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One-pot synthesis of graphene–chitosan nanocomposite modified carbon paste electrode for selective determination of dopamine

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ABSTRACT

Background: A simple, rapid, low-cost and environmentally friendly method was developed to determine dopamine (DA) in the presence of ascorbic (AA) and uric acid (UA) based on a novel technique to prepare a graphene–chitosan (GR–CS) nanocomposite and modify it on the surface of carbon paste electrode (CPE). For our design, CS acts as a media to disperse and stabilize GR, and then GR plays a key role to selective and sensitive determination of DA.
Results: Under physiological conditions, the linear range for dopamine was determined from 1×10^{-4} to 2×10^{-7} mol/L with a good correlation coefficient of 0.9961 in the presence of 1000-fold interference of AA and UA. The detection limit was estimated to be 9.82×10^{-8} mol/L (S/N = 3). In order to study the stability and reproducibility, GR/CS/CPE underwent successive measurements in 10 times and then tested once a d for 30 d. The result exhibited 98.25% and 91.62% activities compared with the original peak current after 10-time measurements and 30-d storage.
Conclusion: The GR/CS/CPE has wide linear concentration range, low detection limit, and good reproducibility and stability, which suggests that our investigations provide a promising alternative for clinic DA determination.

1. Introduction

Since we have known that dopamine (DA) plays a key role in the functions of human being, determinations of DA as an important basis of clinical diagnosis have attracted much interest [1,2]. Various methods have been developed to analysis of DA including chromatography, flow-injection chemiluminescence and electrolysis [3,4,5,6,7,8]. Among the methods mentioned above, electrochemical method presents distinctive advantages, such as quick response, low detection limit, low cost, simple operation and the absence of pretreatment. However, interferences of electroactive impurities, especially the interferences of ascorbic acid (AA) and uric acid (UA) that coexist with DA in body fluids, always affect the accuracy of determinations. In order to eliminate these interferences, the technique of chemically modified electrode has been used to determine DA selectively [9,10,11,12,13,14]. As mentioned from Pardavé's work, only simple modification of the electrode surface with sodium dodecyl sulfate micelles has successfully provided a drastic change in the DA oxidation potential peak, and then the electrochemical oxidation overlap of DA, UA and AA has been separated [15].

Attachment of nanomaterials to the surface of electrode has received wide attention because the modified electrode exhibits more favorable properties than bold electrode even simple chemical modification [16,17,18,19,20]. Graphene (GR), as a prototype two-dimensional carbon system, has been considered as an ideal nanomaterial for modified electrode. Particularly, GR that can provide a friend microenvironment is a promising alternative to bioassay [21,22,23,24,25,26,27,28]. Therefore attractive nature of GR permanently leads to extensive concerns for its synthesis and applications. Currently, several methods have been suggested to prepare GR, such as mechanical cleavage of graphite [29], chemical reduction of graphite oxide [30], thermal expanded graphite [31] and liquid-phase exfoliation [32]. Especially the last method, the liquid-phase exfoliation, is very appealing because it is direct, simple and has subsequent easy applications. Due to the strong π–π stacking and Van der Waals interactions, the solvent choice referring to GR disperse and agglomerate became the key parameter for the technique of liquid-phase exfoliation [25].

The driving force in the investigations and applications of chitosan (CS) is from its satisfying film-forming ability, biodegradability and biocompatibility, especially as a naturally abundant product from deacetylation of chitin [33,34,35]. To the end, CS has been already

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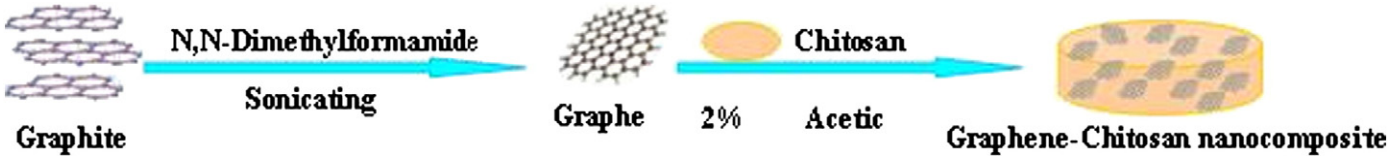

Fig. 1. The procedure for the synthesis of GR–CS nanocomposite.

used to disperse nanomaterials and coat biomacromoleculars [36,37]. Undoubtedly, these intriguing investigations and relative background on the nanomaterials preparation encourage us to continue our efforts for the technique of liquid-phase exfoliation in a convenient and green way. Herein we successfully introduce CS to liquid-phase exfoliation of graphite towards stabilization and solubilization of GR in an aqueous dispersed system. Additionally, the excellent adsorption and viscosity of CS tightens GR modification on the surface of the object.

For this study, hybrid nanocomposite of GR–CS was synthesized by a simple step and then modified on the surface of carbon paste electrode (CPE). The results showed that this modified electrode owns the ability to selectively determine DA with high concentrations of interference. Moreover, this strategy for DA determination was quite accurate and stable. Therefore, the GR–CS modified CPE (GR/CS/CPE) could be a sort of inexpensive and rapid biosensor for DA determination and a good alternative to clinical diagnosis.

2. Experimental

2.1. Reagents and instruments

Dopamine hydrochloride was purchased from Sigma-Aldrich (Germany), and (+)-sodium L-ascorbate, uric acid and chitosan were obtained from Sigma-Aldrich (China). Graphite was obtained from Tianjin Chemical Reagent Factory (China). N,N-dimethylformam was purchased from Sinopharm Chemical Reagent Co., Ltd (China). All the reagents used in this study were of analytical grade, and all solutions were prepared with distilled water. All electrochemical measurements were performed by a CHI650D workstation (Chenhua, Shanghai). A GR/CS/CPE fabricated for a working electrode, a platinum wire and an Ag/AgCl electrode were used to complete the three-electrode system. All the experimental data were the average of three measurements.

2.2. Synthesis of GR–CS nanocomposite

The synthesis of GR–CS nanocomposite is described below: 30 mg graphite was continuously sonicated in 15 mL N,N-dimethylformamide for 30 min, and then 15 mL of 2% acetic acid solution was added for 10 min sonication. The GR–CS mixture was prepared by ultrasonic stirring 60 mg chitosan with the dispersion mentioned above for 1 h. Eventually the resultant dispersion was centrifuged for 90 min at 500 rpm.

2.3. Preparation of GR/CS/GPE

The CPE was prepared by mixing 440 mg graphite powder with 150 mg solid paraffin in an agate mortar and put in incubator until paraffin melted completely. Then the paste was packed into the end of a glass tube, and a copper wire was used as an electrical contact. The surface of the electrodes was polished with Sulfuric acid paper. Eventually, 20 μL nanocomposite of GR–CS was cast to the polished surface of CPE and dried at room temperature.

3. Results and discussion

3.1. Preparation and characterization of GR–CS nanocomposite

GR–CS nanocomposite can be developed in one-pot based on Fig. 1 as described in Section 2.2. While using liquid-phase exfoliation, GR nanosheets show their perfect two-dimensional structure as transparent

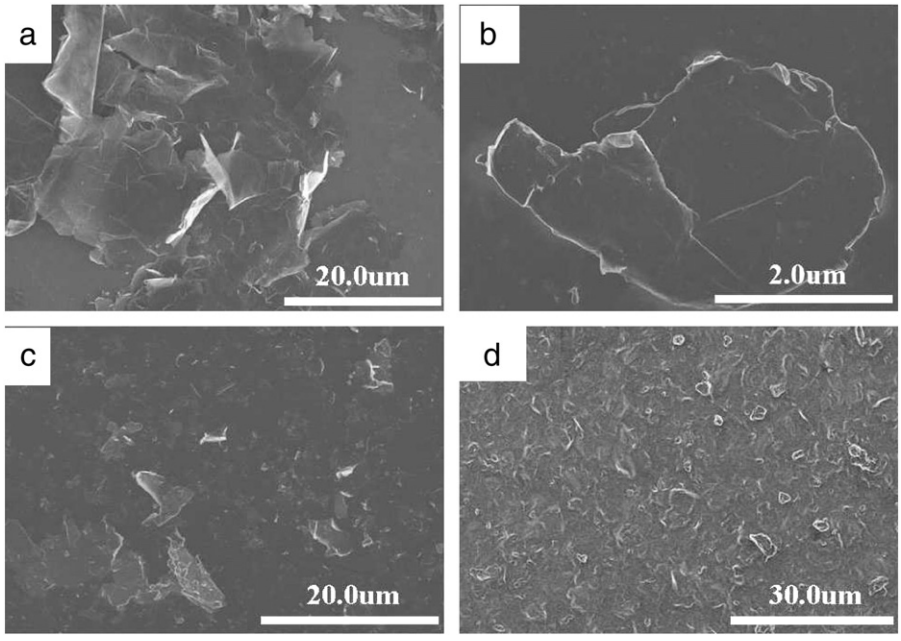

Fig. 2. The images of scanning electron microscope for GR synthesized by liquid-phase exfoliation in N,N-Dimethylformamide (a), one-pot synthesis of GR–CS nanocomposite at low magnification (b) and high magnification (c), GR–CS nanocomposite modified on the surface of CPE (d).

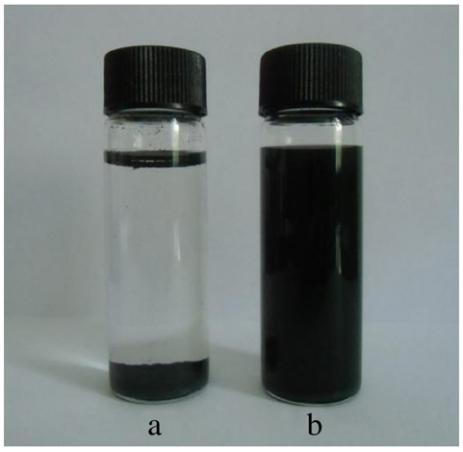

Fig. 3. Photographs of GR dispersed in N,N-dimethylformamide after 3 d store in room temperature (a), GR–CS nanocomposite solution after 21 d store at room temperature (b).

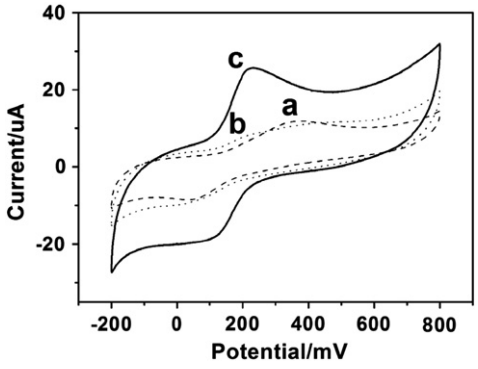

Fig. 4. CVs of bare carbon paste electrode (a: ——), CS/CPE (b: .....), GR/CS/CPE (c: ——) in 0.10 M phosphate buffer (pH 7) containing 1×10^{-4} mol/L DA. Scan rate: 100 mV s^{-1}.

paper (Fig. 2a). Unfortunately, the GR starts to re-agglomerate and settle down just for 3 d (Fig. 3a). Then CS was introduced into the process of exfoliation, and the GR–CS composite still exhibits its classical two-dimensional structure and disperses homogeneously (Fig. 2b). Moreover, the CS solution effectively avoids agglomeration and settlement of GR even during storage in 21 d (Fig. 3b). These results could be attributed to the special structure of CS. The intramolecular structure of CS that has both hydrophilic group and hydrophobic group facilitates itself and can be dissolved in dilute acid solution. Then the protonated amines make CS positively charged groups increasing in diluted acid solution. Owing to a large amount of p electrons in sp^2 hybrid orbital of carbon atom in GR, GR can be dispersed in CS solution homogeneously and stably. However, all sizes of GR–CS composite are obviously smaller than Fig. 2a (Fig. 2c). Fig. 2d proves the membrane of GR–CS nanocomposite coated on the surface of CPE evenly and tightly.

3.2. Electrochemical response of DA at the GR/CS/GPE

In order to study the advantages of the GR/CS/GPE for DA determination, the cyclic voltammograms (CVs) were used to compare the performance of the bare carbon paste electrode, CS/CPE and GR/CS/CPE, respectively. As shown in Fig. 4, the redox peak current of DA on the surface of CS/CPE is smaller than bare carbon paste electrode, but the redox peak current of DA significantly increases when the electrode surface is wrapped with GR–CS nanocomposite. Additionally, the ΔEp of the GR/CS/CPE is 119.23 mV which is smaller than other tested electrodes in our investigations. All results indicate that GR is the dominant factor of accelerating electron transfer on the electrode surface. However, CS plays a mass transfer barrier layer that inhibits the diffusion of the dopamine towards the electrode surface. According to another investigation, CS layer has been proven that it has its owns ability to inhibit and even eliminate the response of the concomitants for dopamine determination [38].

3.3. Effects of pH

Differential pulse voltammograms (DPVs) were employed to detect the electrochemical behaviors of DA at the surface of GR/CS/CPE in 0.10 M phosphate buffer with different pH values (Fig. 5a). According to the results of DPVs, the largest current is obtained at pH 7 which closes to the physiological pH conditions. Therefore, pH 7 was chosen for the subsequent experiments and provided the possibility of real samples' determination. Fig. 5b shows the relationship of peak potential and pH. It can be seen that the peak potential of DA decreases obviously with the increase of pH, and the linear regression equation is Epa(mV) = 552.38–49.45 pH (R = -0.9911). A slope of -49.45 mV/pH indicates that the proportion of the electron and proton involved in the reactions is equal according to Nernst equation.

3.4. Effects of scan rate

Fig. 6a shows the CVs of 1×10^{-4} mol/L DA at the surface of GR/CS/CPE with different scan rates in the range of 20–200 mV s^{-1}. Fig. 6b shows a good linear relationship between the anodic peak current and the square root of the scan rate. The linear regression equation is I(uA) =-7.32 + $3.45v^{1/2}$ (mV $s^{-1})^{1/2}$, and the correlation coefficient is 0.9979. According to the relationship gotten above, we can deduce that the reaction of electron transfer on the surface of GR/CS/CPE is controlled by a diffusive process.

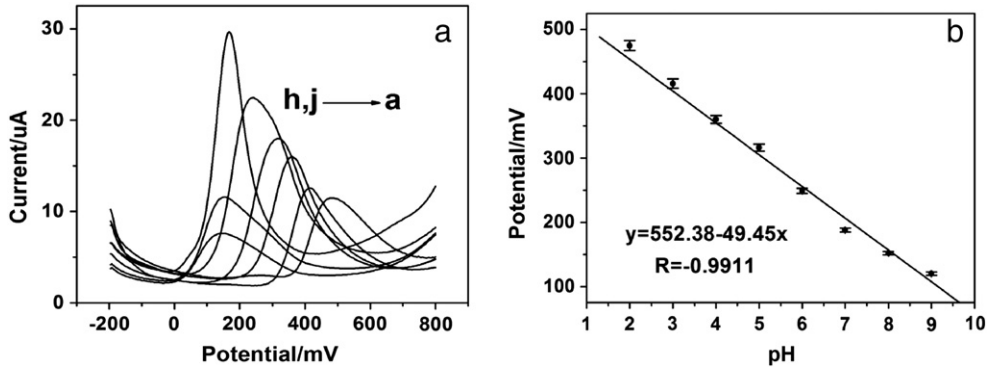

Fig. 5. DPVs of GR/CS/CPE in 0.1 M phosphate buffer containing 1×10^{-4} mol/L DA at different pH (a–h): 2.00, 3.00, 4.00, 5.00, 6.00, 7.00, 8.00, 9.00 (a); plot of Epa versus pH changes (b).

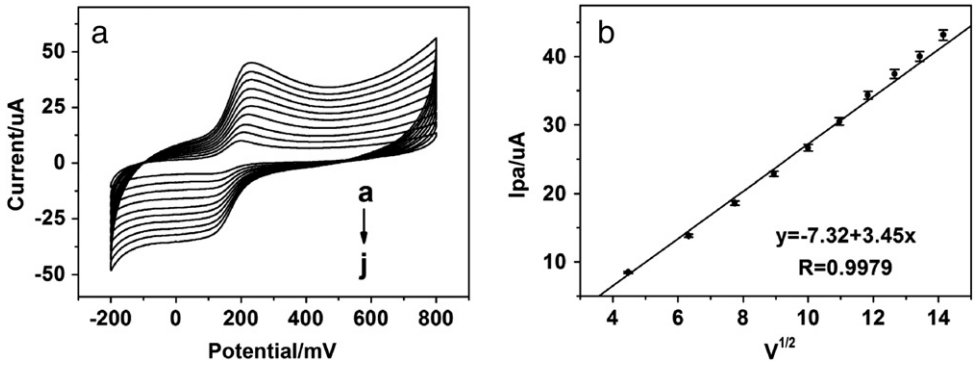

Fig. 6. CVs of GR/CS/CPE in 0.10 M phosphate buffer containing 1×10^{-4} mol/L DA at different scan rate: 20, 40, 60, 80, 100, 120, 140, 160, 180 and 200 mV s^{-1} (a); Graph of anodic peak current versus the square root of the scan rate (b).

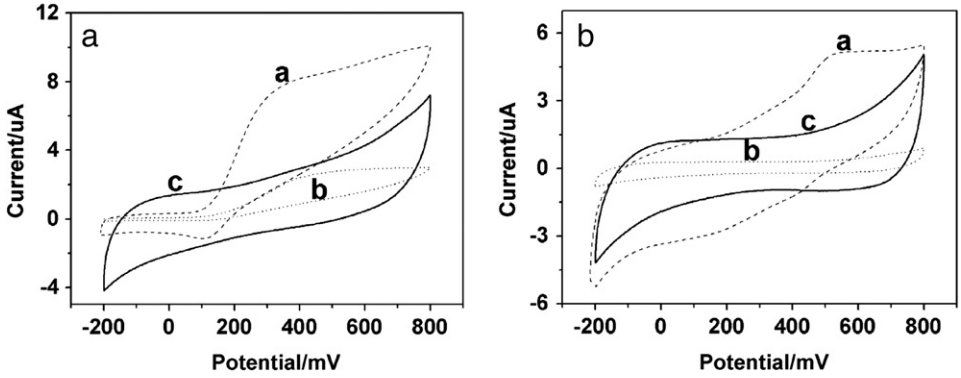

Fig. 7. CVs of bare carbon paste electrode (a: ——), CS/CPE (b: .....), GR/CS/CPE (c: ——) in 0.10 M phosphate buffer (pH 7) containing 0.10 mol/L AA (a); 0.10 mol/L UA (b). Scan rate: 100 mV s^{-1}.

3.5. Electrochemical response of AA and UA at the GR/CS/GPE

In body fluids, AA and UA show 100–1000 times of concentrations compared with that of DA. So the selectivity of electrochemical determinations for DA is very important for clinic diagnosis. Fig. 7 shows the respective CVs of 0.10 mol/L AA and UA at bare CPE, CS/CPE and GR/CS/CPE. As shown in Fig. 7a and b, AA and UA obviously show oxidation peaks at 361 mV and 538 mV on the bare CPE, respectively. Then the chitosan was modified on the CPE, and the oxidation peak current of AA declines sharply and just appears as a relatively wide oxidation peak at 463 mV (Fig. 7a). The oxidation peak of UA even disappears (Fig. 7b). Following the modification of GR, the oxidation peak of AA can not be observed completely (Fig. 7a). The chemical modification is an effective way to eliminate inherent interference of AA and UA. A plausible explanation for this behavior can be in the references [15,39]. According with a lot of investigations, Pardavé considers that the changing of chemical behavior is relative to standard rate constants, k°, for UA and AA, which must be drastically diminished on the modified surface of electrode.

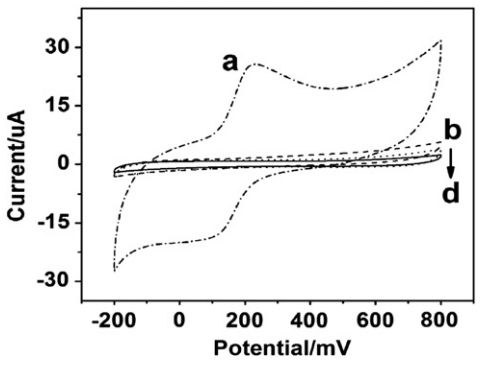

Fig. 8. CVs of scanned by GR/CS/CPE in the presence of 1×10^{-4} mol/L DA, 0.1 mol/L AA and UA. (a: -.-.-), 0.10 mol/L AA (b: - - -), 0.10 mol/L UA (c: —) in 1.00 mol/L phosphate buffer (d: …) at scan rate of 100 mV s^{-1}.

Fig. 8 shows the CVs of 1×10^{-4} mol/L DA with impurities of 0.10 mol/L AA and UA (a), 0.10 mol/L AA (b), 0.10 mol/L UA (c) and blank phosphate buffer (d) at the modified electrode with a scan rate of 100 mV s^{-1}. In 1000 times presence of AA and UA compared with the DA, DA shows remarkable response with negligible background at the surface of GR/CS/CPE. The oxidation peak current (Ipa) and Epa of DA are 25.83 μA and 231.91 mV which are extremely close to only the presence of DA (Ipa is 25.66 μA and Epa is 231.05 mV).

Fig. 9a shows the DPVs of different concentrations of DA in the presence of AA and UA. The DPV peak currents increase linearly with the increase of DA concentration over the range of 0.20–100 μmol/L. The linear regression equation is I(μA) = 1.81 + 0.28C (μmol/L), and the correlation coefficient is 0.9961. Then the detection limit is 9.82×10^{-8} mol/L with noise–signal ration in 3.

3.6. Stability and reproducibility

The stability and reproducibility of the GR/CPE and the GR/CS/GPE were investigated in the PBS solution containing 1×10^{-4} mol/L DA, and 0.10 mol/L AA and UA. According to successive measurements in 10 times, the relative standard deviation of the oxidation peak currents gotten by the GR/CPE and the GR/CS/GPE are 2.36% and 1.75%, respectively. The electrodes are stored in 4°C and tested once a d for 30 consecutive d, and the anodic peak currents of the GR/CPE

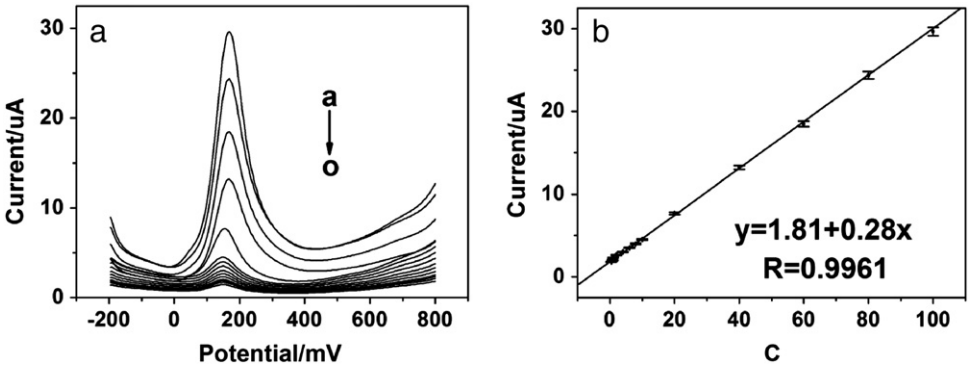

Fig. 9. DPVs of GR/CS/CPE in 0.1 M phosphate buffer containing different concentrations of 0.20, 0.40, 0.60, 0.80, 1, 2, 4, 6, 8, 10, 20, 40, 60, 80, 100 μmol/L DA (a–o) in the presence of 1000-fold concentration of AA and UA (a); Calibrating plots of the anodic peak current versus DA concentrations (b).

exhibited only 72.32% activities compared with initial value, but those of the GR/CS/GPE exhibited more than 91.62% activities (Fig. 10). These mean that CS introduced to our modified electrode indeed improves the performance of stability.

4. Concluding remarks

In this work, we overcome the traditional difficulties of GR preparation and storage, and a novel method of one-step to prepare GR–CS nanocomposites was developed. The GR–CS nanocomposite in our investigations shows a good two-dimensional carbon system and stability for 21 d storage. Then the GR–CS nanocomposite modified CPE exhibits an excellent performance for DA determination in the 1000 times interference of AA and UA at physiological pH. The brilliant characters of GR/CS/CPE, such as wide linear concentration range, low detection limit, and good reproducibility and stability, suggest that our investigations provide a promising alternative for clinic DA determination.

Conflict of interest

There are no conflict of interest.

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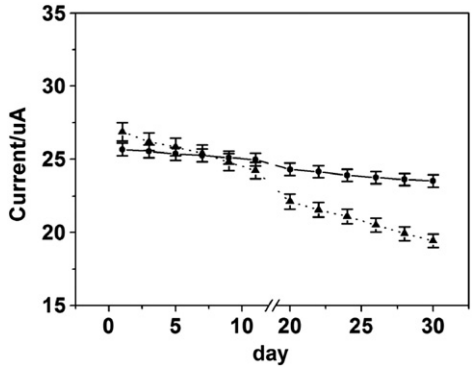

Fig. 10. Plot of the anodic peak current versus d (1–30 d) of GR/CPE (a:…..), GR/CS/CPE (b: —).

Acknowledgments

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Author contributions

Proposed the theoretical frame: DL; Conceived and designed the experiments: CL; Software development: LC; Contributed reagents/materials/analysis tools: JY; Wrote the paper: CL; Performed the experiments: JZ; Analyzed the data: YE.

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