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Sopit Phetsang

Chiang Mai University

Pinit Kidkhunthod

Synchrotron Light Research Institute

Narong Chanlek

Synchrotron Light Research Institute

Jaroon Jakmunee

Chiang Mai University

Pitchaya Mungkornasawakul

Chiang Mai University

Kontad Ounnunkad (✉ suriyacmu@yahoo.com)

Chiang Mai University

Research Article

Keywords: electrochemically, copper, electrode, serum

Posted Date: March 4th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-264254/v1>

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Version of Record: A version of this preprint was published at Scientific Reports on April 29th, 2021. See the published version at <https://doi.org/10.1038/s41598-021-88747-x>.

An electrochemically reduced copper/reduced graphene oxide film modified electrode for sensitive non-enzymatic glucose detection in human serum

Sopit Phetsang^{1,2#}, Pinit Kidkhunthod³, Narong Chanlek³, Jaroon Jakmunee^{1,4,5}, Pitchaya Mungkornasawakul^{1,4,6}, and Kontad Ounnunkad^{1,4,5,7*}

¹Department of Chemistry, Faculty of Science, Chiang Mai University, Chiang Mai, 50200, Thailand

²The Graduate School, Chiang Mai University, Chiang Mai, 50200, Thailand

³Synchrotron Light Research Institute (Public Organization), Nakhon Ratchasima, 30000, Thailand

⁴Center of Excellence for Innovation in Chemistry, Faculty of Science, Chiang Mai University, Chiang Mai, 50200, Thailand

⁵Research Center on Chemistry for Development of Health Promoting Products from Northern Resources, Chiang Mai University, Chiang Mai, 50200, Thailand

⁶Environmental Science Research Center (ESRC), Faculty of Science, Chiang Mai University, Chiang Mai, 50200, Thailand

⁷Center of Excellence in Materials Science and Technology, Chiang Mai University, Chiang Mai, 50200, Thailand

[#]Present address: National Institute of Technology (KOSEN), Nagaoka College, 888 Nishikataki-machi, Nagaoka-shi, Niigata, 940-8532, Japan

*Emails: kontad.ounnunkad@cmu.ac.th, suriyacmu@yahoo.com

ABSTRACT

Numerous studies suggest that modification with functional nanomaterials can enhance the electrode electrocatalytic activity, sensitivity, and selectivity of the electrochemical sensors. Here, a highly sensitive and cost-effective disposable non-enzymatic glucose sensor based on copper(II)/reduced graphene oxide modified screen-printed carbon electrode is demonstrated. Facile fabrication of the developed sensing electrodes is carried out by the adsorption of copper(II) onto graphene oxide modified electrode, then following the electrochemical reduction. The proposed sensor illustrates good electrocatalytic activity toward glucose oxidation with a wide linear detection range from 0.10 mM to 12.5 mM, low detection limit of 65 μM , and high sensitivity of 172 $\mu\text{A mM}^{-1} \text{cm}^{-2}$ along with satisfactory anti-interference ability, reproducibility, stability, and the acceptable recoveries for the detection of glucose in a human serum sample (95.6–106.4%). The copper(II)/reduced graphene oxide based sensor with the superior performances is a great potential for the quantitation of glucose in real samples.

Introduction

Electrochemical glucose sensor has been extensively used in the medical field, illustrated as two classifications based on enzymatic^{1,2} and non-enzymatic sensors^{3,4}. Although enzymatic based glucose sensor exhibits high specificity and sensitivity, the use of enzyme presents disadvantages, including high fabrication cost and poor stability. Additionally, the enzyme is environmentally sensitive to temperature, pH, humidity, and chemical substances, which can reduce its activity⁵. Therefore, the non-enzymatic glucose sensor has been rapidly developed in recent years in order to overcome some disadvantages of the enzymatic based glucose sensor⁶. Diverse nanostructured electrocatalysts toward glucose oxidation, including transition metals⁷⁻⁹, noble metals^{10,11}, bimetallic systems¹², and their nanocomposite based-carbon nanomaterials¹²⁻¹⁴ have been widely investigated for the construction of enzyme-free

electrochemical glucose sensors. Among these materials, some of the transition metals have been paid considerable attention because of their low cost, high conductivity, and good catalytic activity¹². Copper (Cu) is one of the most attractive materials and various kinds of Cu nanostructures, including Cu nanoparticles (CuNPs)^{8,15}, Cu nanowires (CuNWs)¹⁶, Cu oxide (CuO)¹⁷, and Cu(II) form^{5,18} have been demonstrated with their high electrocatalytic activity toward glucose oxidation. The glucose oxidation at the Cu electrode surface is highly dependent on the redox couple of Cu(II)/Cu(III) in alkaline medium, and the Cu(III) species act as an active oxidizer for the oxidation of glucose⁴. Furthermore, the nanocomposites based on nanocarbon materials; CuNPs/nitrogen-doped graphene (NGP)¹⁹, CuO NPs/NGP³, Cu₂O NPs/reduced graphene oxide (rGO)²⁰, CuO NPs/rGO¹³, CuO/graphene oxide (GO)²¹ and Cu(II)-doped carbon nitride/multiwalled carbon nanotube (MWCNT)⁵ illustrated excellent electrocatalytic activities. The use of nanocarbons as material supports can increase the surface area which effectively promote the high loading of electrocatalysts, resulting in the high reactivity and high sensitivity toward glucose oxidation^{13,22}. Nevertheless, the preparation of these nanocomposites is a complicated task which may require the instrument, skill, and strong reducing agents.

As aforementioned, we are interested in the fabrication of a sensitive electrochemical glucose sensor based on Cu(II)/rGO nanocomplexes prepared by the adsorption technique. Although rGO exhibits excellent electrical conductivity, high mechanical strength, and large surface area, it is insoluble and hard to be dispersed in the solvent because of its high hydrophobicity and strong van der Waals interaction^{23,24}. On the other hand, GO provides abundance of the oxygenated functional groups on the surface, offering hydrophilicity and a highly negative charge density²⁵, which can efficiently bind the heavy metal ions to form the metal complexes on the GO surface via strong electrostatic interactions and the coordination of metal ions to oxygenic functional groups²³. Therefore, the utilization of adsorptive property

of GO to adsorb Cu(II) ion and the electrochemical reduction of Cu(II)/GO have been proposed to synthesize the Cu(II)/rGO and surface-reduced Cu(0)/rGO complexes.

In this study, a facile preparation of Cu(II)/rGO complexes with respect to electrocatalytic oxidation of glucose is demonstrated. The GO modified SPCE is used as catalysts support for Cu(II) adsorption. After the complexation of Cu(II)/GO was formed on the surface of GO, the Cu(II)/GO modified SPCE was subsequently reduced by electrochemical technique to give the Cu(II)/rGO nanocomplexes that employed as a glucose sensing platform. A schematic diagram of the device fabrication is shown in Figure 1. The proposed sensor showed good electrocatalytic activity towards glucose. A fast electron transfer between the electrode and the electroactive species would enhance due to the high electrical property of rGO. Additionally, a wide linear range, a good sensitivity, and a low limit of detection (LOD) are observed, and the proposed sensor was applied for the determination of glucose in a biological sample.

Results

Optimal conditions for the fabrication of Cu(II)/GO modified electrodes. Under the adsorption period of 60 min, the concentration of Cu(NO₃)₂ (0.00–100 mM) in an adsorption solution was optimized to acquire the sensing platform with high electrocatalytic performance. To study the effect of Cu(II) concentration on the electrochemical property, cyclic voltammetry (CV) of each Cu(II)/GO modified electrode was performed over the potential range between –0.80 to 1.0 V in 0.20 M acetate buffer (pH 4.5) (supporting information, Figure S1(a)). The redox peak currents of Cu(II) increase accordingly and the peaks potential gradually shift negatively and positively with the increment of Cu(NO₃)₂ concentration from 0.25 to 100 mM. This behavior resulted from the presence of a large amount of electroactive species at the electrode surface. At higher concentrations of Cu(NO₃)₂, the amount of Cu(II) adsorbed on GO modified SPCE was also high. When the Cu(II) complexes are formed, more electrons are transferred led to the anodic and cathodic potential shifts their position to a more positive direction. In this work, the anodic peak current was considered for the optimization of Cu concentration. The correlation graph between anodic current response of Cu(II) and

concentration of $\text{Cu}(\text{NO}_3)_2$ is shown in supporting information, Figure S1(b) curve(a). The anodic peak current of $\text{Cu}(\text{II})$ increases markedly with increasing the concentration of $\text{Cu}(\text{NO}_3)_2$ from 0.25 to 25.0 mM, and the constant current value is subsequently observed at higher concentrations (50.0–100 mM). A raising concentration of $\text{Cu}(\text{II})$ from 0.25 to 25.0 mM can enlarge the adsorption of $\text{Cu}(\text{II})$ on the GO surface; however, at higher concentrations above 25.0 mM, the adsorption could be limited by the amount of GO on the electrode surface. The result indicates that the GO modified SPCE can be used to adsorb the $\text{Cu}(\text{II})$ onto the electrode surface. Moreover, the surface coverage (Γ) of the $\text{Cu}(\text{II})/\text{GO}$ modified electrode was calculated by integration of the anodic peak area using the following equation²⁶.

$$\Gamma = \text{peak area}/nFAv \quad (1)$$

where n is the number of electrons transferred in the electrode ($n = 2$), F is the Faraday constant ($96,487 \text{ C mol}^{-1}$), A is the surface area of the electrode ($7.07 \times 10^{-2} \text{ cm}^2$), and v is the scan rate (V s^{-1}). The estimated surface coverage of the modified electrode with the maximum uptake of $\text{Cu}(\text{II})$ is $5.20 \times 10^{-10} \text{ mol cm}^{-2}$, and the amount of $\text{Cu}(\text{II})$ in the GO-modified SPCE is of $4.90 \mu\text{mol g}^{-1} \text{ GO}$ ($\text{Cu}(\text{II}) = 3.68 \times 10^{-11} \text{ mol}$ and $\text{GO} = 7.5 \mu\text{g}$). Furthermore, the relationship between the $\text{Cu}(\text{NO}_3)_2$ concentration and the peak current of glucose oxidation at each $\text{Cu}(\text{II})/\text{GO}$ modified SPCE prepared from different $\text{Cu}(\text{NO}_3)_2$ concentrations is displayed in supporting information, Figure S1(b) curve(b). The peak current significantly increases when the amount of $\text{Cu}(\text{II})$ catalyst increase with adsorption concentration from 0.25 to 2.50 mM while the current response with insignificant change occurs at higher concentration (5.00–100 mM). This result suggests that an increase in the amount of $\text{Cu}(\text{II})$ provides more active sites, facilitating better electrooxidation of glucose at the electrode surface⁵. However, the electrocatalysis for glucose oxidation is not enhanced by a larger number of $\text{Cu}(\text{II})$ on the electrode surface. It is caused by the limitation of the electrode, which would involve the accessibility of glucose into catalytic $\text{Cu}(\text{II})$ centers inside the $\text{Cu}(\text{II})/\text{GO}$ nanostructure of the electrode²⁷. The $\text{Cu}(\text{II})/\text{GO}$ -modified SPCE prepared using 2.50 mM $\text{Cu}(\text{NO}_3)_2$ (a $\text{Cu}(\text{II})$ uptake = $1.44 \mu\text{mol g}^{-1} \text{ GO}$ ($\text{Cu}(\text{II}) = 1.08 \times 10^{-11} \text{ mol}$ and $\text{GO} = 7.5 \mu\text{g}$) and surface

coverage = 1.52×10^{-10} mol cm⁻²) exhibits sufficient electrocatalytic activity for the oxidation of glucose. Therefore, this concentration is considered to be the optimal concentration, and is further employed for the construction of the sensing platform in this work. In addition, Cu(II) sitting on the surface of GO is also depended on the adsorption time, which influences the electrocatalytic performance of the modified electrode (supporting information, Figure S2(a) and (b)). The adsorption time of 60 min is the optimal adsorption period in term of ability to catalyze glucose oxidation. The amount of GO could also affect the electrochemical property of the modified electrode (supporting information, Figure S3). In this work, a 3.0 mg mL⁻¹ GO dispersion solution for the fabrication of Cu(II)/GO complex exhibits the highest current response of glucose oxidation, which is considered to be the optimal GO concentration.

Characterization of morphological surface of modified electrodes. The field emission scanning electron microscope (FE-SEM) was employed to characterize the surface morphology of the modified SPCEs as shown in Figure 2(a-e). The surface morphology of the bare electrode (Figure 2(a)) significantly changes after modification with the GO. As shown in Figure 2(b), the entire electrode surface is covered by the GO nanosheet, and the typical crumpled and wrinkled nanosheet structure is clearly observed. The large surface area of GO could facilitate the adsorption of Cu(II), which can increase the active sites or catalytic centers on the electrode surface. Although, the Cu(II)/GO-modified SPCE surface has no obvious change from the GO-modified SPCE as shown in Figure 2(c), the existence of Cu element in the nanocomposite can be proved by the energy dispersive X-ray spectroscopic technique (EDS) and the EDS spectrum is showed in supporting information, Figure S4(c). The rGO-modified SPCE (Figure 2(d)) manifestly exhibits a pattern of wrinkled nanostructure and becomes more rough compared to the GO-modified SPCE. Also, more stacking and aggregation of rGO is observed which is the characteristic structure of rGO²⁸. The electrochemical reduction can eliminate the oxygenated functional group on GO surface, resulting in the formation of rGO due to the increased π - π

interaction between the rGO layer²⁹. Likewise, the morphology of Cu(II)/rGO nanocomplex has no change as compared with that of the rGO sheet as shown in Figure 2(e) and the remaining of Cu on the Cu(II)/rGO- modified SPCE is clearly observed in the EDS spectrum (supporting information, Figure S4(d)). This result is expected that the reduction of Cu(II)/GO at the electrode surface would give a high electroactive surface area and high conductivity, enhancing the electron transfer and the electrocatalytic activity.

Furthermore, the X-ray photoelectron spectroscopy (XPS) was employed to further analyze the element composition of Cu(II)/GO- and Cu(II)/rGO-modified electrodes. The XPS survey spectral region and illustration of the difference in the intensities of characteristic peaks are shown in Figure 3(a-c). The wide scan XPS spectra of Cu(II)/GO and Cu(II)/rGO platforms reveal the characteristic peaks at the binding energies approximately of 285.0, 532.5, and 932.5 eV corresponding to C 1s, O 1s, and Cu 2p, respectively (Figure 3(a)). The presence of C, O, and Cu contents can prove the successful adsorption of Cu(II) onto the GO/rGO surface. The peak intensities in the two spectra were different, especially the C 1s spectrum. As seen in Figure 3(b), the XPS C 1s spectrum of Cu(II)/GO exhibits four characteristic peaks at 284.5 (15.35%), 286.0 (42.47%), 287.7 (35.24%), and 289.3 eV (7.15%), which are corresponding to C–C/ C=C (sp^2 hybridized C atoms), C–O (epoxy or alcohol groups), C=O (carbonyl groups), and O=C–O (carboxyl groups) bonds, respectively³⁰. On the other hand, the XPS C 1s spectrum of Cu(II)/rGO obviously shows differences from those of Cu(II)/GO as shown in Figure 3(c). Five characteristic peaks at 283.0 (36.90%), 284.4 (20.68%), 286.1 (18.44%), 287.4 (15.59%), and 288.8 eV (8.39%) are belonging to C–C/ C=C, C–O, C=O, and O=C–O bonds, respectively. The peak intensities of oxygen functional groups, especially C–O, C=O, and O=C–O, significantly decrease, suggesting that most of oxygen functional groups on the GO surface were removed during the electrochemical reduction process. Furthermore, the peak intensities of sp^2 hybridization of C atoms distinctly increase, which it can be proved that the

GO was successfully reduced to be rGO under the optimal condition. Additionally, synchrotron-based X-ray absorption spectroscopy (XAS) technique was employed to determine the valence state of Cu in the prepared Cu(II)/GO- and Cu(II)/rGO-modified electrodes. The Cu K-edge X-ray absorption near-edge structure (XANES) spectra were recorded in transmission modes using a 4-element Si drift detector^{31,32}, as the result shown in Figure 3(d). From Cu K-edge XANES spectra, the edge energies of both Cu(II)/GO and Cu(II)/rGO nanocomposites are similar to the edge energy of Cu(II) ion standard sample (CuSO₄). As compare to the previous report³², our results also exhibit the related absorption peak to the Cu K-edge XANES spectrum of CuO referring to the Cu(II) species. The shoulder peak of Cu(II) (8995.0 eV) is clearly observed for both devices while the shoulder peaks of Cu metal (8981.3 eV) and Cu(I) (8979.9 eV) are not observed. This implies that most of Cu in nanocomplex is to be in an oxidation state of +2 (Cu²⁺), which is related with the presence of high proportion of Cu(II) species in the Cu 2*p* XPS spectra as shown in [supporting information, Figure S5](#). From the simulation of the XPS spectrum of Cu(II)/rGO-modified SPCE, it is agreed well with the presence of the three species (Cu(0) metal, CuO, and Cu(OH)₂ forms), suggesting two oxidation states of copper (0 and +2). These results could be indicated that the majority of copper deposited in the nanocomplexes is Cu(II) species, and a small number of Cu(0) can be found after electrochemical reduction process. The Cu²⁺ possibly coordinates with oxygenated functional groups of GO such as carboxylic group, carbonyl group, or hydroxyl group, forming the other species as CuO, Cu(OH)₂, or -COO-Cu (Cu(II) coordinating with -COOH) in the nanocomplexes providing the Cu(II) species in various forms^{25,27,33}. Also, the Cu(II) species probably remained from the physisorption of Cu²⁺ precursors on the graphenic carbon site due to metal interactions with π -electrons in the graphene layer^{33,34}. These adsorptive properties of GO contributed to having an abundance of Cu(II) species located in the nanocomposite. Furthermore, some Cu²⁺ in the nanocomplex can be reduced during the electrochemical

reduction to form Cu^0 or Cu nanoparticles³⁵; therefore, the electrocatalysis activity at the modified electrode could be involved by two forms of active species including Cu(0) and Cu(II), which are located in the proposed sensing platform.

Electrochemical activity and analytical performance of proposed sensing. The electrochemical activities of different modified electrodes, including the bare SPCE and GO-, Cu(II)/GO-, rGO-, and Cu(II)/rGO-modified SPCEs, were investigated in 0.10 M KCl containing 5.0 mM potassium ferricyanide ($\text{K}_3[\text{Fe}(\text{CN})_6]$). The CVs are shown in supporting information, Figure S6(a). A pair of well-defined redox peaks located at *ca.* 0.32 and 0.20 V (a peak-to-peak separation (ΔE_p) of *ca.* 0.12 V) for oxidation and reduction processes, respectively, corresponding to the quasi-reversible redox reaction of $[\text{Fe}(\text{CN})_6]^{3-/4-}$ is observed at the modified electrodes. The different interfacial structures significantly affect the electrochemical responses. As compared with a current of the bare SPCE (supporting information, curve a, Figure S6(a)), the redox current response is slightly lowered after the modifications with both GO and Cu(II)/GO onto the electrode surface (supporting information, curves b and c, Figure S6(a)). The GO has an abundance of oxygenate functional groups and shows low electrical property³⁶, which could increase the resistance of electron transfer kinetics at the electrode, resulting in the lower current response. In addition, a small electrical conductivity of Cu(II)/GO modified electrode might result from the ionic insulator layer and GO itself. On the other hand, the peak currents of redox probe significantly increase at the rGO modified SPCE, indicating that rGO enhances the electrochemical reversibility of the electrochemical process as shown in curve d (supporting information, Figure S6(a)). The oxygenic functionalities on GO surface can be eliminated by the electrochemical reduction process, forming homogeneous rGO. Interestingly, the Cu(II)/rGO modified SPCE (supporting information, Figure S6(a), curve e) exhibits the highest peak current and the additional redox peak of copper can be observed. Due to an excellent electrical property of Cu(II)/rGO complex,

it can induce the acceleration of the electrochemical reaction which can improve the sensitivity of the proposed electrode for the glucose detection.

Furthermore, The resistances of electron transfer at these modified electrodes were studied by electrochemical impedance spectroscopy (EIS) and Nyquist plots are shown in Figure S6(b) (supporting information). The modified electrodes exhibit similar characteristics impedance spectra consisting of a semicircle in the high-frequency region related to the electron transfer-controlled process and an inclined line in the low-frequency region corresponding to diffusion-controlled process^{37,38}. The equivalent circuit is shown in the inset of Figure S6(b) (supporting information), representing the resistance of the electrolyte solution (R_s), the charge transfer resistance (R_{ct}), the Warburg impedance (Z_w) resulting from the ions from electrolyte solution to the electrode, and the double layer capacitance (C_{dl})^{38,39}. The R_{ct} value, corresponding to the electrical properties of each modified electrode, can be quantified using the diameter of the semicircle in the Nyquist plots⁴⁰. The R_{ct} values of rGO- and Cu(II)/rGO-modified SPCEs (curves d and e, $R_{ct} = 120 \Omega$ and 100Ω , respectively) obviously decreased when compared to those of GO- and Cu(II)/GO-modified SPCEs (curves b and c, $R_{ct} = 500 \Omega$ and 556Ω , respectively). The Cu(II)/rGO nanocomplex modified SPCE shows the smallest semicircle, implying the lowest resistance of the electron transfer of the redox probe at the electrolyte/electrode interface². From the result, the Cu(II)/rGO complex displays a superior electroactivity due to the synergistic effect of rGO and copper (Cu(II) or Cu(0)) which can improve the conductivity, increase the electroactive surface area and accelerate the electron transfer within the sensing electrode.

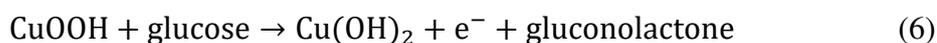
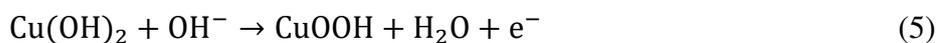
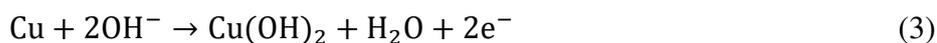
In order to verify the possibility of the proposed electrodes for glucose determination, the catalysis responses of different modified electrodes, including bare SPCE and GO-, and Cu(II)/GO-modified SPCEs, were examined in the 0.10 M NaOH solution in the absence and presence of glucose using CV. The CV was operated over the potential range from -0.2 to 0.8

V at scan rate of 50 mV s^{-1} and the CV curves are shown in Figure 4(a). It is seen that no oxidation peaks were observed in NaOH solution without addition of glucose for all electrodes in the potential scan (curve a-c, Figure 4(a)), suggesting that there is no electrochemical activity toward glucose detection⁴¹. After the addition of glucose, the GO- and Cu(II)/GO-modified SPCE (curve e and f, Figure 4(a)) distinctly exhibits an anodic peak current of glucose oxidation. Especially, Cu(II)/GO-modified SPCE shows apparent anodic current response starting at about 0.35 V with a shoulder peak of oxidation at 0.60 V, while no oxidation peak at the potential scan was observed at bare SPCE (curve d, Figure 4(a)). The increasing of anodic current is attributed to the electrooxidation of glucose with Cu(II) ion sitting on the electrode surface, which acts as an electrocatalyst and promotes a great potential for the electrochemical oxidation corresponding to Cu(II)/Cu(III). To increase the sensitivity of Cu(II)/GO modified SPCE for measuring glucose, the amperometric technique was employed to reduce the Cu(II)/GO of nanocomposite film on electrode. The electrochemical reduction process was performed in phosphate buffer solution (0.01 M, pH 7.4) at the constant potential of -1.50 V and the Cu(II)/rGO modified SPCE with an electrochemical reduction time of 50 s displays the best sensitivity, being use to prepare the sensing platform for the whole glucose detection experiment ([supporting information, Figure S7\(a\) and \(b\)](#)).

The CVs of bare SPCE and rGO-, and Cu(II)/rGO-modified SPCEs in the 0.10 M NaOH solution with the absence and presence of glucose (5.0 mM) are shown in Figure 4(b). It is obviously seen that no electrochemical peak response was observed at unmodified SPCE in both solutions (curves a and d, Figure 4(b)). As compared to the bare SPCE, CVs of rGO- and Cu(II)/rGO-modified SPCEs in NaOH solution exhibit a considerable enhancement in background current due to their higher conductivity and the observed current response of Cu(II)/rGO-modified SPCE (curve c, Figure 4(b)) is larger than that of the rGO modified SPCE (curve b, Figure 4(b)) due to its better conductivity of the nanocomplex. Furthermore, the peak

of Cu(II)/Cu(III) redox couple in the alkaline solution could not be observed at the Cu(II)/rGO-modified SPCE in the working potential ranges. Additionally, the current responses of rGO- and Cu(II)/rGO-modified SPCEs in basic glucose solution (curves e and f, Figure 4(b), respectively) obviously increase and the Cu(II)/rGO modified SPCE shows the highest electrocatalytic property with the current response difference of *ca.* 208 μA at the potential of +0.60 V (curve f, Figure 4(b)) due to the electrooxidation of glucose. Therefore, the good electrocatalysis of glucose at the surface of Cu(II)/rGO nanocomposite is attributed to the synergistic effect of highly conductive rGO and Cu(II), which regularly catalyzes glucose oxidation⁵. The comparison of electrocatalytic performances of different modified electrodes toward glucose oxidation, it is obviously seen that the Cu(II)/rGO-modified electrode exhibits the best electrocatalytic activity (supporting information, Figure S8). Although a well-defined peak of glucose oxidation at Cu(II)/GO-modified SPCE can be clearly observed, rGO- and Cu(II)/rGO-modified electrodes present very high current responses at the oxidation potential of +0.60 V. In addition, the response at the Cu(II)/rGO modified SPCE in a basic glucose solution is 15-fold higher than that of Cu(II)/GO-modified SPCE at this potential.

The mechanism of electrocatalytic oxidation of glucose on the Cu(II)/rGO modified electrode could be a multi-step process due to the presence of Cu(0) metal, CuO, and Cu(OH)₂ species in the nanocomplex. According to the previous reports^{4,41-44}, the commonly accepted mechanism of the glucose electrooxidation in the NaOH solution can be described as the following process. First, Cu is electrochemically oxidized to Cu(II) species in an alkaline medium, including CuO and Cu(OH)₂ (equations (2)–(3)). Subsequently, the Cu(II) species (CuO and Cu(OH)₂) are oxidized to highly oxidizing agents of Cu(III) species or CuOOH (equations (4)–(5)). Then, glucose is catalytically oxidized by Cu(III) species to produce gluconolactone and hydrolyzation to gluconic acid (equation (6) and (7)), while Cu(III) compounds return to Cu(II) compounds:



Although various forms of Cu can be observed on our platform confirming by the XPS spectrum, the formation of Cu(III) species is essential for the electrocatalytic glucose oxidation. The different forms of Cu including Cu(0) or Cu(II) in the nanocomposite can be electrochemically oxidized into Cu(II) and eventually into Cu(III) species (equation 2–5). Therefore, the electrocatalytic glucose oxidation on Cu-based modified electrode could be generated under the similar mechanism as represent in equation (6). The Cu(II)/rGO-modified SPCE was further employed as a sensing platform for the detection of glucose. The anodic peak current for sensing platform increases with increasing in the glucose concentration as shown in [supporting information, Figure S9](#). This mechanism promoted by Cu(III) species; therefore, more Cu (III) would be consumed at high concentration, leading to the large oxidation peak current⁴⁵. To study the electrochemical behavior of the Cu(II)/rGO-modified SPCE, the CV was performed at different scan rates from 10 to 150 mV s⁻¹ in 0.10 M NaOH solution containing 5.0 mM of glucose as shown in [supporting information, Figure S10](#). The anodic peak current of glucose oxidation at the modified electrode is directly proportional to the square root of the scan rate. This suggesting that an electrochemical oxidation process of glucose at the Cu(II)/rGO-modified SPCE is a diffusion-controlled mechanism.

The chronoamperometric technique is widely employed for the analysis of glucose because of its high sensitivity and this electrochemical experiment was performed under the potential of +0.50 V for 50 s. Although the CV of Cu(II)/rGO modified SPCE in glucose

solution illustrated the great anodic current of glucose oxidation at the potential higher than +0.50 V, this potential was selected to evaluate the different concentrations of glucose in this work in order to avoid the interference effect from other electroactive species co-existing in the sample. The chronoamperometric responses of proposed glucose sensor are shown in Figure 4(c) and (d). A Cu(II)/rGO based sensor responds to the glucose solution and reaches to the steady-state current within a short time (35 s). The result shows that the current response increases with increasing glucose concentration (Figure 4(c)). Furthermore, the plot of catalytic oxidation current versus glucose concentration is shown in Figure 4(d). The Cu(II)/rGO modified electrode exhibits a wide linear relation between current response and glucose concentration in the range from 0.10 to 12.5 mM and the corresponding linear regression equation is expressed as $\Delta I (\mu A) = 12.23[\text{Glucose}](\text{mM}) + 5.66$, with a correlation coefficient of $R^2 = 0.99$. From the slope of the calibration curve, the sensitivity and the limit of detection (LOD) values are estimated to be $172 \mu A \text{ mM}^{-1} \text{ cm}^{-2}$ and $65 \mu M$ ($n=3$), respectively. The Cu(II)/rGO based glucose sensor exhibits high sensitivity, low detection limit, and fast response, which is attributed to good electrocatalytic activity. Large surface area and high conductivity of rGO facilitate the electron transfer of the electrochemical probe at the electrode surface. A comparison of the electrochemical sensor performance between our Cu(II)/rGO and different modified electrodes from literatures is summarized in Table 1. It is found that the sensitivity of our sensor is better than those of nafion/CuNPs-nitrogen doped GP⁴⁶ and CuNPs-GP nanoflowers⁴⁷. Also, it provides a good dynamic range, which is wider than those of some previous reports, including CuNPs-MWCNTs⁴⁸, CuNPs-GP⁴⁹, and CuNPs-DMG⁸. Although Cu(II)-C₃N₄/ MWCNTs/GCE, GP/CuNPs/GCE, and CuNPs-MWCNTs/ GCE reveal very high sensitivity, some use higher operating potentials and also complex fabrication processes. From the literatures, they show ultralow detection limit values whilst our sensor presents higher LOD and is sufficient for clinical glucose detection. To evaluate the reproducibility of the proposed

sensor, the Cu(II)/rGO-modified electrodes were fabricated under the optimized condition. The electrocatalytic oxidation of glucose over seven individual electrodes was examined using CV measurement in 0.10 M NaOH containing 5.0 mM glucose and the obtained result is shown in [supporting information, Figure S11](#). The current responses of seven different Cu(II)/rGO-modified SPCEs give the relative standard deviation (R.S.D.) of 3.03%, demonstrating a good reproducibility.

Anti-interference ability during electrochemical oxidation of glucose is very important for the development of the electrochemical non-enzymatic glucose sensor. A number of electroactive species, including ascorbic acid (AA), uric acid (UA), and dopamine (DA) commonly coexisting with glucose in a human fluid sample, and they often interfere the determination of glucose⁴⁶. Therefore, the selectivity study of Cu(II)/rGO-modified electrode was subsequently investigated using chronoamperometry under the same condition above. Solutions of several interference species consisting of AA, DA, UA, sucrose (Su) KCl, and NaCl at the concentration of 0.10 mM with the presence and absence of 1.00 mM glucose were evaluated for studying the interference effect. The effect of presence of such interferences on current response of fabricated glucose sensor is shown in Figure 5(a). It is found that the interfering species, especially AA, UA, and Su, can be oxidized at the operating potential (+0.50 V). The current responses from the interferences oxidation are in a range of 1.84–14.80 %, which is significantly high; however, when they coexist in glucose solution (1.00 mM), slight changes from the current responses of glucose alone for AA (1.38%), DA (3.27%), UA (5.58%), Su (1.54%), KCl (1.54%), and NaCl (0.01%) are observed. Although the common coexisting metabolites resulted in the change of analytical signal, the ten-time higher concentration of glucose was employed in our study. It is well known that the normal range of glucose level in human blood is approximately of 3–8 mM⁴⁹ while the UA concentration of 0.13–0.46 mM¹ and AA concentration of 23 μ M⁵⁰ are found in the human blood. The

concentrations of these interference species are significantly lower than that of glucose in human blood. Furthermore, the concentrations of blood glucose are much higher in the diabetic patients. Therefore, the additional current responses from such interferences can be considered as being negligible and the interference species would not affect the analytical signal of the glucose oxidation in the clinical sample analysis. The result suggests that the Cu(II)/rGO modified electrode provides a satisfactory selectivity and is sufficient for the determination of glucose in real samples. Additionally, the stability test of the Cu(II)/rGO based non-enzymatic glucose sensor was investigated by chronoamperometric technique. After fabrication, the proposed sensors were stored at room temperature for 20 days, and the change in current response toward 2.5 mM glucose solution was measured as shown in Figure 5(b). It was found that the 87% current response of the initial response was obtained after storage for 20 days. The result indicates good stability of the developed sensor, which can be applied for glucose detection in the real samples. To prolong the electrode shelf life, the electrodes might require the storage in a proper atmosphere such as inert atmosphere, low temperature, low humidity atmosphere, and low pressure atmosphere or vacuum.

In order to study the practical feasibility of the developed sensor, the Cu(II)/rGO nanocomplex-modified electrode was subsequently employed for the determination of glucose in human serum sample. The human serum sample was diluted with 0.10 M NaOH for 50 times and then glucose stock solutions (0.05, 1.00, 2.50, 4.00, and 5.00 mM) were spiked into the diluted serum solution. The chronoamperometry was carried out to investigate the glucose oxidation at a Cu(II)/rGO modified electrode and each measurement was performed for three times ($n = 3$). As seen in Table 2, the percent recovery values for glucose determination by our electrode are in the range from 95.6% to 106.4% and the R.S.D. values are found to be less than 5%. This suggests that the proposed sensing platform offers a good selectivity and repeatability for determination of glucose in real sample matrix (human serum). Hence, it

demonstrated that the proposed modified electrode can be applied for the assay of glucose in real sample.

Discussion

we successfully prepared Cu(II)/rGO nanocomplex on the SPCE by a facile adsorption process and electrochemical reduction. The cost-effective Cu(II)/rGO based glucose sensor exhibits excellent electrocatalytic activity for glucose oxidation, which shows a wide linear range of 0.10–12.5 mM with a relatively low LOD of 65 μM ($n = 3$), rapid response time, and great sensitivity of 172 $\mu\text{A mM}^{-1} \text{cm}^{-2}$. The highly conductive rGO improves glucose oxidation over electrocatalyst (Cu(II)), resulting in a good sensitivity in the detection. Furthermore, the Cu(II)/rGO nanocomplex-modified electrode possesses satisfactory anti-interference ability, great reproducibility, and good stability, which is suitable for measurement of glucose in serum sample. Accordingly, this Cu(II)/rGO sensing platform can be considered as a potential electrode for the construction of glucose and other kinds of sensors in clinical diagnosis.

Methods

Chemicals and materials. All chemicals used were of analytical grade. Ascorbic acid (AA, 99%), disodium hydrogen phosphate (Na_2HPO_4 , 99.5%), dopamine (DA, 99%), D-(+)-glucose (Glu, $\geq 99.5\%$), uric acid (UA, $\geq 99\%$), L-potassium chloride (KCl, 99.5%), sodium chloride (NaCl, 99.5%), sodium dihydrogen phosphate (NaH_2PO_4 , 99.5%), and human serum from human male (AB plasma, USA origin) were purchased from Sigma-Aldrich, Singapore. Carbon ink was purchased from Acheson, Singapore. Natural graphite ($\geq 98\%$) was purchased from Bay Carbon Inc., USA. Sucrose (Su, extra pure), sodium hydroxide (NaOH, 98%), and copper (II) nitrate ($\text{Cu}(\text{NO}_3)_2$, 99.5%) were purchased from Loba Chemie, India. Potassium ferricyanide ($\text{K}_3[\text{Fe}(\text{CN})_6]$, $\geq 99\%$), and diethylene glycol monobutyl ether ($\text{HOCH}_2\text{CH}_2\text{OCH}_2\text{CH}_2\text{O}(\text{CH}_2)_3\text{CH}_3$, $\geq 98.0\%$) were obtained from Merck, Germany.

Deionized water (DI water, 18.2 M Ω cm at 25 °C) was collected from a purification system (Millipore systems, USA).

Apparatus and instrumentation. Electrochemical measurements, i.e., cyclic voltammetry (CV) and chronoamperometry, were carried out with an EmStat3 potentiostat (PalmSens, Netherlands). Home-made screen printed carbon electrode (SPCE) with a diameter of 3.0 mm was employed as a working electrode (WE). The SPCEs were prepared following the optimal condition as reported by Reanpang *et al.*⁵¹ and the procedure is displayed in the supporting information. The platinum wire was employed as a counter electrode (CE), and the silver/silver chloride (Ag/AgCl, 3 M NaCl) from BASi (USA) was used as a reference electrode (RE). Electrochemical impedance spectroscopy (EIS) measurement was performed by Autolab type PGSTAT302N (Metrohm, Netherland). The electrochemical experiments were carried out at room temperature. A field-emission scanning electron microscope (FE-SEM, JEOL JSM-6335F) combined with energy dispersive X-ray spectroscopy (EDS) was employed for studying the surface morphology and elemental analysis of the electrode surfaces. In addition, synchrotron-based X-ray absorption spectroscopy (XAS) technique was employed to determine the valence state of Cu on the modified electrodes. Also, the X-ray photoelectron spectroscopy (XPS) technique was employed to analyze elemental composition of prepared electrodes. Both experiments were measured at the SUT-NANOTEC-SLRI XAS Beamline (BL5.2) and the SUT-NANOTEC-SLRI XPS Beamline (BL5.3) at the Synchrotron Light Research Institute (Public Organization), Thailand.

Preparation of Cu(II)/rGO modified electrodes. Graphene oxide (GO) was prepared according to a modified Hummers method⁵². The GO powder was dispersed in DI water (3.0 mg mL⁻¹) using the ultrasonic cleaner for 2 h. Then, 2.5 μ L of GO solution was dropped on the plasma-treated SPCE, and the modified SPCE was dried in the air. The GO-modified SPCE was subsequently immersed in 2.5 mM Cu(NO₃)₂ aqueous solution for 60 min to adsorb the

copper(II) ion (Cu(II)). After that, the modified electrode was rinsed with DI water several times and dried at room temperature to obtain Cu(II)/GO modified electrode. The Cu(II)/GO film on the electrode surface was reduced by amperometric technique, at the constant potential of -1.50 V in phosphate buffer solution (0.01 M, pH 7.4) for 50 s. Then, the modified electrode was rinsed and dried to give the Cu(II)/rGO modified SPCE. In addition, the concentration of $\text{Cu}(\text{NO}_3)_2$ (0.25–100 mM), the concentration of GO (1.0–5.0 mg mL^{-1}), the adsorption time and electrochemical reduction time were investigated in order to achieve the best electrocatalytic activity.

Electrochemical measurement. To study the electrochemical behavior and the electron transfer of different modified electrodes, the cyclic voltammograms (CVs) of bare SPCE and the GO-, Cu(II)/GO-, rGO-, and Cu(II)/rGO- modified SPCEs were evaluated in 0.10 M KCl electrolyte solution containing 5.0 mM $\text{K}_3[\text{Fe}(\text{CN})_6]$. The CV was performed by scanning the applied potential from 0.80 to -0.40 V at a scan rate of 50 mV s^{-1} . Furthermore, the electrocatalytic activity for glucose oxidation of different modified SPCEs was investigated using CV. The study of glucose electrooxidation was performed in 0.10 M NaOH solution containing 5.0 mM of glucose by CV under scanning potential from -0.20 to 0.80 V at a scan rate of 50 mV s^{-1} . Moreover, the effect of scan rate on the electrochemical response at Cu(II)/rGO-modified SPCEs was investigated. In order to study the electrocatalytic activity of Cu(II)/rGO-modified SPCEs obtained from different reduction times, the amperometry was conducted for the successive addition of glucose solution into 0.10 M NaOH under stirring at a constant applied potential of $+0.60$ V. Additionally, the Cu(II)/rGO-modified SPCEs were applied to determine glucose using chronoamperometry. The experiment was performed in 0.10 M NaOH with various concentrations of glucose (0.10–12.5 mM). The working potential of $+0.50$ V was applied to the electrochemical system for 50 s in order to reduce the interfering from common electroactive species in this work. To analyze the target molecule in serum

sample, the glucose was spiked into a 50-fold diluted solution of human serum sample, and the quantification of glucose was carried out under the same experimental condition. The preparation of glucose solution is presented in supporting information.

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Acknowledgements

S.P. thanks an award of the Science Achievement Scholarship of Thailand (SAST). This work was supported by Chiang Mai University (CMU) and CMU Mid-Career Research Fellowship program. The authors gratefully acknowledge the Graduate School (CMU), Research Center on Chemistry for Development of Health Promoting Products from Northern Resources, Center of Excellence for Innovation in Chemistry (PERCH-CIC), Center of Excellence in Materials Science and Technology and Department of Chemistry, Faculty of Science, CMU. The authors would like to thank the support from the Program Management Unit for Human Resources & Institutional Development, Research and Invitation, NXPO [Frontier Global Partnership for Strengthening Cutting-edge Technology and Innovations in Materials Science].

Author contributions

S.P. prepared the electrode, performed experiments, analyzed the data, and wrote the manuscript. P.K. and N.C. performed XPS and XAS experiments and analyzed the data. K.O. designed and supervised the research, analyzed the data, and wrote the manuscript. All the electrochemical measurements, SPCE fabrication, sample preparation, data analysis were performed with the help of J.J., P.M., and K.O. All authors discussed and reviewed the manuscript.

Competing interests

The authors declare no competing interests.

Figures

Figure 1.

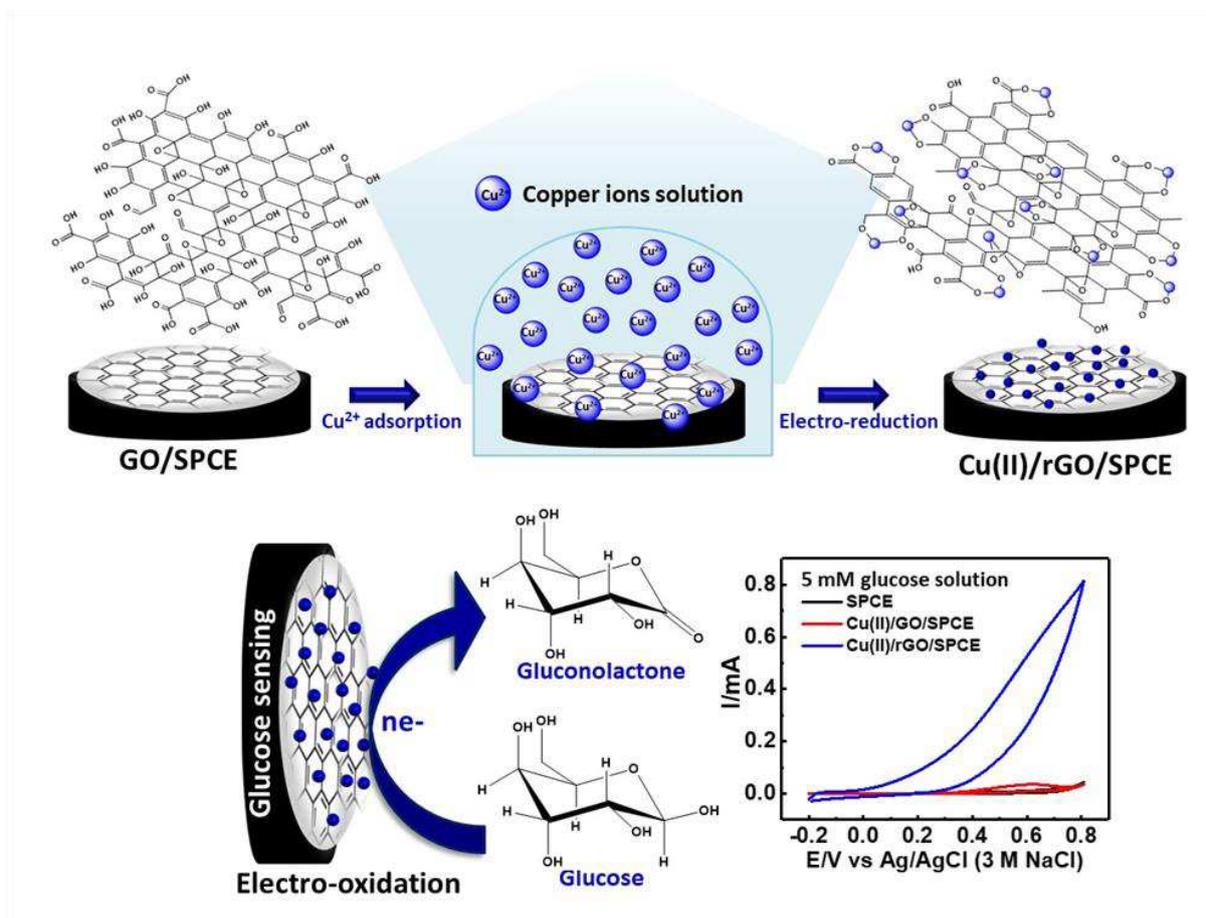


Figure 2.

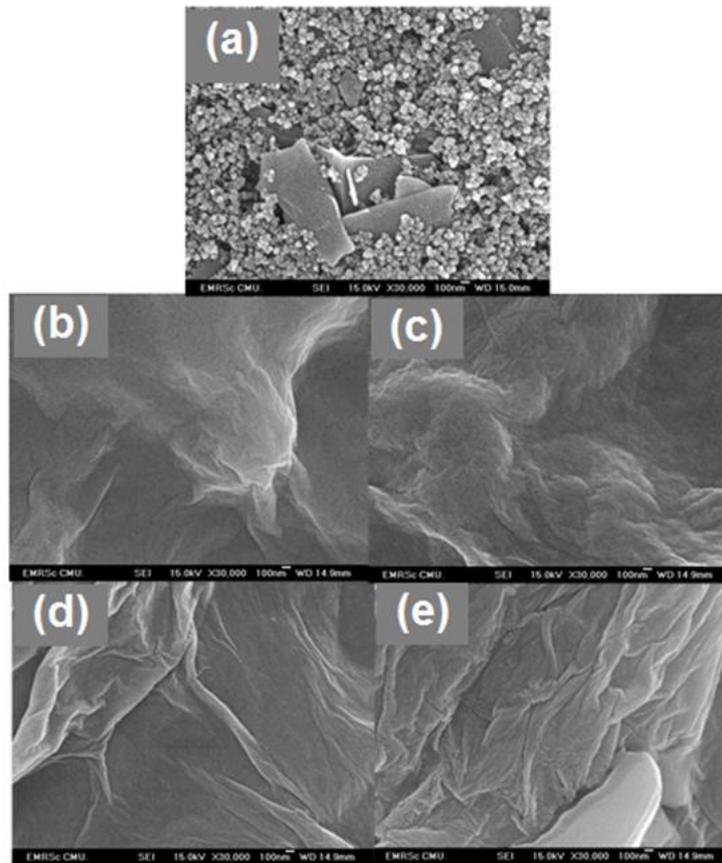


Figure 3.

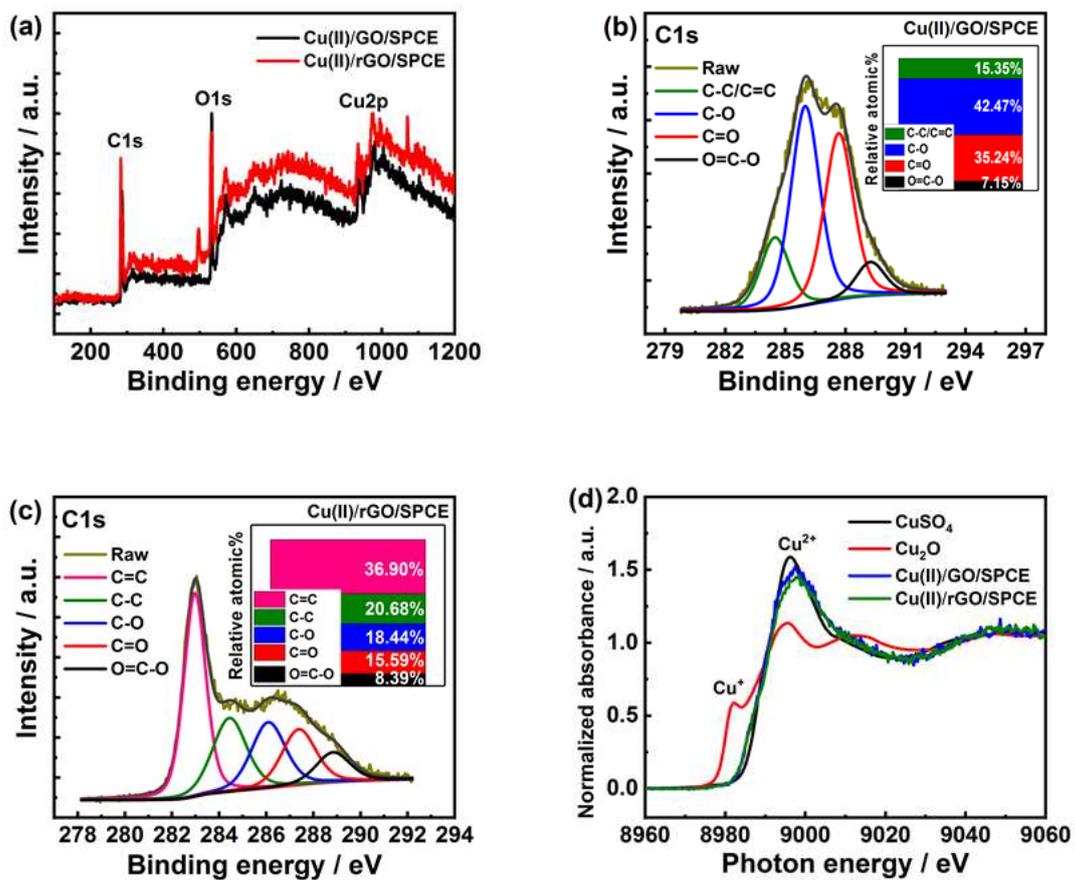


Figure 4.

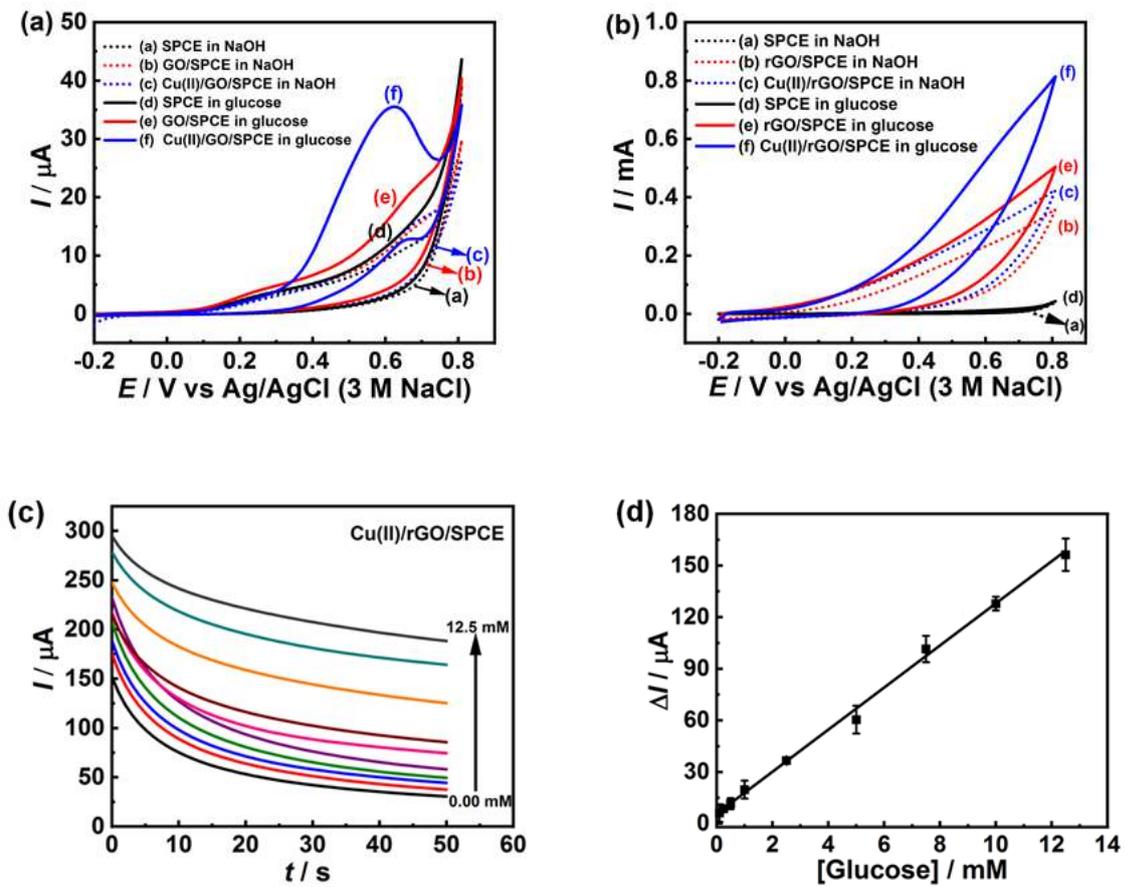


Figure 5.

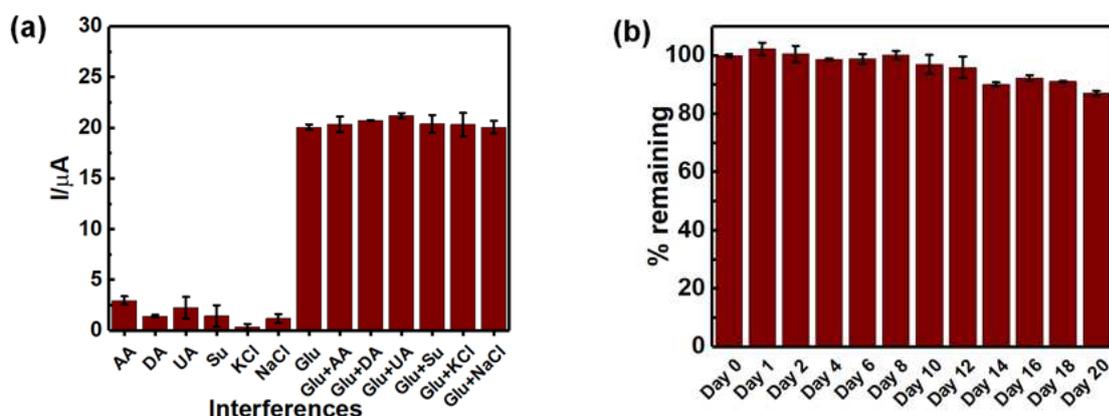


Figure Captions

Figure 1. Schematic illustration. Fabrication of non-enzymatic glucose sensor based on the Cu(II)/rGO nanocomplex-modified SPCE and its application in glucose detection.

Figure 2. FE-SEM images of bare SPCE (a) and GO- (b), Cu(II)/GO- (c), rGO- (d), and Cu(II)/rGO- (e) modified SPCEs.

Figure 3. XPS and XAS analysis. (a) Wide scan XPS spectra of Cu(II)/GO- and Cu(II)/rGO-modified electrodes, (b) C1s XPS spectrum of Cu(II)/GO-modified electrode, (c) C1s XPS spectrum of Cu(II)/rGO-modified electrode, and (d) normalized Cu K-edge XANES spectra of standard Cu(II) (CuSO_4), standard Cu(I) (Cu_2O), and Cu(II)/GO- and Cu(II)/rGO-modified electrodes.

Figure 4. Non-enzymatic detection of glucose. (a) CVs of different modified electrodes; bare SPCE, and GO-, and GO/Cu(II)-modified SPCE and (b) CVs of bare SPCE, and rGO-, and rGO/Cu(II)-modified SPCE in 0.10 M NaOH solution with the absence and presence of glucose at a scan rate of 50 mV s^{-1} . (c) Chronoamperograms from different glucose concentrations (0.10–12.50 mM) using Cu(II)/rGO-modified electrode at an operating potential of +0.50 V in 0.10 M NaOH solution and (d) the corresponding calibration graph.

Figure 5. Anti-interference study and stability test. (a) Interference study of glucose sensor; the responses of 0.10 mM AA, DA, UA, Su, KCl, and NaCl in the presence and absence of 1.0 mM glucose at the fabricated glucose sensor, applied working potential of +0.50 V and (b) stability test of Cu(II)/rGO based glucose sensor; current responses of 2.5 mM glucose detection.

Table 1. Comparison of electrocatalytic performances of Cu-based electrodes for electrochemical non-enzymatic glucose sensors

Electrodes	Potential (V)	Sensitivity ($\mu\text{A mM}^{-1} \text{cm}^{-2}$)	Linear range (mM)	Detection limit (μM)	Ref.
Cu(II)-C ₃ N ₄ /MWCNTs/GCE	+0.60	929	0.0005–12	0.35	5
DMG-CuNPs/GCE	+0.65	–	0.001–3.0	0.5	8
Nafion/CuNPs-N-GP/GCE	+0.50	48.13	0.004–4.5	1.3	46
CuNPs-GP nanoflowers/GCE	+0.30	11.3	0.005–0.90 and 0.90–11.0	1.0	47
CuNPs-MWCNTs/GCE	+0.55	1096	Up to 7.5	1.0	48
CuNPs/GP/GCE	+0.50	607	0.005–1.4	0.2	49
Cu(II)/rGO/SPCE	+0.50	171.95	0.10–12.5	65	This work

CuNPs: copper nanoparticles, Cu(II): copper(II) ion, GCE: glassy carbon electrode, MWCNTs: multi-walled carbon nanotubes, C₃N₄: graphitic carbon nitride, CuNPs-N-GP: copper nanoparticles decorated nitrogen-doped GP, DMG: dimethylglyoxime, SPCE: screen-printed carbon electrode

Table 2. Determination of glucose in human serum sample by our proposed Cu(II)/rGO film modified electrode

Glucose concentration (mM)		Recovery (%)	R.S.D (%)
Spiked amount	Found		
0.05	0.52±0.57	103.5	1.53
1.00	1.06±0.84	106.4	1.51
2.50	2.53±0.88	101.1	1.19
4.00	4.20±1.49	105.0	1.58
5.00	4.78±5.01	95.63	4.96

Figures

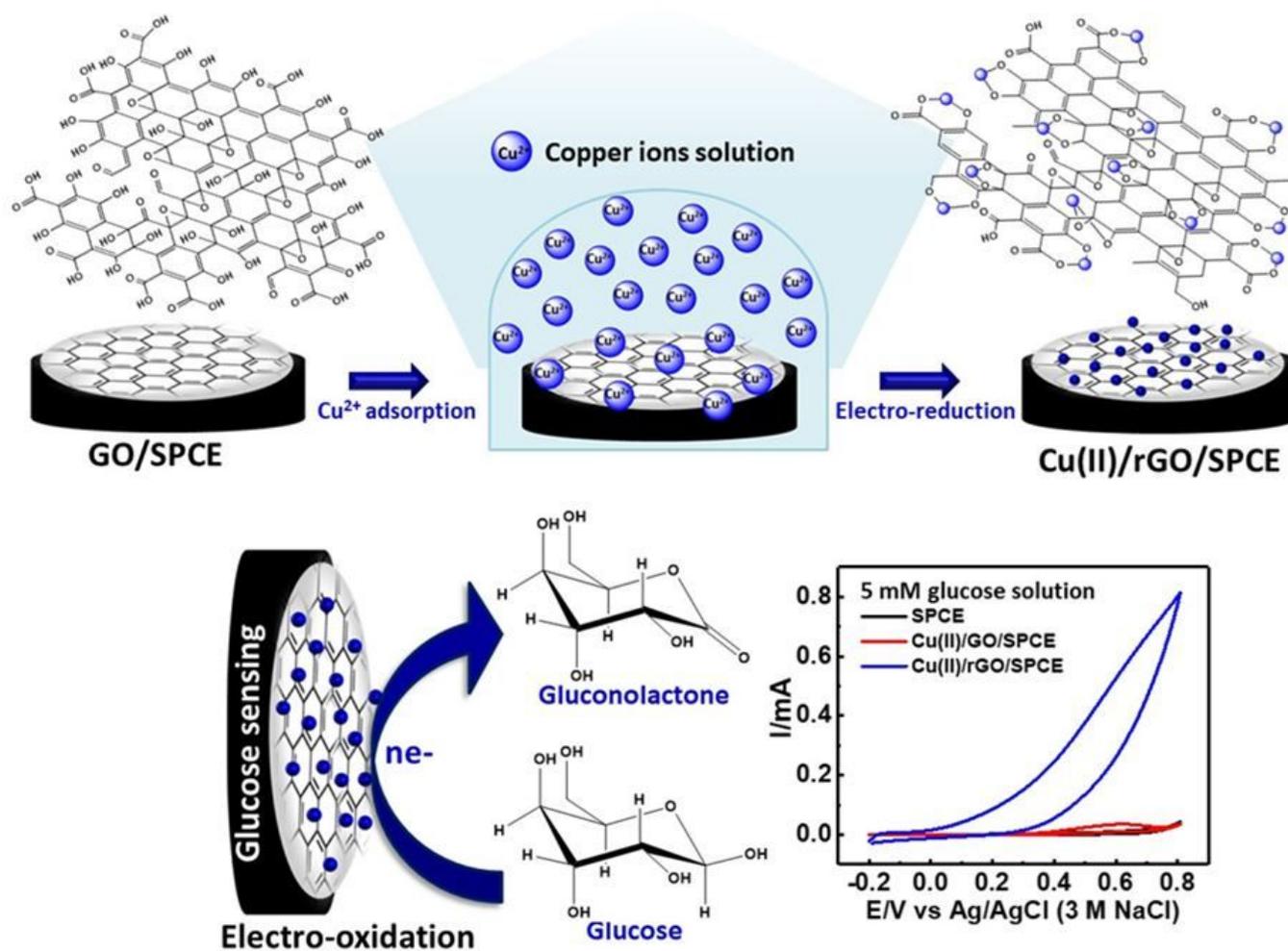


Figure 1

Schematic illustration. Fabrication of non-enzymatic glucose sensor based on the Cu(II)/rGO nanocomplex-modified SPCE and its application in glucose detection.

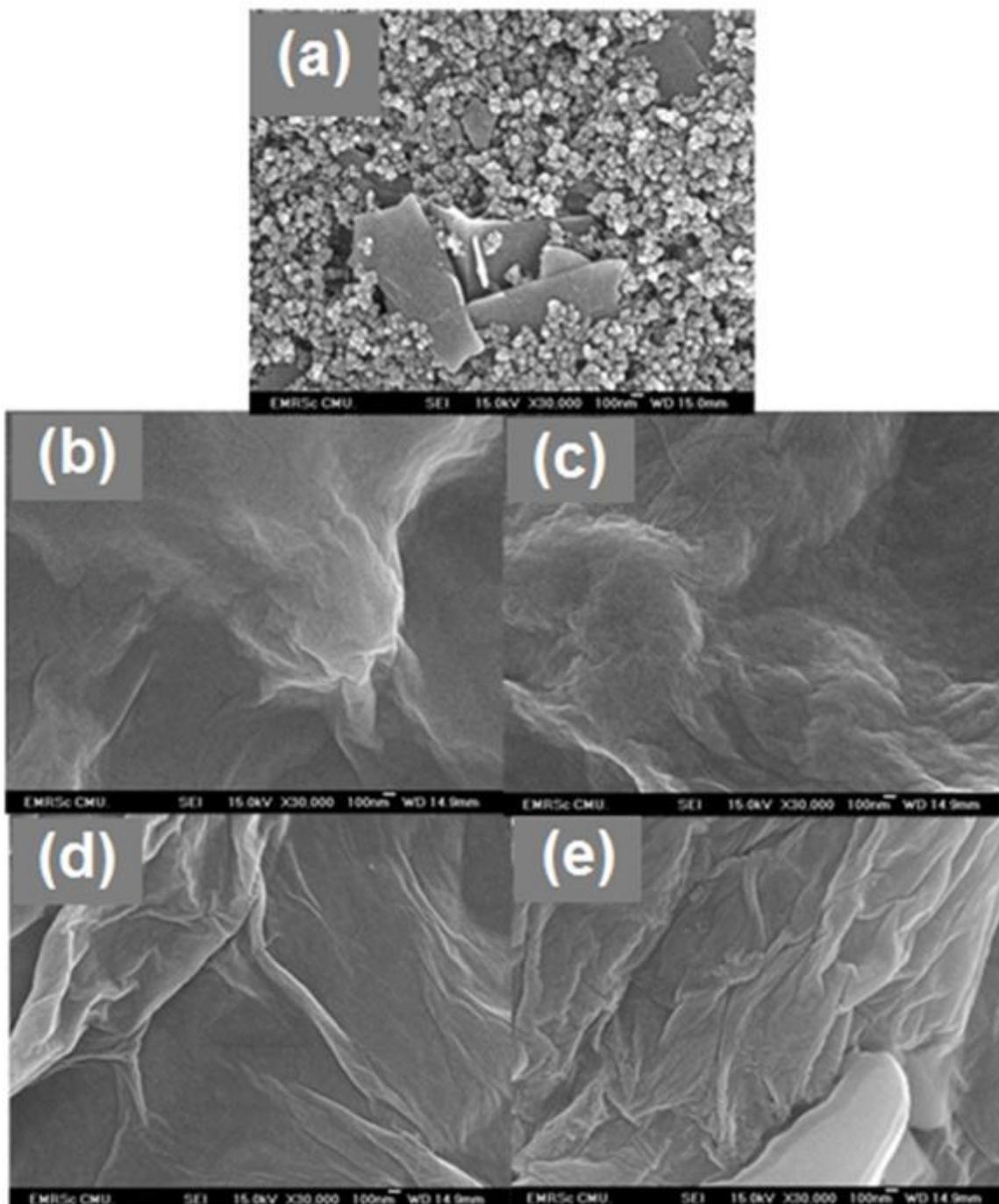


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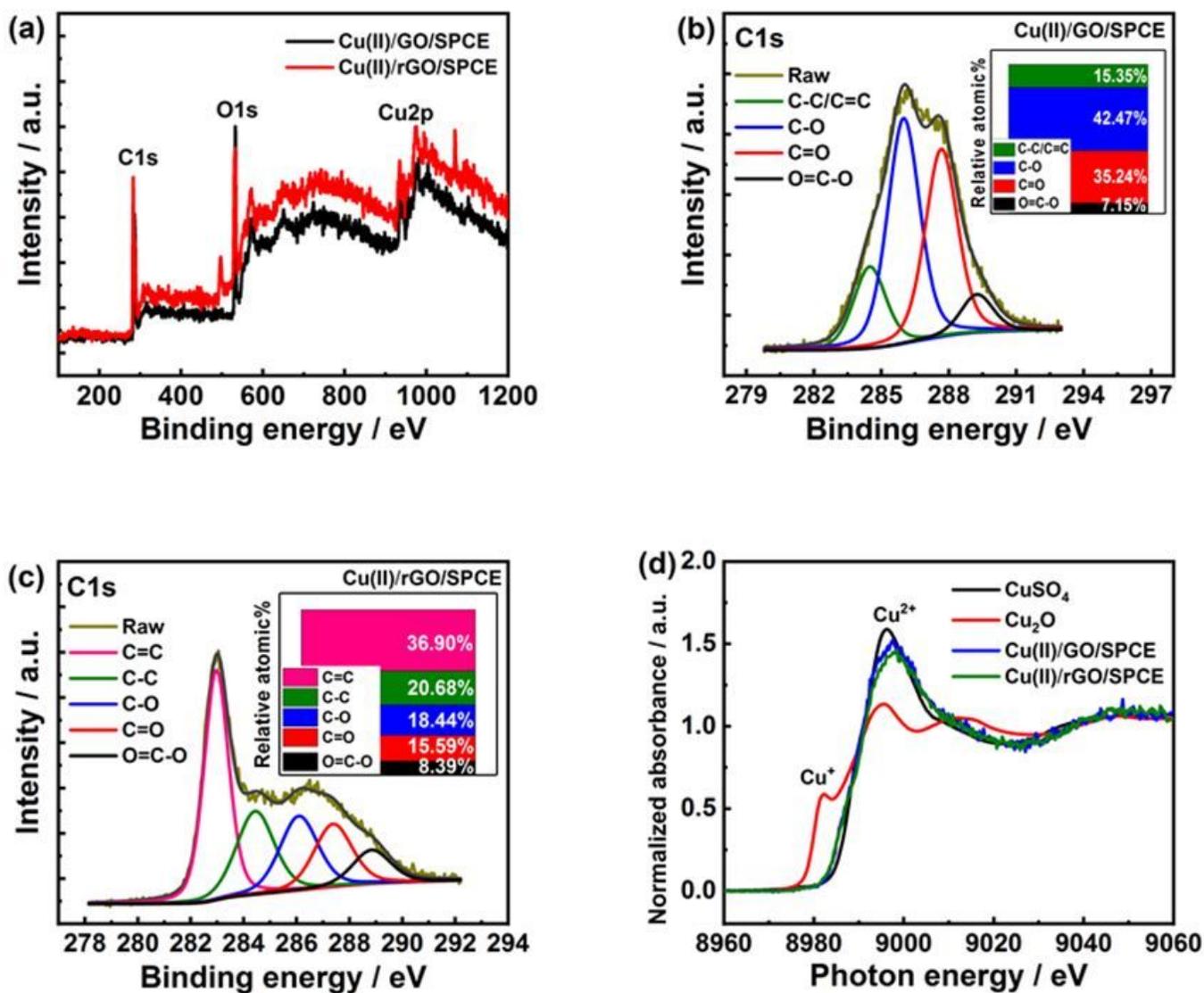


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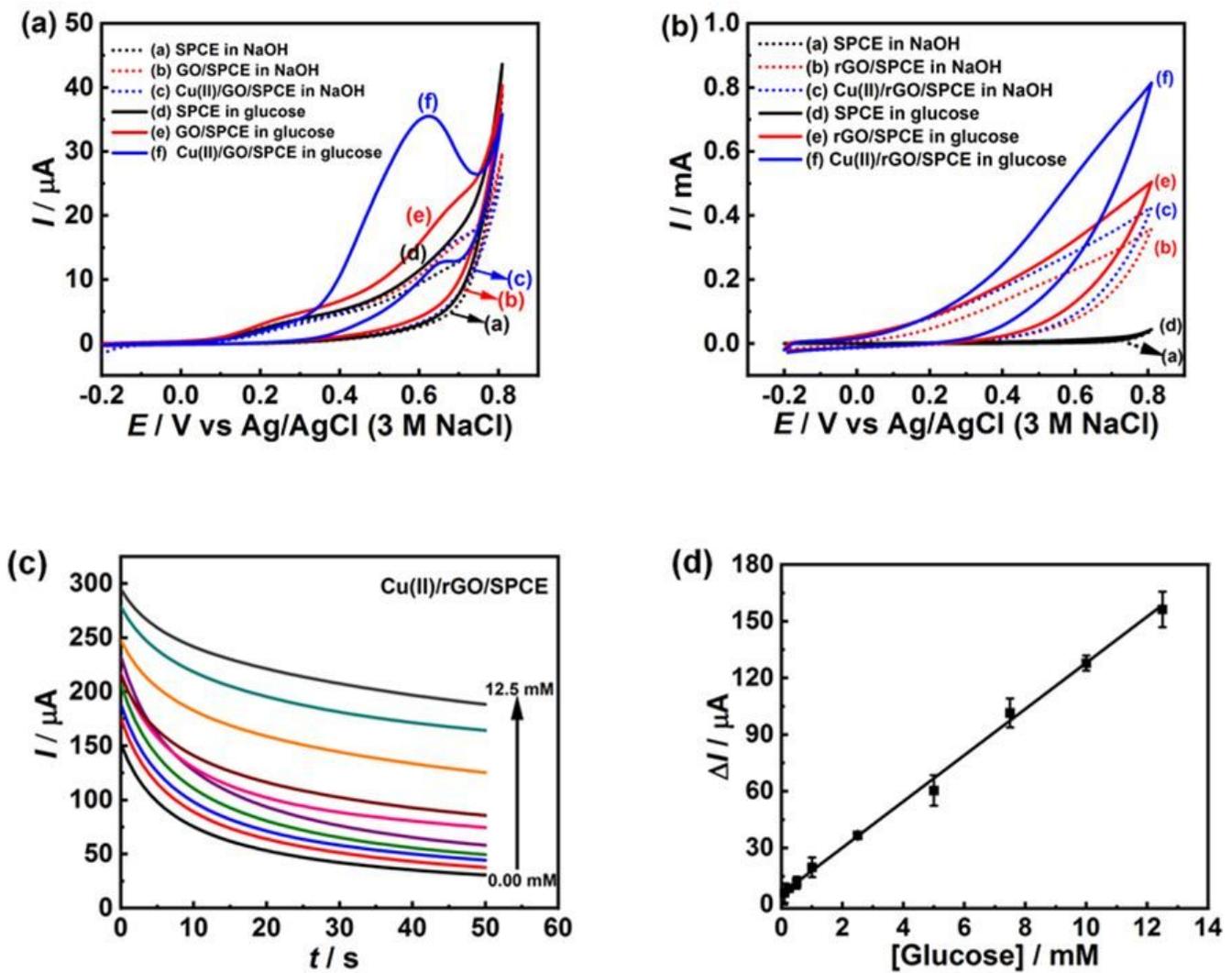


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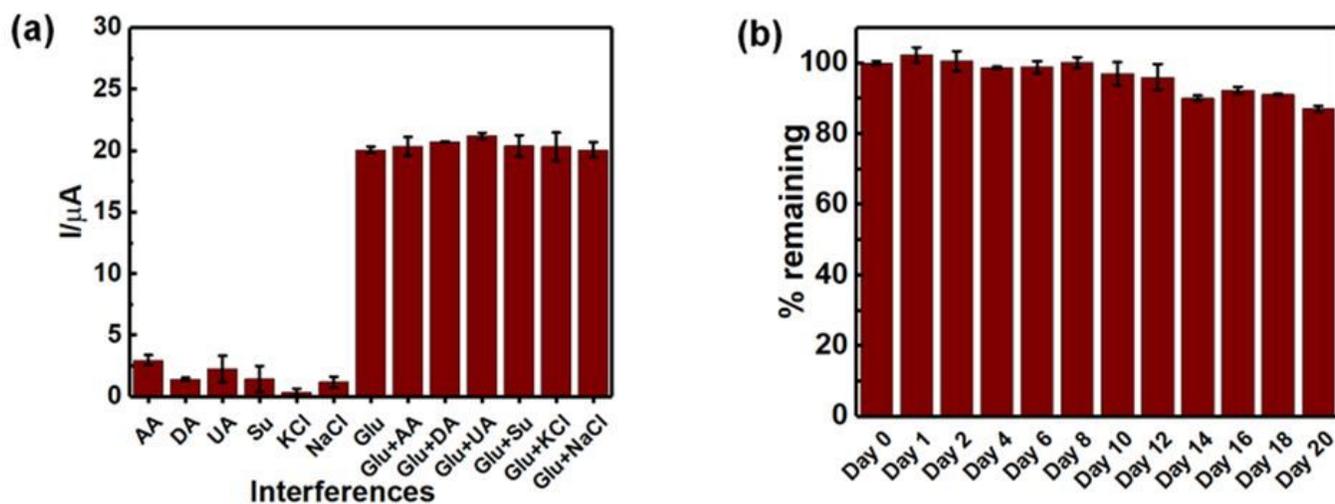


Figure 5

Anti-interference study and stability test. (a) Interference study of glucose sensor; the responses of 0.10 mM AA, DA, UA, Su, KCl, and NaCl in the presence and absence of 1.0 mM glucose at the fabricated glucose sensor, applied working potential of +0.50 V and (b) stability test of Cu(II)/rGO based glucose sensor; current responses of 2.5 mM glucose detection.

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