Application of Graphene Nanocomposite in Motion Sensing of Human Body

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Abstract: *For exploring the application of graphene nano-composite, graphene is combined with other nano-materials to develop a new graphene nano-composite with high conductivity, good biocompatibility and strong affinity for enzymes, which improved the working performance of sensors in human motion sensing. Graphene oxide (GO) is prepared by using Hummers and offeman methods, and graphene is prepared from graphene oxide with glucose as a reducing agent. On the basis of electro-deposition of Prussian blue chitosan (PB-CS) film modified gold electrode, a new nano material graphene is introduced, and glucose oxidase as a model enzyme, a glucose biosensor based on RGO/PB-CS nano-composite is constructed. The research results showed that the sensor constructed has higher sensitivity, lower detection limit and smaller apparent Michaelis constant. To sum up, the combination of graphene and Prussian blue chitosan effectively promoted the electron transfer between the electrode surface and the analytical substrate, and improved the working performance of the sensor, which has potential application value in human motion sensing.*

Keywords: *Graphene nano-composite, Human body, Motion sensing, Biosensors.*

Introduction

Since its discovery in 2004, graphene has aroused strong concern. It has excellent thermal conductivity, mechanical properties and electron transfer properties. Graphene has been used in various fields such as nanocomposites, supercapacitors and electrochemical sensors [1]. Graphene is a new type of nanomaterial formed by the close accumulation of sp^2 hybrid carbon atoms, which has a large specific surface area and good biocompatibility. It can be combined with biomolecules, polymers and organic drug molecules through covalent and non-covalent bonds, and increases the capacity of enzymes in biosensors. In addition, it can provide a good microenvironment for the enzyme. Due to its excellent electrical conductivity and high electron mobility, graphene is extremely sensitive to the electrical response of the external molecules [7]. However, van der Waals forces and static electricity exist between the graphene nanosheets. The irreversible agglomeration of graphene is easy to occur in the solution, which will seriously affect the further application of graphene. Therefore, it is especially important to modify graphene to prevent its agglomeration. Biosensors are composed of molecular recognition originals (such as enzymes, antigens and tissues) and various transducers (such as electrochemical electrodes, photoelectric transducers, etc.). According to the different original,

biosensors can be divided into enzyme sensors, immune sensors, DNA sensors and microbial sensors [6]. Enzyme biosensors are the most widely used biosensors [10]. In recent years, researchers combined different nanomaterials with graphene to prevent the agglomeration of graphene and give better performance of graphene, so as to provide high-quality materials for biosensor construction [11]. In this study, graphene was prepared by chemical reduction method. Glucose oxidase was used as a model enzyme to investigate its application in biosensors. On this basis, each graphene – AuNG-β-CD/Prussian blue nanocomposites were prepared by one-step electrodeposition technique. Chitosan has good biocompatibility and it is used to immobilize acetylcholinesterase. Among them, the introduction of Prussian blue can oxidize thiocholine, and reduce the working potential of the sensor. Nano gold can not only effectively prevent the graphene from aggregating, but also enhance the conductivity of graphene [9]. The β-cyclodextrin can be combined with graphene through hydrogen bonds and can produce non-covalent bonds with acetylcholine. This can improve the sensor selectivity, increase the rate of acetylcholine enrichment and improve sensitivity [5]. It has potential application value in the motion sensing of human body.

Preparation and characterization of graphene and its application in glucose biosensor

Instruments and reagents

CHl750 electrochemistry workstation; the three-electrode system: the working electrode is a gold electrode, the reference electrode is Ag/AgCl (3MKCl), and the counter electrode is platinum wire; X-ray diffractometer, Electric field emission scanning electron microscopy, T1901 dual-beam UV-visible spectrophotometer; DV215CD precision electronic balance; 79HW-1 constant temperature heating magnetic agitator; KQ2200DE ultrasonic cleaner; Ultra-pure water system. Glucose oxidase; Graphite powder; Glucose. The other reagents are all analytical and pure, and the experiment water is high pure water.

Preparation of graphene

Hummers and Offeman method were used to prepare graphene oxide [2]. 5.00 g $K_2S_2O_8$ and 5.00 g P_2O_5 are added to 12.0 mL concentrated sulfuric acid and stirred evenly. Then, 2.00 g graphite powder is added and stirred for 30 minutes. It was slowly warmed to 80 °C for 4 hours. Then, it is reduced to room temperature and is repeatedly washed to neutral with secondary water. After filtration, it was dried at 60 °C to get the preoxidation product. 1 g preoxidation product was added to the concentration of concentrated sulfuric acid at 0 °C of 36.0 mL and stirred for 30 minutes. Then, 5.00 g $KMnO_4$ is slowly added. The temperature does not exceed 10 °C. Reagent was stirred for 30 minutes. The temperature was slowly raised to 35 °C and the reagents were allowed to react for 8 hours. 360 mL of secondary water and 5.00 mL of 30% H_2O_2 were added and stirred for 30 minutes and centrifuged at 4,000 rpm for 20 minutes. It is washed 3 times with 5% aqueous hydrochloric acid. Finally, the secondary water is used to wash it to neutral. Then, it is dried, and the graphite oxide is obtained. Graphite oxide is mixed with a suitable amount of secondary water. Then, the supernatant was sonicated in an ultrasonic cleaner for 2 hours to disperse the graphene oxide. It is centrifuged at 3000 rpm for 30 minutes. Graphite oxide which has not peeled off in the lower layer was removed and a graphene oxide dispersion was obtained and stored at 4 °C for use.

The preparation of graphene is as follows: A 25.0 mL dispersion was added to a round bottom flask. 200 mg of glucose was added and stirred for 40 minutes. Then, 100 μL of NH_3 water was added and stirred for 5 minutes and reacted at 95 °C for 1 hour. Finally, it was cooled to room temperature and stored at 4 °C for use.

Preparation of glucose biosensors

The gold electrodes were polished to a mirror surface with 1.0, 0.3 and 0.05 μm $A1_2O_3$ powders, respectively, and the distilled water was ultrasonically cleaned. The electrodes were subsequently sonicated in acetone, Piranha solution (H_2SO_4:H_2O_2 = 3:1 v/v). After washing with water, it is dried. The clean gold electrode was placed in a mixed solution of 0.5 mM $K_3[Fe(CN)_6]$ + 0.5 mM $FeCl_3$ + 0.01% chitosan (CS) (containing 0.1 M KCl + 0.01 M HCl). The cyclic voltammetric sweep of 10 cycles at room temperature was carried out at the rate of 20 mVs^{-1} in the range from -0.1 to 0.45 V, and dried at room temperature [3]. 5 μL of graphene dispersions were added to the surface of the electrode. After drying at room temperature, 5 μL 10.0 $mg \cdot mL^{-1}$ glucose oxidase was added. After drying at 4 °C, 5 μL chitosan solution (1 wt%, 1% acetic acid buffer solution) was added and crosslinked with 0.25% glutaraldehyde for 20 minutes. Then, it was washed with water to obtain CS/GOD/RGO/PB-CS/Au.

Biosensor based on Prussian blue/graphene nano gold – β cyclodextrin hybrid membrane

Instruments and reagents

CHl750 electrochemistry workstation (Shanghai Chen Hua Instrument Company); the three electrode system: the working electrode is a gold electrode, the reference electrode is Ag/AgCl (3MKCl), and the counter electrode is platinum wire; X-ray diffractometer (XRD, D/MAX.2500, Japan), Electric field emission scanning electron microscopy (SEM, S-4800, Japan), T1901 dual-beam UV-visible spectrophotometer (Beijing Purkinje General Analysis Instrument Co., Ltd.); DV215CD precision electronic balance (OHAUS company); 79HW-1 constant temperature heating magnetic agitator (Jiangsu Rong Hua Instrument Manufacturing Co., Ltd.); KQ2200DE ultrasonic cleaner (Kunming Ultrasonic Instrument Co., Ltd.); Ultra-pure water system (HFNW10-UV).

Acetylcholinesterase (AChE, 518 unit mg^{-1}, Sigma); Graphite powder (pure spectrum, Sinopharm Chemical Reagent Co., Ltd.); Tetrachloroauric acid ($HAuCl_4$, Sigma); Acetylthiocholine chloride (ATCL, analytical pure, Sigma); Malathion standards (analytical grade, Sigma); Carbaryl standard (analysis pure, Sigma); Iodide phosphorodide (analysis pure, Sigma). The other reagents are all analytical and pure, and the experiment water is high pure water.

Test method

Three-electrode system: the working electrode is a modified glassy carbon electrode, the counter electrode is a platinum wire, and the reference electrode is Ag/AgCl (3 M KCl). 0.1 M PBS (pH 6.5) is a supporting electrolyte. The enzyme electrode assembly is divided into three steps. In the first step, RGO-AuNPs-β-CD modified films were assembled by potentiostatic deposition. In the second step, cyclic voltammetry was used to assemble the Prussian blue chitosan (PB-CS) membrane. In the third step, acetylcholinesterase and chitosan are dropped on the surface of the modified electrode. In the experiment, the electrodes were characterized by scanning electron microscopy (SEM) and cyclic voltammetry (CV) [4]. Cyclic voltammetry and time-current (i-t) curves were used to study the current response of the sensor to acetylcholine chloride. Differential pulse voltammetry (DPV) was used to investigate the effect of inhibition time on human motion sensing [8].

The glassy carbon electrode was polished to a mirror surface by 1, 0.3 and 0.05 μm $A1_2O_3$ powders. Each step is cleaned with distilled water. The electrodes were placed in nitric acid

(HNO_3:H_2O = l:1 v/v), ethanol and water for ultrasound. After cleaning the electrode with the secondary water, the electrode is dried.

(1) The assembly of RGO-AuNPs-β-CD modified membrane
15 μL 2.00 mg·mL^{-1} GO dispersions were dripped to the surface of a clean glass carbon electrode (GCE) and dried at room temperature. Then, it was placed in a mixed solution of 0.1 M PBS (containing 1.25 mM $HAuCl_4$ + 0.15 mg·mL^{-1} β-CD). RGO-AuNPs-β-CD/GCE was deposited for 720 s under a magnetic stirring potential of -1.4 V and dried at room temperature.

(2) The assembly of PB-CS film
The modified electrode RGO-AuNPs-β-CD/GCE was placed in a mixed solution of 0.100 M KCl + 10.0 mM HCl + 0.500 mM $K_3[Fe(CN)_6]$ + 0.500 mM $FeCl_3$ + 0.01% CS. Cyclic voltammetry was performed 10 turns in a potential range from -0.1 to +0.45 V. PB0-CS was deposited on the electrode surface to obtain a PB-CS/RGO-AuNPs-β-CD/GCE modified electrode and dried at room temperature.

(3) Immobilization of enzymes
On the surface of the PB-CS/RGO-AuNPs-β-CD/GCE electrode, 10 μl of 0.500 mg·mL^{-1} acetylcholinesterase was added dropwise and allowed to dry at 4 °C. Then, 10 μL CS solution (1 wt%, 1% acetic acid solution) was dropped on the surface of the modified electrode. Acetylcholinesterase sensor is obtained, that is, CS/AChE/PB-CS/RGO-AuNPs-β-CD/GCE.

Results and analysis

The application of graphene in glucose biosensor

The graphene, prepared with glucose as a reducing agent, is dispersed uniformly and can be directly applied to the construction of the sensor without any treatment. Glucose can reduce graphene oxide as a kind of green non-toxic reductant, and the oxidation product can be used as a stabilizer to disperse graphene uniformly and stably, so as to ensure the excellent performance of graphene. In this experiment, graphite powder, graphite oxide and graphene were firstly characterized by X-ray diffraction. In the UV spectrum of graphene oxide and graphene, the maximum absorption peak of graphene oxide was shifted from 228 nm to 266 nm after being reduced. It shows that the oxygen-containing functional groups on the surface of graphene oxide are gradually reduced and the conjugated system of π-π is gradually repaired to obtain highly conductive graphene.

As shown in Fig. 1, the UV spectrum of aqueous graphene oxide showed the largest peak at 228 nm. With the progress of the reduction reaction, the maximum absorption peak of graphene oxide gradually shifts and finally appears at 266 nm. It shows that graphene oxide is reduced to graphene.

For the application of graphene in glucose biosensors, a new type of glucose biosensor was constructed by using RGO/PB-CS composite membrane to immobilize GOD. The CV method was used to investigate the sensor assembly process. As shown in Fig. 2, the response of the sensor to 0.5 mM glucose in the potential range from -0.3 to 0.2 V was investigated by the time-current (i-t) curve method. The experimental results show that the catalytic current increases significantly with the increase of working potential in the potential range from -0.3 to 0.0 V. In the range of 0.0-0.2 V, the catalytic current is gradually decreased. Therefore, this experiment chose 0.0 V as the working potential of glucose sensor.

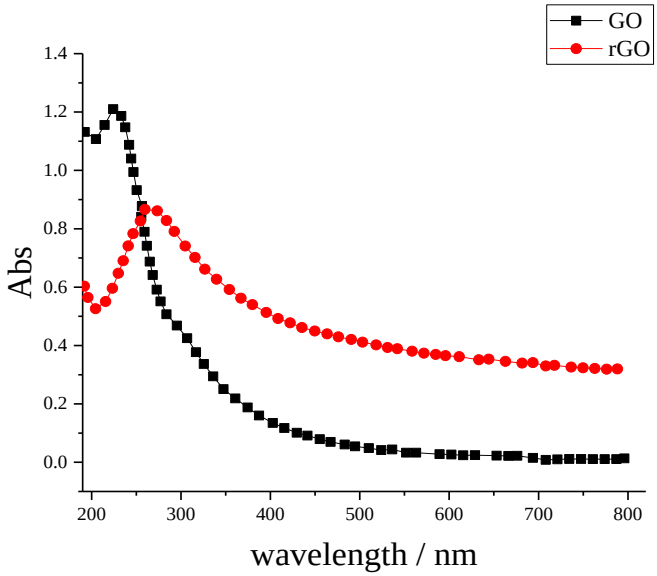

Fig. 1 UV-Vis absorption spectra (Abs) of reduced graphene oxide (RGO) and GO

Compared with the bare gold electrode, PB-CS modified electrode showed a pair of obvious redox peaks near 0.13 V and 0.23 V due to the mutual conversion of Prussian blue (PB) and Prussian white (PW). When the electrode surface is further covered with RGO, the peak current of the electrode increases. RGO greatly improves the sensor's conductivity and electron transfer process. After GOD is modified, as a non-conductive substance, GOD hinders the electron transfer. The current response is significantly reduced. After modification of chitosan, the current response increased again, which may be due to the weak conductivity of chitosan. The above results show that PB, RGO, GOD and CS were successfully assembled on the gold electrode.

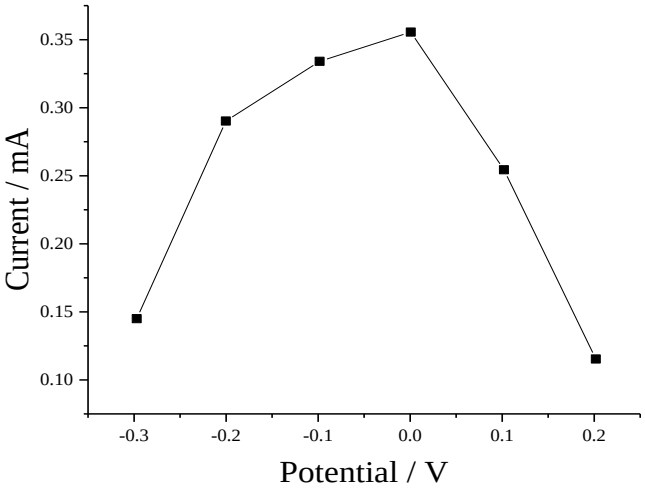

Fig. 2 Effect of the applied potential on the stable currents response of the enzyme biosensor in 0.1 M PBS containing 0.5 mM glucose

Prussian blue stability and glucose oxidase activity are affected by the pH value. Therefore, it is of great significance to investigate the pH value. The response of the sensor to 0.5 mM glucose at pH 5.0 to 8.0 was investigated. The results are shown in Fig. 3. As pH increases from 5.0 to 6.5, the sensor response current increases. However, as the pH continues to increase,

the response current decreases. When the pH value is 6.5, the catalytic current value is the largest. Therefore, the pH of the PBS test solution selected in this experiment was 6.5.

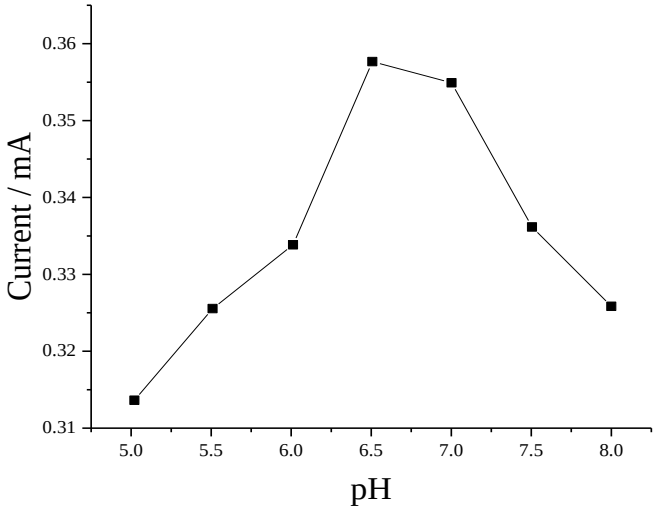

Fig. 3 Effect of the solution pH on the stable currents response of the enzyme biosensor in 0.1 M PBS containing 0.5 mM glucose, applied potential, 0.0 V *vs*. Ag/AgCl (3 M KCl)

As shown in Fig. 4, the response of the sensor to 0.600 mM glucose was examined experimentally with GOD concentrations of 5.00 mg·mL^{-1}, 10.0 mg·mL^{-1}, 15.0 mg·mL^{-1} and 20.0 mg·mL^{-1}, respectively. When GOD concentration increased from 5.00 mg·mL^{-1} to 10.0 mg·mL^{-1}, the current response was significantly increased. However, when the GOD concentration continues to increase, the response current tends to be flat. Therefore, the GOD concentration in this experiment was 10.0 mg·mL^{-1}.

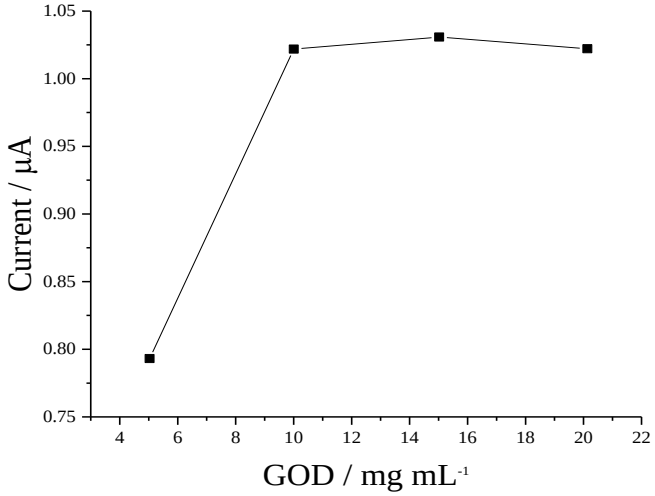

Fig. 4 Effect of GOD on the stable currents response of the enzyme biosensor in 0.1 M PBS (pH 6.5) containing 0.6 mM glucose

In the experiment, the response of the sensor to 0.6 mM glucose in the range from 4.00 to 10.0 of the graphene reaction solution was investigated. As can be seen from Fig. 5, the current response increases significantly as the amount of graphene increases from 4.00 μL to 5.00 μL. However, when the amount of graphene continues to increase, the response current is significantly reduced. Therefore, the optimal amount of graphene is 5.00 μL.

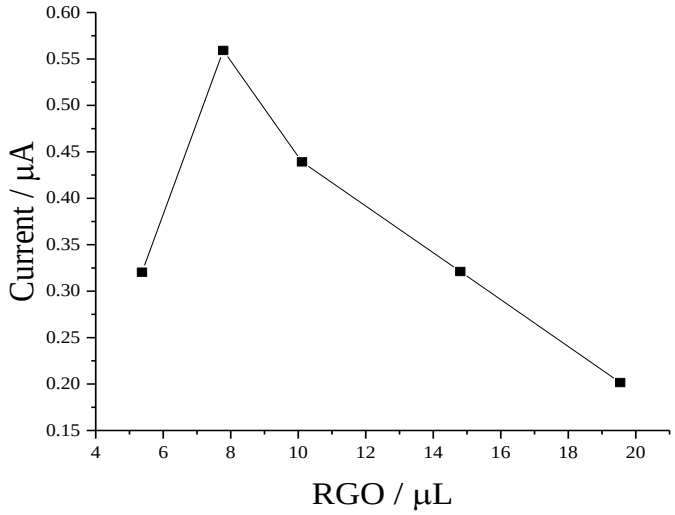

Fig. 5 Effect of the amount of the RGO on the stable currents response of the enzyme biosensor in 0.1 M PBS containing 0.6 mM glucose, applied potential, 0.0 V *vs*. Ag/AgCl (3 M KCl)

Through the sensor application conditions optimization experiments, it was found that when the PBS test solution pH is 6.5, the catalytic current value is the largest. This is determined by both the enzyme and the electronic nanoparticle Prussian blue. On the one hand, GOD has the highest catalytic activity on glucose at pH 3.5-6.5. On the other hand, nano-Prussian blue has better stability in acidic environment. In this study, 0.0 V was used as the working potential. It has greater sensitivity at 0.0 V. Under the high working potential, the reductive substance in the solution can easily interfere with the determination. Therefore, in order to obtain better selectivity and sensitivity, 0.0 V is used as the working potential of the sensor. In addition, the concentration of GOD was also investigated. When the concentration of GOD increases, the greater the density of the enzyme on the surface of the electrode, the more the active center is, which is beneficial to the enzymatic reaction. The current response is significantly increased. As the GOD concentration continues to increase, the response current tends to be gentle. The amount of enzyme increases the impedance of the sensor, which is detrimental to electron transfer. Therefore, the optimal GOD concentration is 10 $mg \cdot mL^{-1}$.

Chit/GOD/RGO/PB-CS modified electrode has a larger current response to glucose than the graphene-free modified electrode. After adding glucose solution, the reduction current rapidly increased to 95% of the steady-state current in 5 s, indicating that the sensor responds rapidly to glucose. The effect of graphene on the performance of biosensor was investigated by CV method. The results show that after graphene modification, the peak current is about 2 times higher than that of the modified electrode without graphene. The addition of RGO significantly improves the sensor's electrochemical response to glucose. The reason is as follows. First, RGO's unique physicochemical properties help to enhance the biosensor's electrochemical response signal, while high surface area contributes to increased GOD loading. Second, the synergistic effect between RGO and PB-CS can promote the electron transfer between the PB and Au electrodes and promote the electrochemical reduction of hydrogen peroxide on the electrode surface. Third, the PB-CS/RGO nanocomposite film provides a suitable microenvironment for GOD, which helps to maintain the enzyme activity. All of these can increase the biosensor electrochemical response to glucose and improve the sensitivity.

Three Chit/GOD/RGO/PB-CS modified electrodes were made to detect 0.100 mM glucose respectively. The reproducibility of the sensor was investigated. The results showed that the relative standard deviation was 6.3%. At the same time, the repeatability of the sensor was examined. The 0.100 mM glucose was detected by the same electrode for 6 times continuously, and the relative standard deviation was 4.2%. The modified electrode was placed in 0.1 M PBS and stored at 4 °C to investigate the stability of the electrode. The results showed that after 13 days, the biosensor reduced the current response to 0.500 mM glucose only by 5%. The experimental results show that the biosensor has good selectivity, repeatability and stability. This is due to the following reasons. First, the introduction of PB-CS reduces the working potential (0.0 V) of the sensor and reduces the interference of some bioactive substances. In addition, the cross-linked CS film with a compact network structure on the surface of the electrode can not only prevent the leakage of the electroactive substances and improve the selectivity of the sensor, but also effectively prevent the leakage of the enzyme. Finally, the PB-CS/RGO nanocomposite film provides a suitable microenvironment for immobilization of the enzyme, thereby preserving the enzyme activity.

The experiment also investigated the biosensor in the actual sample. To verify the sensor's response to glucose in the actual sample, standard addition was used. The sensor will be constructed to detect the body's blood glucose concentration after exercise. Table 1 shows that the recovery of glucose obtained by this method is between 93.9% and 100.0%.

Table 1. Results of glucose testing in serum of diabetic patients (n = 3)

Sample	1	2	3	4	5
$C_{original\ sample}$ ± SD, (mM)	12.5 ± 0.0	14.5 ± 0.3	15.5 ± 0.3	17.6 ± 0.5	10.9 ± 0.9
Diluted sample, (mM)	7.50	8.60	9.30	10.6	6.50
Glucose added, (mM)	20.0	20.0	20.0	20.0	20.0
Found ± SD, (mM)	26.3 ± 2.5	28.6 ± 1.5	28.9 ± 1.0	29.8 ± 1.1	26.1 ± 0.9
Recovery, (%)	93.9	100.0	98.0	96.2	97.8

Sensor based on Prussian blue/graphene nano gold – β cyclodextrin hybrid membrane

In this study, the effects of graphene oxide, chloroauric acid, the concentration of β-CD, deposition time and deposition potential on the performance of the electrode were investigated. The influence of the concentration of graphene oxide (Fig. 6), chloroauric acid (Fig. 7) and β-CD (Fig. 8) on the performance of the counter electrode was investigated by CV method. The RGO-AuNPs-β-CD modified electrode will be deposited under different conditions. Scanning was performed in 0.1 MPBS (containing 5.00 mM $K_3[Fe(CN)_6]$/ $K_4[Fe(CN)_6]$) in the range from 0.5 V to 0.15 V. As can be seen from Figs. 6-8, the peak current was highest when the concentrations of graphene oxide, chloroauric acid and β-CD were 2.00 mg·mL^{-1}, 1.25 mM, and 0.150 mg·mL^{-1}, respectively. Therefore, deposition was carried out with graphene oxide, chloroauric acid and β-CD concentrations of 2.00 mg·mL^{-1}, 1.25 mM and 0.150 mg·mL^{-1}, respectively.

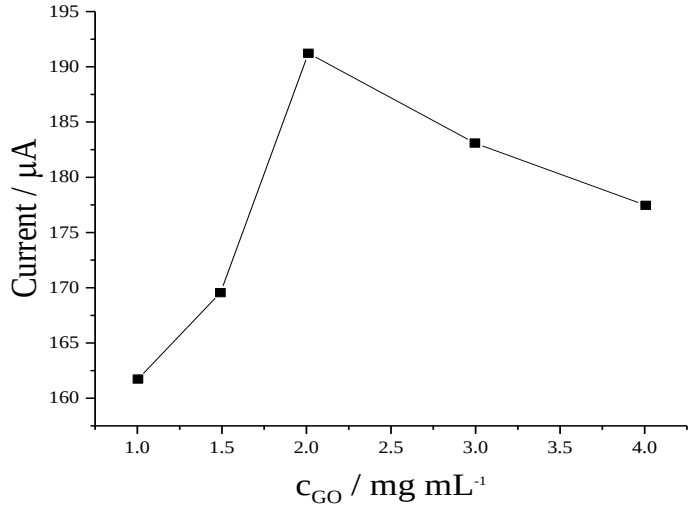

Fig. 6 Effect of concentration of GO on the response currents of the RGO-AuNPs-β-CD/GCE electrode in 0.1 M PBS (pH 6.5) containing 5 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$

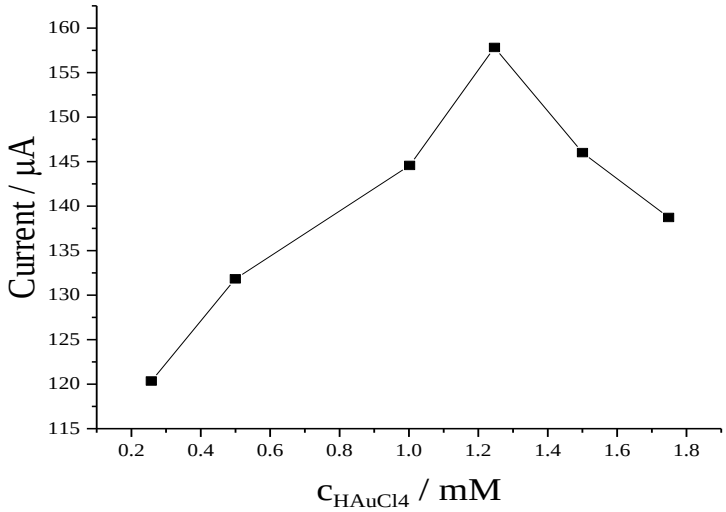

Fig. 7 Effect of concentration of $HAuCl_4$ on the response currents of the RGO-AuNPs-β-CD/GCE electrode in 0.1 M PBS (pH 6.5) containing 5 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$

The CV method was used to investigate the effect of deposition time on the sensor response current. In 0.1 M PBS (containing 1.25 mM $HAuCl_4$ + 0.150 mg·mL^{-1} β-CD), different deposition times were chosen for electrodeposition. The RGO-AuNPs-β-CD modified electrode deposited under different conditions was then placed in 0.1 M PBS (containing 5.00 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$), and scanned in the range from 0.5 V to -0.15 V. As shown in Fig. 9, when the deposition time is 720 s, the peak current of CV plot is the highest and the deposition effect is best. Therefore, the optimal deposition time of RGO-AuNPs-β-CD film is 720 s. Finally, the effect of deposition potential on the electrode performance was investigated by CV method. As shown in Fig. 10, when the deposition potential is -1.4 V, the peak current is the maximum, and the RGO-AuNPs-β-CD film has the best performance. The best deposition potential is -1.4 V.

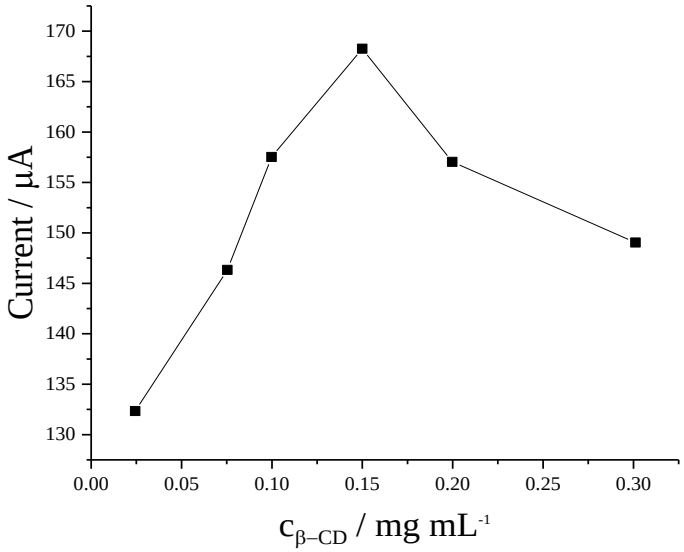

Fig. 8 Effect of concentration of β-CD on the response currents of the RGO-AuNPs-β-CD/GCE electrode in 0.1 M PBS (pH 6.5) containing 5 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$

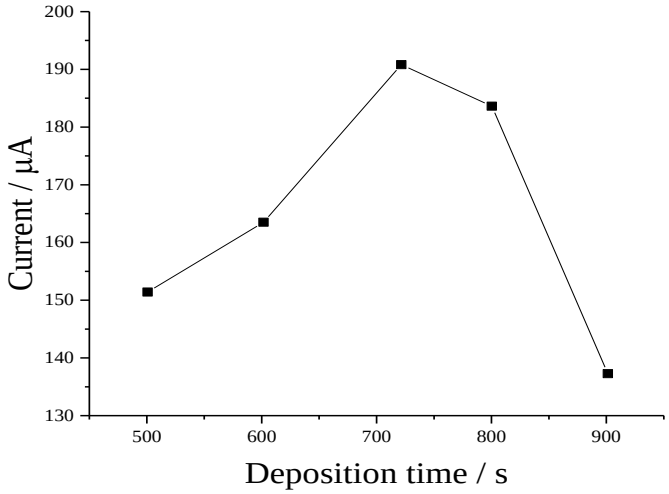

Fig. 9 Effect of RGO-AuNPs-β-CD deposition time on the response currents of the RGO-AuNPs-β-CD/GCE electrode in 0.1 M PBS (pH 6.5) containing 5 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$

According to the above experimental results, the optimal conditions for the deposition of RGO-AuNPs-β-CD film are as follows. 15 μL of 2.00 mg·mL^{-1} GO dispersion was dropped on the electrode surface. After drying, it was deposited in a mixture of 0.1 M PBS (1.25 mM $HAuCl_4$ + 0.150 mg·mL^{-1} β-CD) at -1.4 V for 720 seconds.

In order to investigate the effect of RGO-AuNPs-β-CD on the biosensor current response, two different modified electrodes of CS/AChE/PB-CS/RGO-AuNPs-β-CD/GCE and CS/AChE/PB-CS/GCE were prepared. Compared with the modified electrode without RGO-AuNPs-β-CD nanocomposites, the sensitivity and the response speed of graphene nanocomposites increased obviously after the graphene nanocomposite was added.

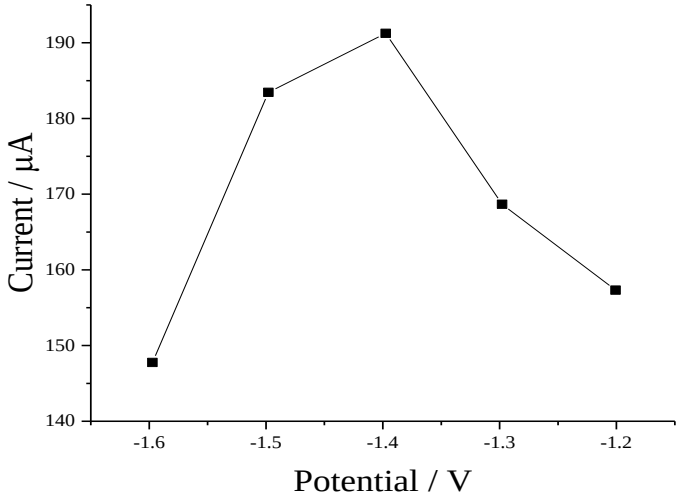

Fig. 10 Effect of RGO-AuNPs-β-CD deposition potential on the response currents of the RGO-AuNPs-β-CD/GCE electrode in 0.1 M PBS (pH 6.5) containing 5 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$

The amount of acetylcholinesterase immobilization is an important factor affecting the performance of biosensors. As shown in Fig. 11, the effect of different concentrations of AChE on 50.0 μM ATCl response current was investigated by i-t curve in this study. As the AChE concentration increased, the response current of the sensor also gradually increased and reached the maximum at a concentration of AChE of 0.500 $mg \cdot mL^{-1}$. After that, as the concentration of acetylcholinesterase continues to increase, the response current of the sensor tends to be gentle. Therefore, the optimal amount of immobilized AChE is 0.500 $mg \cdot mL^{-1}$.

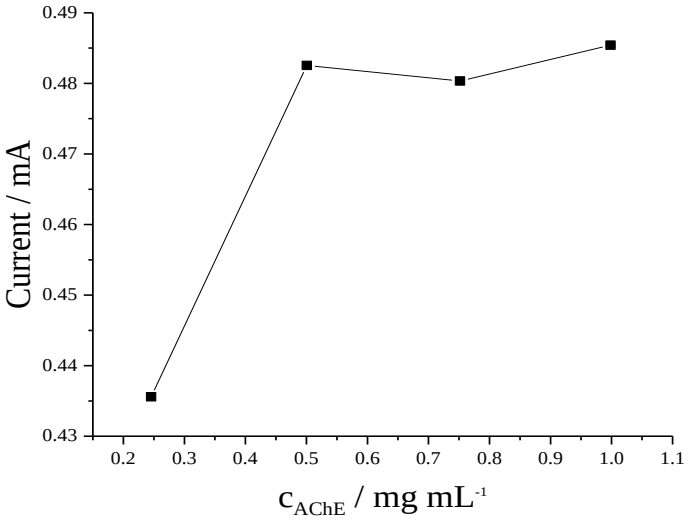

Fig. 11 Effect of AChE concentration on the response currents of the CS/AChE/PB-CS/RGO-AuNPs-β-CD/GCE electrode in 0.1 M PBS (pH 6.5) containing 50.0 μM ATCl

Under the best experimental conditions, the biosensor current response to ATC1 was investigated. After adding ATCl solution, the oxidation current increased rapidly and reached 95% of the steady state current in 5 s. It shows that the sensor responds quickly to ATCl. The concentration of ATCl was in the range of 1.50-269 μM and 344-2.22×10^3 μM, respectively.

The response current has a linear relationship with the concentration of ATCl, as shown in Fig. 12. The correlation coefficients were 0.9992 and 0.9942, respectively. By using the Lineweaver-Burk formula, the Michaelis constant is calculated to be 0.106 mM. It is smaller than 3-carboxyphenylboronic acid/graphene/nanogold (0.16 mM), Prussian blue-chitosan/multi-walled carbon nanotubes/hollow gold nanospheres (0.21 mM) and the Michaelis constant of the acetylcholinesterase biosensor constructed by cadmium sulphide-graphene nanocomposite (0.24 mM). The smaller Michaelis constant indicates that the affinity between acetylcholinesterase immobilized on the modified membrane and acetylcholine chloride is relatively high, which shows a high catalytic activity.

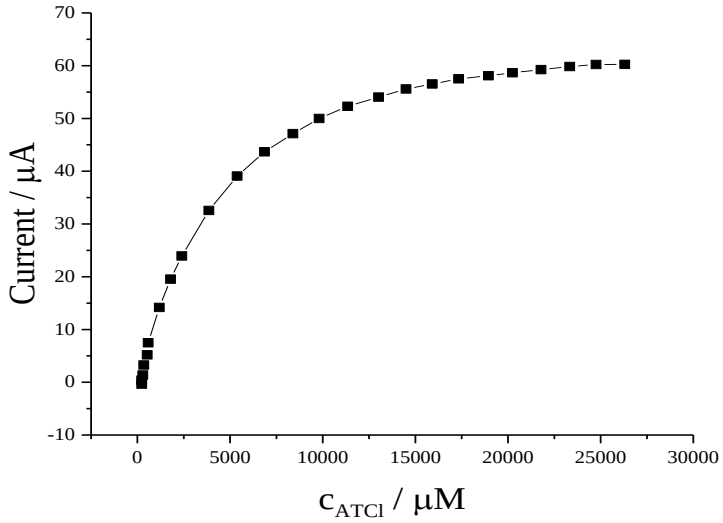

Fig. 12 Linear calibration between the response currents and ATCl concentration

The experimental results show that the biosensor has good repeatability, selectivity and stability, which is attributed to the excellent properties of PB, CS, AuNPs, RGO and β-CD in nanocomposites. First, nano gold particles have a large specific surface area, strong adsorption capacity and larger surface free energy. Enzymes can be strongly immobilized on the surface of nanoparticles and cannot leak easily. Secondly, graphene can provide more reactive sites and maintain the enzyme activity well. The large specific surface area and good biocompatibility provide a good microenvironment for the enzyme. β-CD reversibly binds to acetylcholine, which improves the selectivity of the sensor. The introduction of PB-CS can effectively oxidize thiocholine, which reduces the working potential of the sensor from 0.65 V to 0.2 V, and reduces the interference of some bioactive substances. In addition, the CS film on the surface of the electrode network can not only prevent the leakage of electroactive substances and improve the selectivity of the sensor, but also effectively prevent the leakage of the enzyme.

Conclusion

Graphene was prepared by chemical reduction method to characterize the graphene structure and surface morphology. Using glucose oxidase as a model, the application of graphene in biosensors was investigated. PB-CS/RGO-AuNPs-β-CD nanocomposite films were prepared by one-step electrochemical reduction. An enzyme inhibitory biosensor based on AChE was constructed. The results show that the introduction of graphene greatly improves the performance of the sensor and improves the sensitivity of the sensor. RGO-AuNPs-β-CD composite films were obtained by one-step electrodeposition. Graphene is combined with nanoscale gold. The synergy between the two is fully brought into play. It enhanced the electrochemical response signal of thiocholine, and increased the sensitivity and selectivity of

the sensor. The PB in nanocomposites makes the two sensors have lower working potential and improve the selectivity of the sensor. It has potential application value in the motion sensing of human body.

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