explore the CuO microsphere/GC electrode for practical applications, the modified electrode was evaluated for the determination of glucose in human blood serum sample. 50 μL of serum sample was added to 15 ml 0.1 M KOH solution, and current response was recorded at 0.35 V. Glucose concentration in serum was found to be 12.27 mM, which was compared with lab reports (Fig. 8). The lab report value was

Table I. Comparison of sensitivities and operation potentials of different non-enzymatic glucose sensors (from amperometric i-t response).

Electrode	Sensitivity	Reference	Operation Potential (V)
Porous Au	$11.8 \mu\text{A mM}^{-1} \text{cm}^{-2}$	Li et al. (2007) ⁶¹	+ 0.35 vs Ag/AgCl
Cu/MWCNTs	$251.4 \mu\text{A mM}^{-1} \text{cm}^{-2}$	Kang et al. (2007) ⁶²	+ 0.65 vs Ag/AgCl
CuO/CuOx	$1890 \mu\text{A mM}^{-1} \text{cm}^{-2}$	Babu et al. (2010) ⁶⁰	+ 0.7 vs Ag/AgCl
Ni nano wires	$1043 \mu\text{A mM}^{-1} \text{cm}^{-2}$	Lu et al. (2009) ⁶³	+ 0.55 vs Ag/AgCl
CuO nanowires	$0.49 \mu\text{A mM}^{-1} \text{cm}^{-2}$	Zhang et al. (2008) ⁶⁴	+ 0.49 vs Ag/AgCl
MnO ₂ /MWCNTs	$33.19 \mu\text{A mM}^{-1} \text{cm}^{-2}$	Chen et al. (2008) ⁶⁵	+ 0.3 vs Ag/AgCl
CuO nano particles- Modified carbon Nanotube	$2596 \mu\text{A mM}^{-1} \text{cm}^{-2}$	Jiang et al. (2010) ⁶⁶	+ 0.55 vs Ag/AgCl
Porous CuO microcubes	$-70.8 \mu\text{A mM}^{-1}$	Zhang et al. (2009) ⁶⁷	+ 0.61 vs Ag/AgCl
CuO nano spheres	$404.53 \mu\text{A mM}^{-1} \text{cm}^{-2}$	Reitz et al. (2008) ⁶⁸	+ 0.6 vs Ag/AgCl
Porous CuO	$2900 \text{ mA M}^{-1} \text{cm}^{-2}$	Cheverko (2010) ⁶⁹	+0.65 vs Hg/HgO
Dendritic CuO	$2970 \text{ mA M}^{-1} \text{cm}^{-2}$	Our work	+ 0.5 vs NCE
CuO microspheres	$709.345 \text{ mA M}^{-1} \text{cm}^{-2}$	Our work	+ 0.35 vs NCE

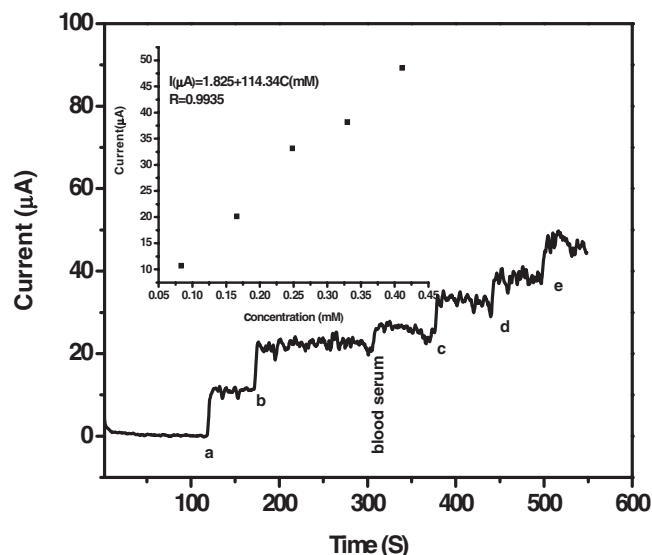


Figure 8. Amperometric response of CuO microspheres/GC to standard glucose solution and serum sample at a potential of 0.35 V in 0.1 M KOH. a,b,c,d,e refer to standard glucose addition and each addition corresponds to 1.499 mg/dL (83.28 μ M). Inset shows current vs concentration plot.

13 mM. This shows that values we obtained from amperometry experiment are 94% agreement with lab reports. The value was calculated from the calibration graph obtained between current and known concentrations of glucose.

- b) Analysis of glucose consumption in the anolyte of microbial fuel cell (MFC) – Performance of modified electrode for glucose sensing is also tested by removing samples from the anolyte of the MFC. We tried to replace the spectrophotometric method described in experimental section by the modified electrode to check the fuel consumption. 2 mL of the sample from the anode compartment of the fuel cell was taken out and centrifuged at 7500 rpm at 20°C for about 10–15 minutes and the supernatant is used for glucose detection. 100 μ L of the sample is added to 0.1 M KOH solution and current response is monitored. Amperometric experiment at constant applied potential was performed with standard additions of glucose. A calibration graph of current vs concentration was obtained for standard additions of glucose from which the unknown concentration of glucose in the anolyte of the MFC was calculated. Results show that recovery of standard glucose is >96% (SI Fig. 4).⁵⁹ The plot of current vs concentration gives regression equation as $I \text{ (mA)} = 0.2706 + 0.28C \text{ (mM)}$ with $R = 0.9941$. This regression equation can be used for calculating unknown concentration. The obtained results are in good agreement with those obtained from spectrophotometric method. The results demonstrated here reveal the potential applications of the CuO microspheres/GC for determination of glucose.

Reproducibility, stability and anti-interference property of CuO/GC.— Reproducibility of the electrode was tested by preparing CuO/GC five times and evaluated their amperometric current response at 0.35 V. Results showed deviation of less than 2%, which means the performance of the electrode, is highly reproducible. The modified electrode is kept in a closed container at room temperature when not in use. Response was checked every two days over a period of four weeks. Sensitivity of the electrode shows a deviation less than 3%. This shows that electrode prepared in this method is highly reproducible and stable.

Anti-interference property is also important for sensors. Because easily oxidisable species such as dopamine, ascorbic acid, uric acid and other carbohydrate compounds usually co-exist with glucose in the biological systems. Electrochemical response of interference is checked with CuO microspheres (Figure 9) and dendritic CuO in

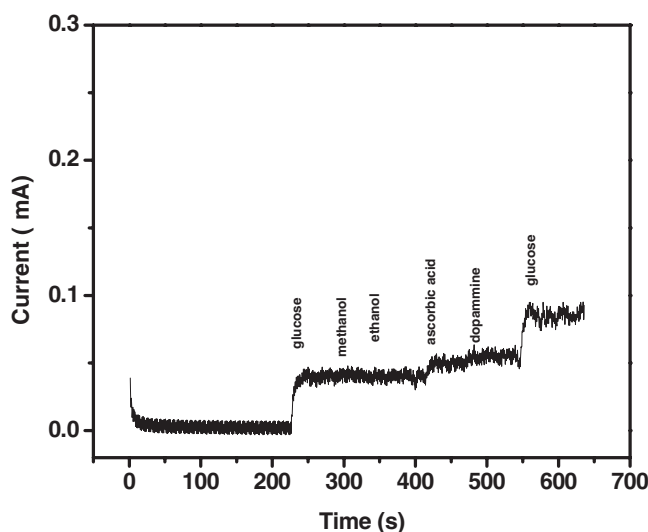


Figure 9. Amperometric response of CuO microspheres/GC in the presence of 0.1 mM of glucose, 0.1 mM methanol, 0.1 mM ethanol, 0.1 mM ascorbic acid and 0.1 mM dopamine at a potential of 0.35 V in 0.1 M KOH.

0.1 M KOH. The concentration of glucose is usually greater than 30 times than the concentration of interfering species in body fluids.^{50,60} The current responses for the interfering species were found to be 0.352% for dopamine and 0.8% for ascorbic acid in the case of CuO microspheres. The comparison was made with the amperometric currents obtained for glucose whose concentration levels are 30 times more compared to interferents. We also checked the interference of methanol and ethanol and it was found to be 0.116% for methanol, 0% for ethanol. So the percentage of interference was found to be less than 1% for CuO microspheres. But for dendritic CuO the interference observed was above 1% for methanol, 1.28% for dopamine, 0.66% for ascorbic acid, 1.13% for methanol, and 0.006% for ethanol. It can be concluded that the interference is negligible. Workability of the modified electrode was tested by spiking a known concentration of glucose into 15 mL 0.1 M KOH solution and the recovery of the sample was calculated from the current response, sample recovered was found to be greater than 95% (SI table-1).⁵⁹ This shows that the CuO microspheres/GC electrode discussed in this study shows excellent sensitivity and selectivity for glucose oxidation at low over potential.

Conclusions

We have shown a simple and cost effective method to modify glassy carbon surface using CuO microspheres in order to find a potential application in direct electrochemical oxidation of glucose in alkaline medium. Cu was deposited on glassy carbon surface using galvanostatic method. These electrodes after conversion to corresponding hydroxide layer exhibit good electrocatalytic behavior for glucose oxidation in alkaline medium. Morphological characterization done using SEM, shows that CuO have spherical appearance with micrometer dimension. The overpotential for glucose oxidation was reduced by 200 mV for CuO microspheres when compared with dendritic appearance. CuO modified electrode shows good catalytic current response when compared with unmodified and Cu deposited electrode. A possible mechanism involving hydroxide-oxy-hydroxide redox couple for the oxidation was proposed. Also we have validated the modified electrode for sensing of glucose in human blood serum and analytes of microbial fuel cells and the results show good agreement with the standard value. The sensitivity of detection was 709 $\text{mA M}^{-1} \text{cm}^{-2}$. Compared to other non-enzymatic electrode our work shows good sensitivity with lower operation potential. The response time was less than 7 s. More over this electrode shows high reproducibility and stability. So this shows that CuO/GC modified in this way can be applied

as electrocatalyst for glucose fed fuel cell applications and for invitro sensing of glucose.

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