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Polymer-assisted *in Situ* Growth of Copper Nanoparticles on Graphene Surface for Non-Enzymatic Electrochemical Sensing of Glucose

Zonghua Wang [1*], *Jianfei Xia* [1], *Xiaoling Qiang* [1], *Yanzhi Xia* [1*], *Guoyu Shi* [1], *Feifei Zhang* [1,2], *Guangting Han* [1], *Linhua Xia* [1] *and Jie Tang* [2]

[1] Laboratory of Fiber Materials and Modern Textile, the Growing Base for State Key Laboratory, College of Chemical and Environment Engineering, Qingdao University, Shandong, 266071, P. R. China.
[2]National Institute for Materials Science, Sengen 1-2-1, Tsukuba 305-0047, Japan
[*]E-mail: wang_zonghua@yahoo.com.cn

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We describe a facile and effective polymer (polyacrylic acid, PAA)-assisted approach to synthesize a electrochemically active copper nanoparticle-graphene composite (CuNPs/PAA/GR) and also investigate its electrocatalytical activity towards oxidation of glucose in alkaline media. In the presence of PAA, the CuNPs/PAA/GR nanocomposite is prepared by *in situ* chemical reduction of the mixture of graphene oxide (GO) and Cu^{2+} ions using the sodium borohydride as a reductant. PAA acts as a bifunctional molecule for solubilizing GR into solution and chelating Cu^{2+} onto GR surfaces to facilitate the chemical deposition of CuNPs. The morphology of nanocomposite is characterized by scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Using the CuNPs/PAA/GR nanocomposite as a electrode modified material, excellent electrochemical performances are obtained, such as an enhanced electrocatalytic property, high sensitivity, excellent selectivity and satisfactory stability towards oxidation of glucose. This newly developed CuNPs/PAA/GR modified electrode is envisaged as a promising non-enzyme glucose sensor.

Keywords: Copper nanoparticles, Graphene, In situ, Polyacrylic acid, Non-enzyme glucose sensor.

1. INTRODUCTION

Graphene (GR), as a two dimensional and monoatomic thick building block of a carbon allotrope, has received much attention due to its fast electron transportation, high thermal conductivity, excellent mechanical flexibility and good biocompatibility [1-4]. GR has been applied in many

technological fields, such as electronic and photovoltaic devices [5], nanocomposites [6], field-effect transistors [7], energy storage [8] and solar cells [9]. Besides, it has a great potential applicability in the electrochemical sensors field [10-13].

Glucose is a key metabolite for living organisms, especially in the case of patients suffering diabetes. For the sake of instrumentation simplicity and operation processes, electrochemical detection methods have become more and more popular [14, 15]. And the development of novel sensors for fast, sensitive and reliable monitoring of glucose is highly required in medicine and clinical diagnostics fields. Since the first enzyme electrode reported by Clark and Lyons in 1962, glucose enzyme electrodes based on glucose oxidase (GOD) have been widely used to construct various amperometric sensors thanks to their high sensitivity, good selectivity, wide linearity and quick response time [16-19]. However, the enzymes-modified electrodes still have several disadvantages namely critical operational conditions, poor stability, high cost of enzymes, and complicated immobilization procedure [20-22]. Furthermore, the catalytic activity of GOD is easily affected by environmental conditions such as temperature, substrate concentration, pH value, heavy metal salt and humidity. Indeed, simple non-enzymatic glucose sensors are highly desirable with the advantages of stability, reproducibility, simplicity, and low cost [23]. Many efforts have been done to develop non-enzymatic sensors for glucose detection. For instance, electrode modified with noble metal nanoparticles (Au, Pt) [24, 25], metal alloys [26], and metal oxide [27]. Though some satisfactory results were obtained using the electrochemical technique, there is still room to investigate an anti-interference, cheap and excellent sensitive sensor for quantification of glucose. Noteworthy, Luo [28] recently reported CuNPs modified GR via electrodeposition method to create non-enzymatic glucose sensor showing the catalytic activity of CuNPs.

Herein, we developed a moderate and effective *in situ* growth method to load even more CuNPs on the surface of GR with the help of polymer molecule. In our experiment, GR surfaces were firstly noncovalently functionlized with polyelectrolyte (polyacrylic acid, PAA), and then was employed as a template to deposit CuNPs. The modified glassy carbon electrode (CuNPs/PAA/GR/GCE), investigated by cyclic voltammetry and chronoamperometry, showed the promising non-enzymatic electrochemical sensing of glucose.

2. EXPERIMENTAL

2.1 Reagents

Expanded graphite was provided by Qingdao Fujin graphite friendly (Qingdao, China). Glucose was purchased from Tianjin Guangfu Fine Chemical Research Institute (Tianjin, China). Poly acrylic acid (PAA) was purchased from Tianjin Kermel Chemical Reagent Factory (Tianjin, China). Dopamine (DA) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Ascorbic acid (AA) was obtained from Shanghai Reagent Factory (Shanghai China). Uric acid (UA) was purchased from Sinogharm Chemical Reagent. $CuCl_2$, $NaBH_4$, $KMnO_4$ and other reagents were of analytical-reagent grade and used without further purification.

2.2 Synthesis of the CuNPs/PAA/GR nanocomposite

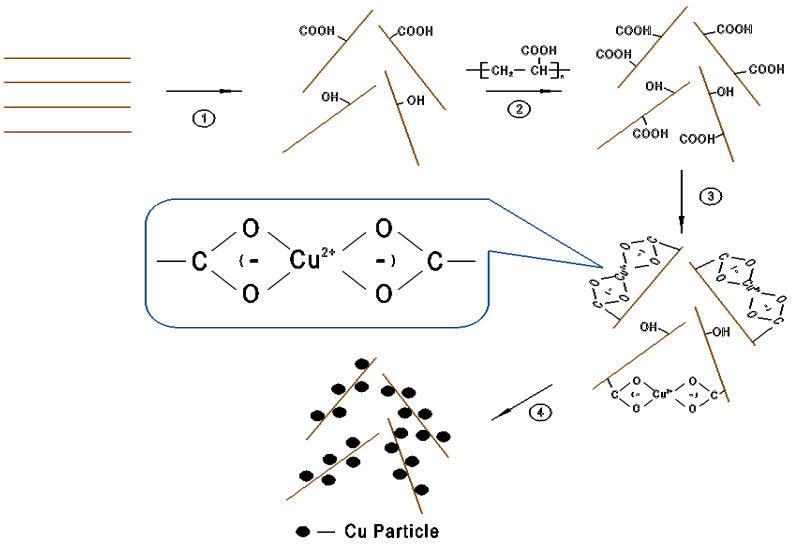

Figure 1. The schematic to synthesize CuNPs/PAA/GR nanocomposite.

Fig. 1 illustrates the schematic to synthesize CuNPs/PAA/GR nanocomposite: (1) exfoliation of oxidized graphite in water by sonication; (2) addition of PAA to GO solution; (3) addition of Cu^{2+} to PAA/GO solution; (4) chemical reduction of Cu^{2+}.

Oxidized graphite was firstly prepared from expanded graphite powder by a modified Hummers method [29]. 30 mg of as-prepared oxidized graphite powder was then dispersed in 30 mL of double-distilled water by ultrasonic cleaner till oxidized graphite completely dispersed. After 20 mL of PAA (15.0 wt %) was added, followed by 2 h successively sonication and then kept overnight at room temperature, PAA noncovalently functionlized graphene oxide (PAA/GO) was obtained. Subsequently, freshly prepared $CuCl_2$ (50 mL, 0.2 mol/L) solution was added into the above PAA/GO dispersion under vigorous agitation, and the mixture was stirred at room temperature for 2 h. Finally, $NaBH_4$ (0.4 mol/L) was added dropwise into the above mixture, followed by another 1 h stirring. As the reaction progressed, its color changed from blue to black brown, indicating the precipitation of CuNPs. The suspension was then centrifugalized, rinsed with CH_3OH and H_2O several times to remove the excess PAA, and dried under vacuum oven at 50℃. CuNPs/GR was fabricated following the same procedure for preparing CuNPs/PAA/GR without PAA.

2.3 Preparation of the CuNPs/PAA/GR/GCE modified electrode

A glassy carbon electrode (GCE) was successively polished with 0.3 μm and 0.05 μm alumina powders, and then ultrasonically cleaned in double-distilled water, and allowed to dry at room temperature. 1 mg of CuNPs/PAA/GR was fully dispersed in 1 mL of double-distilled water to get a suspension. CuNPs/PAA/GR modified GC electrode (CuNPs/PAA/GR/GCE) was prepared by dropping 5 μL of the resulting CuNPs/PAA/GR suspension onto the GC electrode surface and then subsequent evaporation of the solvent.

2.4 Electrochemical measurements

Cyclic voltammetry (CV) measurements were performed using a CHI 660C (Shanghai Chen-hua Instruments Co., Shanghai, China) electrochemical analyzer. A conventional three-electrode system was employed, involving a CuNPs/PAA/GR/GCE as the working electrode, a platinum wire as the counter electrode and a saturated calomel electrode (SCE) as the reference electrode. Electrochemical experiments were carried out with a scan rate of 100 mVs^{-1}, using a 100 mM NaOH solution as the supporting electrolyte. All solutions were prepared with double-distilled water and deoxygenated by bubbling high-purity nitrogen (N_2) for at least 10 min.

2.5 Morphology characterization

The morphology of the nanocomposite was examined using scanning electron microscope (SEM, Quanta-200, FEI, Netherlands) and transmission electron microscopy (TEM, JEM-2100F, Japan).

3. RESULTS AND DISCUSSION

3.1 Morphology characterization

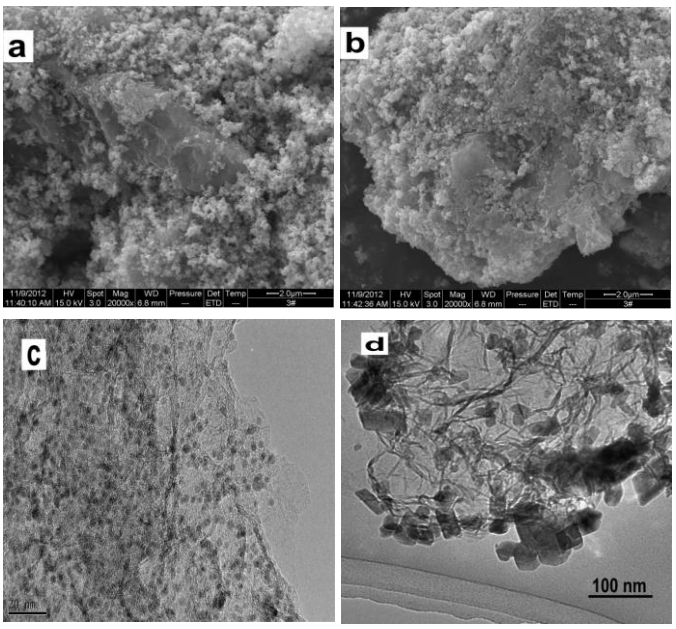

Figure 2. SEM images of CuNPs/PAA/GR (a), CuNPs/GR (b); TEM images of CuNPs/PAA/GR (c), CuNPs/GR (d).

Fig.2 (a, b) shows SEM images of CuNPs/PAA/GR (a) and CuNPs/GR (b), respectively. Compared with Fig.2b, it can be observed that more CuNPs are embedded throughout the PAA/GR

matrix (Fig.2a). TEM images of CuNPs/PAA/GR (c) and CuNPs/GR (d) shown in Fig.2 (c, d) more clearly present the difference. In the absence of PAA for synthesising the composite (Fig.2d), CuNPs are unevenly deposited and aggregated on the surface of GR, and these aggregates are composed of several CuNPs. In the presence of PAA (Fig.2c), CuNPs are more uniformly dispersed on the surface of GR with much smaller average diameter. The results demonstrate that the decoration of GR with PAA palys an important role for the CuNPs growing on the surface of GR. We conclude that the PAA acting as a bifunctional molecule can solubilize GR into solution and protect GR against gathering. Moreover, a large number of carboxyl groups in the PAA chains would coordinate more Cu^{2+} onto GR surfaces preferably.

3.2 Electrocatalysis of glucose at CuNPs/PAA/GR/GCE

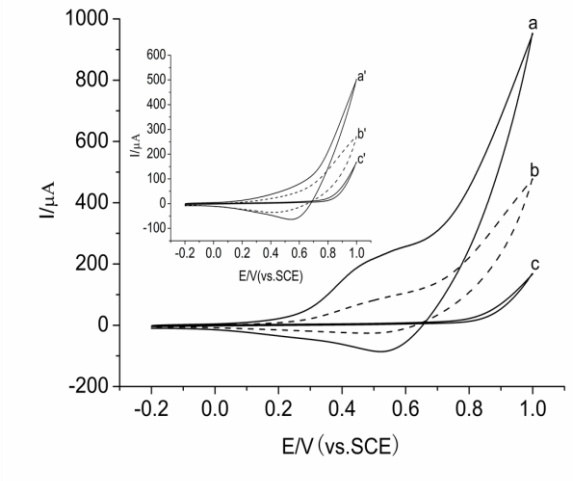

Figure 3. Cyclic voltammograms of CuNPs/PAA/GR/GCE (a, a'), CuNPs/GR/GCE (b, b') and GR/GCE (c, c') with and without of 2.0 mM glucose in 100 mM NaOH solution. Scan rate: 100 mVs^{-1}.

Fig.3 displays cyclic voltammograms of CuNPs/PAA/GR/GCE (a, a'), CuNPs/GR/GCE (b, b') and GR/GCE (c, c') with and without 2.0 mM glucose in 100 mM NaOH solution, respectively. Compared with Fig. 3a' (inset), upon adding 2.0 mM glucose, a dramatic increase of anodic current signal, corresponding to the irreversible glucose oxidation at the CuNPs/PAA/GR/GCE was observed in Fig. 3a. As illustrated in Fig. 3, the peak current for the oxidation of glucose at the CuNPs/PAA/GR/GCE (a) was much higher than that on the CuNPs/GR/GCE (b), which results from more CuNPs deposited on GR due to the exsitence of the polymer PAA. This suggests that plenty of CuNPs played a major role in the oxidation of glucose with its catalytic activity against glucose [30]. Whereas for the GR/GCE without Cu, whatever in the absence or presence of glucose, no peak was observed.

The effect of scan rate on the oxidation of glucose at the CuNPs/PAA/GR/GCE in 100 mM NaOH solution were also investigated by cyclic voltammetry. As shown in Fig. 4, the anodic peak current (I_{pa}) of oxidation of glucose intensified linearly as the scanning rate increased from 50 to 210

mVs^{-1}. A linear correlation (the inset, I_{pa} (μA)=152.0+0.5608 *v*, R=0.9984) was obtained between the peak current and scan rate, indicating that the electrochemical oxidation of glucose at the CuNPs/PAA/GR/GCE was controlled by the adsorption process [31].

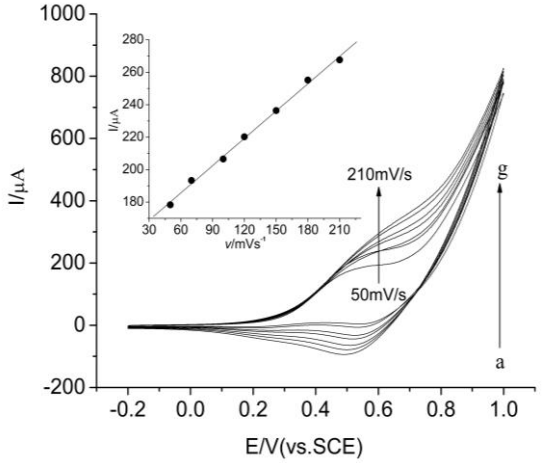

Figure 4. Cyclic voltammograms of 2.0 mM glucose at the CuNPs/PAA/GR/GCE with different scan rates of 50 mV/s~210 mV/s (a→g) in 100 mM NaOH solution. Inset is the plot of the peak current with the scan rate.

3.3 The effect of detection potential

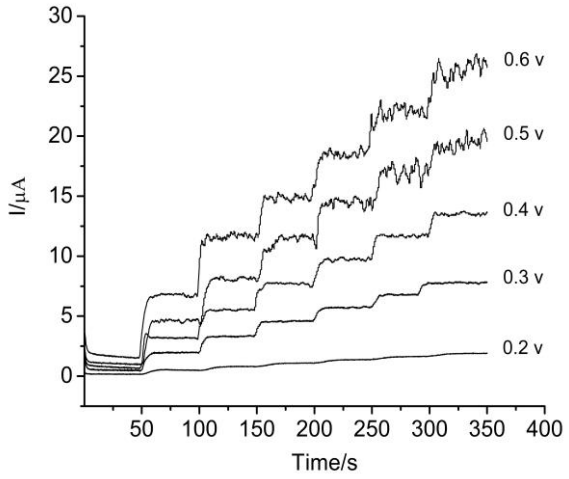

Figure 5. Current-time responses of CuNPs/PAA/GR/GCE at different potentials ranging from 0.20 to 0.60 V in 100 mM NaOH with addition of 40 μM glucose at 50 s interval.

The detection potential strongly affects the amperometric response. So, the optimal detection potential was tested ranging from 0.20 to 0.60 V by dropwise addition of 40 μM glucose into 100 mM NaOH solution at 50 s interval. As we can see from Fig. 5, when the detection potential was below

0.40 V, the response current of glucose increased slowly. However, when the detection potential was higher than 0.40 V (from 0.40 V to 0.60 V), the response current increased rapidly, but the background noise also raised. Considering that the best signal-to-noise ratio and the fact that high potential may oxidize many unwanted species or intermediate interfering, in our case, 0.40 V was selected as the optimal detection potential.

3.4 Amperometric response of the CuNPs/PAA/GR/GCE toward glucose

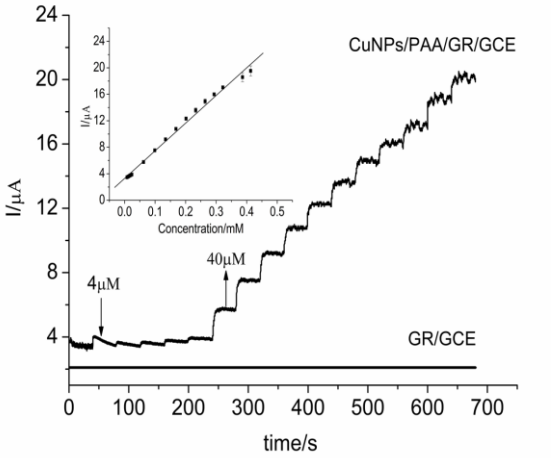

Figure 6. Current-time responses of CuNPs/PAA/GR/GCE and GR/GCE at +0.40 V with successive addition of certain concentration of glucose. (Inset) The plot of current response vs. glucose concentration at CuNPs/PAA/GR/GCE.

The typical current-time response curves of the CuNPs/PAA/GR/GCE and GR/GCE for successive addition of glucose are compared in Fig. 6. The GR/GCE showed almost no current response with the addition of glucose, whereas the CuNPs/PAA/GR/GCE yielded a significantly larger current response, which is consistent with the results of cyclic voltammetry (Fig.3). A well-defined, stable amperometric response could be observed at the CuNPs/PAA/GR/GCE with successive addition of certain concentration glucose into 100 mM NaOH at 40 s interval for 17 steps. In addition, an extremely attractive feature is reflected by its fast response time towards the oxidation of glucose. The calibration curve of glucose at the CuNPs/PAA/GR/GCE is shown in the inset of Fig. 6. The current response curve exhibited a linear dependence on glucose concentration I_{pa}(μA)=3.043+ 44.64 C (mM), with a correlation coefficient of 0.9993. The sensor displays a linear range of 0.3 μM to 0.6 mM glucose and the detection limit is 0.08 μM (at signal/noise=3).

The analytical performances of the proposed electrode and some reported electrode are listed in Table 1. It can be observed that the CuNPs/PAA/GR/GCE has the lowest detection limit for glucose sensing compared with other reported electrodes. This can contribute to PAA, which effectively promotes the uniform growth of CuNPs with much smaller size, consequently enhancing the electroactive surface area for glucose oxidation. Additionally, the cross-linking frameworks integrated into the stacked graphene layers accelerates the mass transportation, which further improves the

accessibility of glucose to the electroactive surface.

Table 1. Comparison of different modified electrodes for glucose determination.

Electrode materials	Linear range	Detection limit	Reference
Cu nanoparticles	1 μM to 5 mM	0.5 μM	32
Cu-Cu_2S nanocomposite	2 μM to 8.1 mM	0.1 μM	33
Cu/MWCNTs	0.7-3.5 mM	0.21 μM	30
CuNPs/GR	Up to 4.5 mM	0.5 μM	28
CuNPs/PAA/GR	0.3 μM to 0.6 mM	0.08 μM	This Work

3.5 Reproducibility, stability and anti-interference property of the CuNPs/PAA/GR/GCE

The reproducibility and stability of the sensor were evaluated. Five as-made electrodes were investigated to compare their amperometric current responses. The relative standard deviation (RSD) was 3.2 %, confirming that the preparation method was highly reproducible. The stability of the sensor was examined by measuring its current response to glucose over two weeks which only 4.5 % loss in the current signal, indicating its good stability.

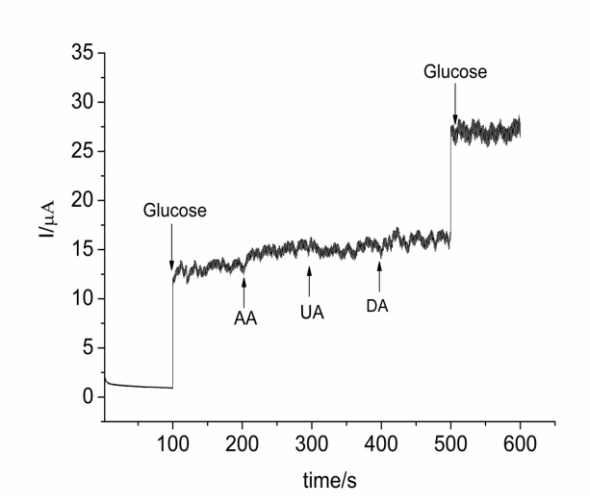

Figure 7. Interference test of the sensor in 100 mM NaOH at +0.40 V with 0.1 mM glucose and 0.01 mM interfering species as indicated.

One of the important analytical factor for an amperometric sensor is its ability to discriminate the interfering species. The specificity of the sensor was investigated using different interfering that normally co-exist with glucose in human blood serum. The normal physiological level of glucose in human blood is about 30 times of interfering species such as AA, UA and DA. In our work, the

interference experiments were carried out by addition of 0.1 mM glucose and 0.01 mM interfering species in 100 mM NaOH, shown in Fig. 7. The measured effects (RSD) of AA, UA and DA along with glucose at + 0.40 V were 1.2 %, 3.5 % and 2.6 %, respectively. It can be seen that the responses of CuNPs/PAA/GR/GCE did not show any significant change in the presence of interfering species. These results indicate that CuNPs/PAA/GR/GCE is highly specific to glucose even in the presence of several interfering species normally found in the biological samples.

3.6 Human serum samples measurement

The CuNPs/PAA/GR/GCE was used to determine glucose in human serum samples for testing its practical application. 100.0 μL of serum sample was added to 10.0 mL of 100 mM NaOH solution. Table 2 displays the determination results of five samples and the recovery. A glucose result of a diabetic people was as high as 11.1 mM, while it was only 3.9~5.7 mM for the four healthy people. The recovery was determined by addition of standard glucose to the serum samples. We can see that the excellent recoveries (≥95%) were obtained at the CuNPs/PAA/GR/GCE. The results, as shown in the table 2, confirm that the proposed method is suitable to the quantitative analysis of glucose.

Table 2. Determination of glucose in human serum samples

Sample	Concentration (mM)	Added (mM)	RSD (%)	Recovery (%)
1	3.9	0.2	4.2	95
2	4.4	0.2	3.5	96
3	5.0	0.2	1.8	98
4	5.7	0.2	2.3	97
5	11.1	0.2	4.4	98

4. CONCLUSIONS

In summary, a novel nanocomposite, CuNPs/PAA/GR, was fabricated by a facile and effective *in situ* chemical reduction of a mixture containing graphene oxide and Cu^{2+} ions assisted by polymer PAA. Characterization by SEM and TEM revealed that the CuNPs were well dispersed and embedded throughout the GR matrix. The novel non-enzymatic glucose sensor CuNPs/PAA/GR/GCE presented kinds of attractive analytical features such as high sensitivity, strong stability, good reproducibility, and excellent selectivity as well as quick response. Besides, the CuNPs/PAA/GR/GCE can be used as an amperometric sensor for routine analysis of glucose in human serum samples with satisfactory results. These experimental results indicate that the CuNPs/PAA/GR/GCE holds the prospect for effective non-enzymatic determination of glucose at low concentrations and detective limit, but with very high anti-interference ability.

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References

1. K. S. Novoselov, A. K. Geim, S. V. Morozov, D. Jiang, Y. Zhang, S. V. Dubonos, *Science.* 306 (2004) 666.
2. A. A. Balandin, S. Ghosh, W. Bao, I. Calizo, D. Tewelderhan, F. Miao, *Nano. Lett*. 8 (2008) 902.
3. C. Lee, X. Wei, J. W. Kysar, J. Hone, *Science*. 321 (2008) 385.
4. X. Y. Zhang, J. L. Yin, C. Peng, W. Q. Hu, Z. Y. Zhu, W. X. Li, C. H. Fan, Q. Huang, *Carbon*. 49 (2011) 986.
5. Z. Y. Liu, D. W. He, Y. S. Wang, H. P. Wu, J. G. Wang, *Synth. Met*. 160 (2010) 1036.
6. H. Kim, A. A. Abdala, C. W. Macosko, *Macromolecules*. 43 (2010) 6515.
7. S. Gilje, S. Han, M. S. Wang, K. L. Wang, R. B. Kaner, *Nano. Lett*. 7 (2007) 3394.
8. D. W. Choi, D. H. Wang, V. V. Viswanathan, I. T. Bae, W. Wang, Z. M. Nie, J. G. Zhang, G. L. Graff, J. Liu, Z. G. Yang, T. Duong, *Electrochem. Commun.* 12 (2010) 378.
9. X. L. Fang, M. Y. Li, K. M. Guo, Y. D. Zhu, Z. Q. Hu, X. L. Liu, B. L. Chen, *Electrochim. Acta*. 65 (2012) 174.
10. L. H. Tang, H. B. Feng, J. S. Cheng, J. H. Li, Chem. *Commun*. 46 (2010) 5882.
11. Y. Bo, H. Y. Yang, Y. Hu, T. M. Yao, S. S. Huang, *Electrochim. Acta*. 56 (2011) 2676.
12. Y. Wang, Y. M. Li, L. H. Tang, J. Lu, J. H. Li, *Electrochem. Commun*. 11 (2009) 889.
13. L. Q. Luo, L. M. Zhu, Z. X. Wang, *Bioelectrochemisty.* 88 (2010) 156.
14. Y. H. Dou, N. Bao, J. J. Xu, H. Y. Chen, *Electrophoresis.* 23 (2002) 3558.
15. J. Wang, Talanta. 56 (2002) 223.
16. L. C. Clark, C. Lyons, A. N. Y. Acad, *Science*. 102 (1962) 29.
17. B. A. Gregg, A. Heller, *Anal. Chem*. 62 (1990) 258.
18. Y. Liu, M.K. Wang, F. Zhao, Z. A. Xu, S. J. Dong, *Biosens. Bioelectron*. 21 (2005) 984.
19. C. S. Shan, H. F. Yang, J. F. Song, D. X. Han, A. Ivaska, L. Niu, *Anal. Chem.* 81 (2009) 2378.
20. H. H. Weetall, W. H. Pitcher Jr, *Science.* 232 (1986) 1396.
21. W. Tischer, F. Wedekind, *Topics in Current Chemistry*. 200 (1999) 95.
22. Y. Asanomi, H. Yamaguchi, M. Miyazaki, *Molecules*. 16 (2011) 6041.
23. R. Wilson, A. P. F. Turner, *Biosens. Bioelectron*. 7 (1992) 165.
24. Y. G. Zhou, S. Yang, Q. Y. Qian, X. H. Xia, *Electrochem. Commun.* 11 (2009) 216 .
25. D. Rathod, C. Dickinson, D. Egan, E. Dempsey, *Sensor. Actuat: B-Chem*. 143 (2010) 547.
26. D. Y. Liu, Q. M. Luo, F. Q. Zhou, *Synth. Met*. 160 (2010) 1745.
27. L. Q. Luo, F. Li, L. M. Zhu, Y. P. Ding, Z. Zhang, D. M. Deng, B. Lu, *Colloid Surface*. B. 102 (2013) 307.
28. J. Luo, S. S. Jiang, H. Y. Zhang, J. Q. Jiang, X. Y. Liu, *Anal. Chimi. Acta*. 709 (2012) 47.
29. C. M. Chen, Q. H. Yang, Y. G. Yang, W. Lv, Y. F. Wen, P. X. Hou, M. Z. Wang, H. M. Cheng, *Adv. Mater*. 21 (2009) 3007.
30. X. H. Kang, Z. B. Mai, X. Y. Zou, P. X. Cai, J. Y. Mo, *Anal. Biochem*. 363 (2007) 143.
31. J. Chen, W. D. Zhang, J. S. Ye, *Electrochem. Commun*. 10 (2008) 1268.
32. Q. Xu, Y. Zhao, J. Z. Xu, J J Zhu, *Sens. Actuat B.* 114 (2006) 379.
33. X. J. Zhang, L. L. Wang, R. Ji, L. T. Yu, G. F. Wang, *Electrochem. Commun*. 24 (2012) 53.