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Functionalized gold nanoparticles/reduced graphene oxide nanocomposites for ultrasensitive electrochemical sensing of mercury ions based on thymine–mercury–thymine structure

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ABSTRACT

A sensitive, selective and reusable electrochemical biosensor for the determination of mercury ions (Hg^{2+}) has been developed based on thymine (T) modified gold nanoparticles/reduced graphene oxide (AuNPs/rGO) nanocomposites. Graphene oxide (GO) was electrochemically reduced on a glassy carbon substrate. Subsequently, AuNPs were deposited onto the surface of rGO by cyclic voltammetry. For functionalization of the electrode, the carboxylic group of the thymine-1-acetic acid was covalently coupled with the amine group of the cysteamine which self-assembled onto AuNPs. The structural features of the T bases functionalized AuNPs/rGO electrode were confirmed by attenuated total reflection infrared (ATR-IR) spectroscopy and scanning electron microscopy (SEM) spectroscopy. Each step of the modification process was characterized by cyclic voltammetry (CV) and electrochemical impedence spectroscopy (EIS). The T bases modified AuNPs/rGO electrode was applied to detect various trace metal ions by differential pulse voltammetry (DPV). The proposed biosensor was found to be highly sensitive to Hg^{2+} in the range of 10 ng/L–1.0 μg/L. The biosensor afforded excellent selectivity for Hg^{2+} against other heavy metal ions such as Zn^{2+}, Cd^{2+}, Pb^{2+}, Cu^{2+}, Ni^{2+}, and Co^{2+}. Furthermore, the developed sensor exhibited a high reusability through a simple washing. In addition, the prepared biosensor was successfully applied to assay Hg^{2+} in real environmental samples.

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1. Introduction

According to highly toxic and bio-accumulative properties, contamination of heavy metal ions has attracted serious concern (Lin et al., 2009b). Mercury ion (Hg^{2+}), as a kind of widely used heavy metal ions, is one of the most toxic elements that impacts the health of human and ecological environment (Li et al., 2014b). If the Hg^{2+} introduced into the environment, inorganic mercury can be easily methylated to organic mercury, methylmercury, which accumulates along the food chain and has more adverse health effects on mammals (Chen et al., 2013; Laffont et al., 2015). Recent studies have shown that exposure to very low level of Hg^{2+} for ages is harmful to the nervous and immune system of humans, that can be implicated in a number of health problems, such as respiratory failure, pulmonary edema and nephrotic syndrome (Morel et al., 1998; Ekino et al., 2007; Zhang et al., 2013; Jiang et al., 2011). Depending on the World Health Organization, the safety value of Hg^{2+} in drinking water is 1 μg/L (Li et al., 2014b). Therefore, it is important to develop novel sensitivity and selectivity detection methods for identification of trace amount of Hg^{2+}.

Until now, various kinds of analytical technique for the detection of Hg^{2+} were reported, including fluorescence (Guo et al., 2009), atomic absorption spectroscopy (Pourreza and Ghanemi, 2010), and inductively coupled plasma-optical emission spectroscopy (Acon et al., 2000). Even though these techniques could determine very low concentrations toward Hg^{2+}, while they all involve requiring expensive instruments and materials, high operating costs and require complicated procedures. Comparatively, electrochemical methods have more attractive in measuring trace heavy metal ions not only in laboratory but also for *in situ* measurements, due to the simplicity, low cost, high sensitivity, and good selectivity (Hezard et al., 2012; Lin et al., 2009a, 2010). Many electrochemical techniques are used for heavy metal ions detection, such as differential pulse voltammetry (DPV) (Xiong et al., 2015), square wave voltammetry (SWV) and electrochemical impedance spectroscopy (EIS) (Lin et al., 2012; Jia et al., 2015). Moreover, the association of a pre-concentration step with these pulsed techniques allows low detection limits reached to picomolar (Salaun, van den Berg, 2006).

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Au nanoparticles (AuNPs) have been widely employed for electrochemical detection of Hg^{2+} (Miao et al., 2009). Hezard and co-workers described an electrochemical sensor for Hg^{2+} based on AuNPs modified electrode (Hezard et al., 2012). AuNPs decorated carbon fiber and graphene quantum dots functionalized AuNPs also have been used to electrochemically detect the low concentration of Hg^{2+} (Li et al., 2014b). Gold is the remarkable matrix for the electrochemical determination of Hg^{2+}, because of a high affinity of Au towards Hg which could enhance the effect of pre-concentration. However, after pre-concentration and stripping processes, the Hg atoms cannot be removed completely from the Au substrate (Lin et al., 2015; Giacomino et al., 2008). On the other hand, Graphene oxide (GO) and reduced graphene oxide (rGO) have been utilized for electrochemical sensor development, attribute to its high electrical conductivity, biocompatibility, and versatile surface modification (Huang et al., 2014; Zhou et al., 2013). In order to enhance the electrochemical performance, it is extremely attractive to development hybridized biosensors based on the synergistic cooperation between GO and AuNPs. For example, a glucose electrochemical biosensor was developed using chitosan/rGO/AuNPs modified Pt electrode by Chen and co-workers (Fang et al., 2015), and an ultrasensitive detection of lead ion based on DNAzyme modified AuNPs/GO nanocomposites was also reported (Li et al., 2014a). Meanwhile, special thymine-thymine (T–T) mismatches show high selectivity for Hg^{2+} against other metal ions to form T–Hg^{2+}–T complexes have been proved (Zhang and Guo, 2012; Tanaka et al., 2007). Based on this principle, various DNA-based biosensors for Hg^{2+} determination were developed (Li et al., 2009; Wang et al., 2008; Tang et al., 2012). Although rarely selectivity and stable results could be achieved through the modification procedure, the labeled probes involve the drawback of costly and complex synthesis are still remained (Xiong et al., 2015; Jia et al., 2015).

To address these issues, we proposed a sensitive, and selective strategy for electrochemical detection of Hg^{2+} based on T bases modified AuNPs/rGO electrode. Firstly, AuNPs were electrochemical deposited onto the surface of rGO, and then the amine group was introduced by a self-assembled monolayer of cysteamine (CA) on the AuNPs which was covalently coupled with the carboxyl group of the thymine-1-acetic acid (T-COOH). The specific binding of T bases and Hg^{2+} forming interaction to T–Hg^{2+}–T complexes in aqueous solution. With the electrochemical signal amplification of AuNPs/rGO nanocomposites, the biosensor satisfactorily detected Hg^{2+} in the range from 10 ng/L to 1.0 μg/L, and a detection limit down to 1.5 ng/L. In the present study, the T bases modified AuNPs/rGO electrode showed greater affinity towards Hg^{2+}, compared to the other heavy metal ions. What is more, the prepared electrochemical biosensor could be regenerated by simple washing in EDTA solution, and successfully implemented for detection of trace Hg^{2+} in real environmental samples.

2. Experimental

2.1. Chemicals and materials

Natural graphite powder (325 meshes) was purchased from Alfa Aesar. 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS), 2-(N-morpholino) ethanesulfonic acid (MES), and cysteamine (CA) were purchased from Sigma-Aldrich. Gold chloride trihydrate ($HAuCl_4 \cdot 3H_2O$) and thymine-1-acetic acid (T-COOH) were obtained from J&K Scientific Ltd. (Shanghai, China). All the metal ion standard stock solutions and other chemicals were purchased from the Aladdin Chemistry Co. Ltd (Shanghai, China). The reagents were of analytical grade, and were used without further purification.

Ultrapure water (>18 MΩ, Pall Corp., USA) was utilized throughout for preparation of all solutions. 10 mM phosphates buffered solution (PBS, 0.5 M NaCl, pH 7.0) prepared by mixing appropriate amounts of Na_2HPO_4 and NaH_2PO_4 was employed as a supporting electrolyte during the analysis of metal ions. 100 mM MES (pH 6.0) was used in further modification steps, and pH was adjusted using either NaOH or HCl solutions. Various concentrations of different metal ions, such as, Zn^{2+}, Cd^{2+}, Pb^{2+}, Cu^{2+}, Ni^{2+}, Co^{2+} and Hg^{2+} were prepared by diluting high concentration standard stock solutions.

2.2. Apparatus

All electrochemical measurements were performed in a conventional three-electrode cell comprising a modified glassy carbon electrode (GCE) (5 mm in diameter) as the working electrode, a platinum plate with a surface area of 4 cm^2 as the counter electrode and a saturated calomel electrode (SCE) as the reference electrode using a CHI750D electrochemical workstation (Chenhua Instruments, Shanghai, China). The solutions were deoxygenated by bubbling with high-purity nitrogen for at least 15 min prior to each experiment. Attenuated total reflection infrared (ATR-IR) spectra were obtained by infrared spectrophotometry (Bruker, Tensor 27). The surface morphology was characterized by field emission scanning electron microscope (FESEM, Hitachi S-4800).

2.3. Biosensor fabrication

Prior to the modification process, GCE was firstly polished carefully with 1.0 μm, 0.3 μm and 0.05 μm alumina slurries until a mirror surface was established, followed by washed ultrasonically with ultrapure water, ethanol and ultrapure water, respectively. The GCE was then electrochemically cleaned by cycling the potential between -0.3 and $+1.5$ V (vs. SCE) at 100 mV/s in a 0.5 M H_2SO_4 solution until a steady-state electrochemical oxidation and reduction wave was obtained (Jiang et al., 2011). Subsequently, the pretreated electrode was rinsed with ultrapure water and dried with nitrogen.

GO was synthesized from natural graphite powder using a modified Hummer's method. The GO nanosheets were obtained through the oxidative treatment of natural graphite by ultrasonic exfoliation (Kovtyukhova et al., 1999). As-purified GO nanosheets were dispersed in water by ultrasonic technique, giving a yellow–brown dispersion with a concentration of 1.0 mg/mL. Then, 5 μL GO solution was drop-casted onto the GCE surface and dried under an infrared lamp for 30 min. In order to form rGO film modified GCE, the GO coated GCE was electrochemically reduced by cyclic voltammograms from -0.2 to -1.6 V at a scan rate of 50 mV/s in an electrolyte of 0.5 M Na_2SO_4 solution (Chen et al., 2013). AuNPs were prepared according to the reported method (Benvidi et al., 2015). Briefly, AuNPs were electrochemical deposited on the surface of rGO film under cyclic voltammetry (CV) scanning from $+0.2$ to $+1.0$ V in 0.25 mM $HAuCl_4$ solution with a scan rate of 50 mV/s for 10 cycles (Lin, 2015). The AuNPs/rGO electrode was rinsed with ultrapure water before and after each modified step.

CA modified AuNPs/rGO electrode was prepared by incubating the AuNPs/rGO electrode into 2 mg/mL CA solution (C_2H_5OH: $H_2O=3:1$) at 4 °C for 10 h. The CA/AuNPs/rGO modified electrode was washed thoroughly with ultrapure water in order to remove the reagents which have weakly interaction with the electrode surface. T-COOH was modified to the surface of CA/AuNPs/rGO electrode through amide bond, which was achieved by immersing the CA/AuNPs/rGO electrode into 1 mg/mL T-COOH MES buffer solution (100 mM, pH 6.0) containing 100 mM EDC/NHS for 10 h.

Then, the T/AuNPs/rGO modified electrode was rinsed with MES buffer and stored at 4 °C in PBS.

2.4. Electrochemical measurements

The standard solutions with different concentrations of Hg^{2+} were prepared by successively diluting the stock solution. The T/AuNPs/rGO modified electrode was immersed in each Hg^{2+} standard solution, leading to formation of the specific T–Hg^{2+}–T complexes. After rinsing thoroughly with PBS, the Hg^{2+} captured T/AuNPs/rGO modified electrode was transferred into the metal ion-free PBS while a potential of −0.4 V was applied for about 120 s to reduce Hg^{2+}, and the electrochemical response was measured by differential pulse voltammetry (DPV) measurement from −0.5 to +0.5 V at an amplitude of 20 mA, a pulse width of 0.05 s, a pulse period of 0.2 s and quiet time of 2 s. In order to study the selectivity of the T/AuNPs/rGO modified electrode towards Hg^{2+}, the sensor performance was investigated against PBS containing various cations (e.g. Cd^{2+}, Pb^{2+}, Cu^{2+}, Ni^{2+}, Zn^{2+}, and Co^{2+}). The detection of Hg^{2+} was repeated at least 3 times, and the measurements for various metal ions were performed under the same conditions. After each electrochemical determination, the used electrode was regenerated by immersing into 100 mM EDTA solution to remove the Hg^{2+} from the electrode surface. Prior to get reused for another assay, the regenerated electrode was washed thoroughly with PBS.

3. Results and discussion

3.1. Preparation and characterization of the electrochemical Hg^{2+} biosensor

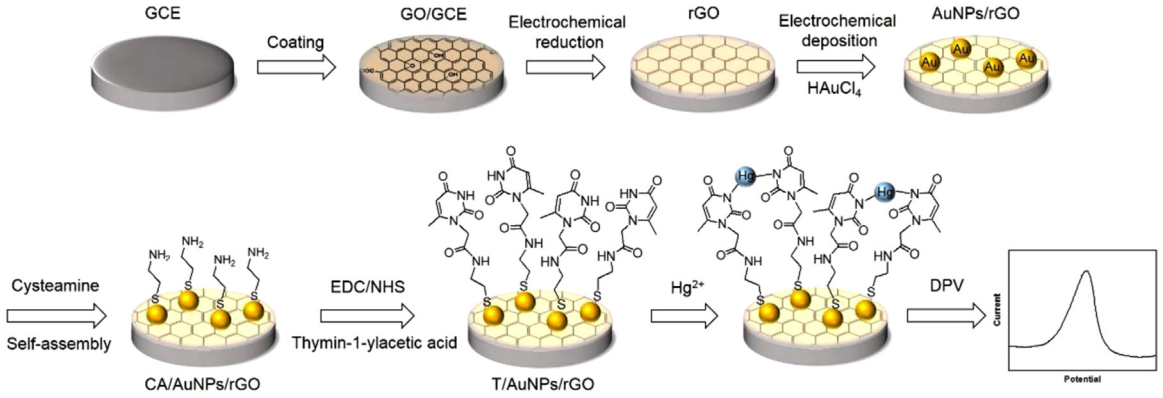
Scheme 1 illustrates the steps involved in the sensing strategy for Hg^{2+} electrochemical detection based on electrochemical signal amplification by the AuNPs/rGO nanostructure and the specific combination between Hg^{2+} and T bases. It is well known that Hg^{2+} ion can selectively bind between two thymine (T) bases with strong affinity and form stable T–Hg^{2+}–T linkage (Xiong et al., 2015). Compared with the T bases chained in the DNA strand, weaker space resistance between the small molecule and Hg^{2+} could lead to the coordination more easily. Amount of Hg^{2+} can be captured through the specific binding on the modified electrode surface. Attribute to the synergistic and amplifying effects of AuNPs/rGO nanocomposites, the electrochemical responses could be enhanced (Zhou et al., 2013).

In order to characterize the modified films, SEM analyses were performed. As shown in Fig. 1a, the rGO film reveals a typical wrinkled and crumpled surface of graphene. Fig. 1b displays the AuNPs layer which produced on the surface of rGO by electrochemical techniques. After the reduction of Au ions, the AuNPs are homogenously distributed onto the rGO surface, and the distances between the NPs are uniform. Attributed to the formation of new nucleation and the growth of particles through the electrochemical deposition process, two distinct morphologies were synthesized (Hezard et al., 2012). The two different particle sizes are found to be 50 ± 5 nm and 10 ± 5 nm, respectively. The structure of the T/CA/AuNPs/rGO film is presented in Fig. 1c. With conjugation of the CA and T bases to the modified electrode, the surface morphology of the T/CA/AuNPs/rGO film shows little departure from the original one presumably due to the small size of the modifiers.

The ATR-IR spectrum of the modified electrodes are displayed in Fig. S1. From the spectra of CA/AuNPs/rGO modified electrode, the strong peak observed at 3034 cm^{-1} corresponds to the N–H stretching, suggesting that the successful incorporation of the amino groups from CA to the AuNPs/rGO surface (Tedsana et al., 2013). In the spectrum obtained after modification of the T bases on the CA/AuNPs/rGO film, a new peak of C=O band appears at 1656 cm^{-1}, indicating that the predicted surface structure of T bases functionalized CA/AuNPs/rGO film through amide bonds and the surface of T/AuNPs/rGO electrode is capped with carboxylates groups (Lin et al., 2010).

To investigate the reduction of GO, XPS measurements for GO and rGO were carried out. The C_{1s} spectrum of GO (Fig. S2a) can be deconvoluted into four peaks by fitting analyses. The peaks at binding energies of 284.5, 286.7, 287.4 and 288.2 eV corresponding to C–C, C–O, C=O and O–C=O, respectively (Akhavan, 2010). As the C_{1s} XPS spectra of rGO (Fig. S2b) shown, the main C_{1s} peak located at 284.8 eV can be attributed to sp^2 and sp^3 hybridized carbon from the reduced graphene oxide (C–C) (Xiang et al., 2011). It is worth noting that the peaks at 286.6, 287.5 and 287.0 eV related to epoxy/hydroxyl (C–O), carbonyls (C=O) and carboxyls groups (O–C=O) are decreased significantly. This indicates that the GO coated on the surface of the GCE has been efficiently reduced into rGO in the electrochemical reduction process by CVs (Li et al., 2015).

The electrochemical characterization for fabricating the electrochemical Hg^{2+} sensor was investigated by EIS (Fig. 2). The Nyquist plots including a semicircle portion at higher frequencies corresponding to the electron-transfer-limited process and a linear part at lower frequency range representing the diffusion-limited process (Su et al., 2012). Charge transfer resistance (R_{et}) of the electrode can be obtained from the diameter of the semicircle. The curves of rGO/GCE and AuNPs/rGO/GCE exhibits gradually smaller radius of semicircles compared with bare GCE, which showed that the presence of AuNPs and rGO could enhance the electron transfer process. Compared with the radius of the semicircle with



Scheme 1. A schematic of the thymidine functionalized biosensor for Hg^{2+} detection.

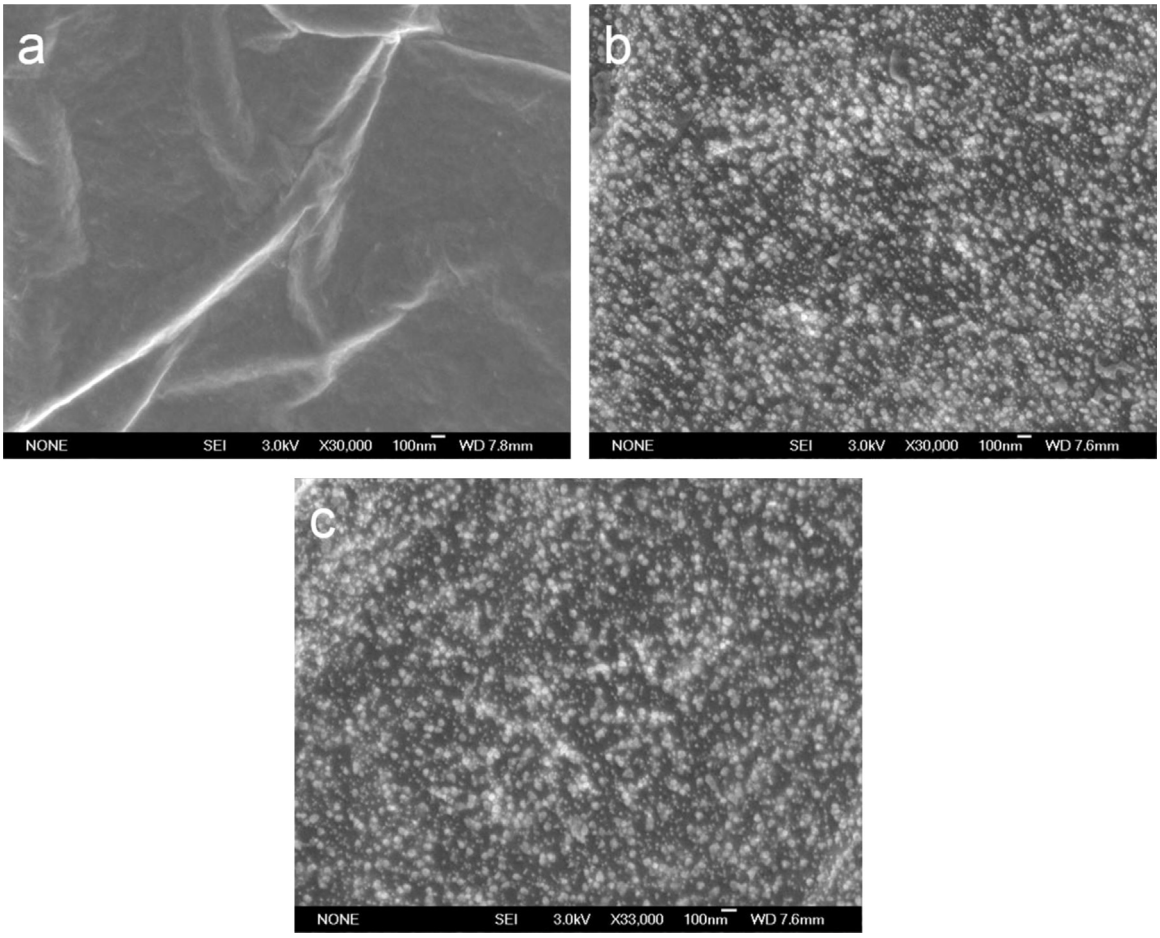


Fig. 1. SEM images of rGO (a), AuNPs/rGO (b) and T/CA/AuNPs/rGO (c) films on the surface of the modified electrode.

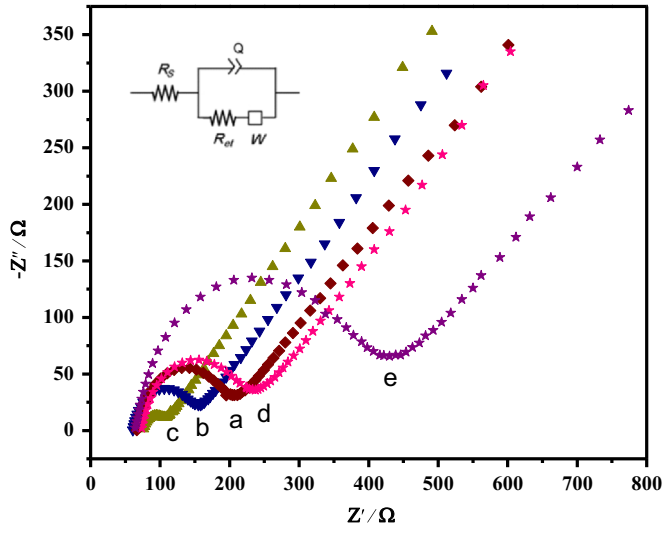


Fig. 2. Nyquist plots of the different electrode in 5 mM $K_3Fe(CN)_6$ aqueous solution containing 0.1 M KCl: (a) GCE, (b) rGO/GCE, (c) AuNPs/rGO/GCE, (d) CA/AuNPs/rGO/GCE, (e) T/CA/AuNPs/rGO/GCE. The inset is the equivalent circuit used to model impedance data in the form of Rs, W, Ret and Q representing the solution resistance, the Warburg diffusion resistance, the electro-transfer resistance and the double layer capacitance, respectively.

AuNPs/rGO/GCE electrode (curve c), the radius of the semicircle with CA/AuNPs/rGO/GCE increased (curve d). The EIS data indicates the successful formation of a CA self-assembled monolayer hindering the electron transfer. When T bases were immobilized on the electrode, the semicircle of the EIS curve obviously increased (curve e), suggesting that the T bases layer on the electrode not only impede the access of redox probe but also block the electron transfer (Wei et al., 2015). The impedance results were in good agreement with the conclusion from the CV scannings (Fig. S3). After recording and analyzing the gold oxidation and reduction process by CV (Fig. S4), the surface coverage of the AuNPs after modification was calculated as 64.3%.

3.2. Optimization of experimental parameters

The effect of accumulation time on peak current was investigated. Fig. 3a depicts the peak currents of the biosensor to 1 μg/L Hg^{2+} at varying incubation time. The peak currents increase rapidly with incubation time from 0 to 15 min, and then they reach a saturation value after 15 min. The result suggests that a period of 15 min is optimal for forming T–Hg^{2+}–T complexes on T/AuNPs/rGO surface through the specific interaction between T bases and Hg^{2+}. Moreover, from the respects of sensitivity and short time analysis, 15 min incubation time is chosen in the following measurements.

The acidity of the incubation solution has a considerable effect on the electrochemical behavior of Hg^{2+} which is important to the sensor sensitivity (Zhou et al., 2013). The effect of pH on the stripping peak current of Hg^{2+} at T/AuNPs/rGO electrode is displayed in Fig. 3b. The peak currents increase gradually in the range of pH 3.0–7.0, and then a notable decrease from 7.0 to 8.0. The reason might be the precipitation of Hg^{2+} at high pH value and T bases quaternarization at low pH value. Therefore, pH 7.0 is selected for the electrochemical texts throughout subsequent

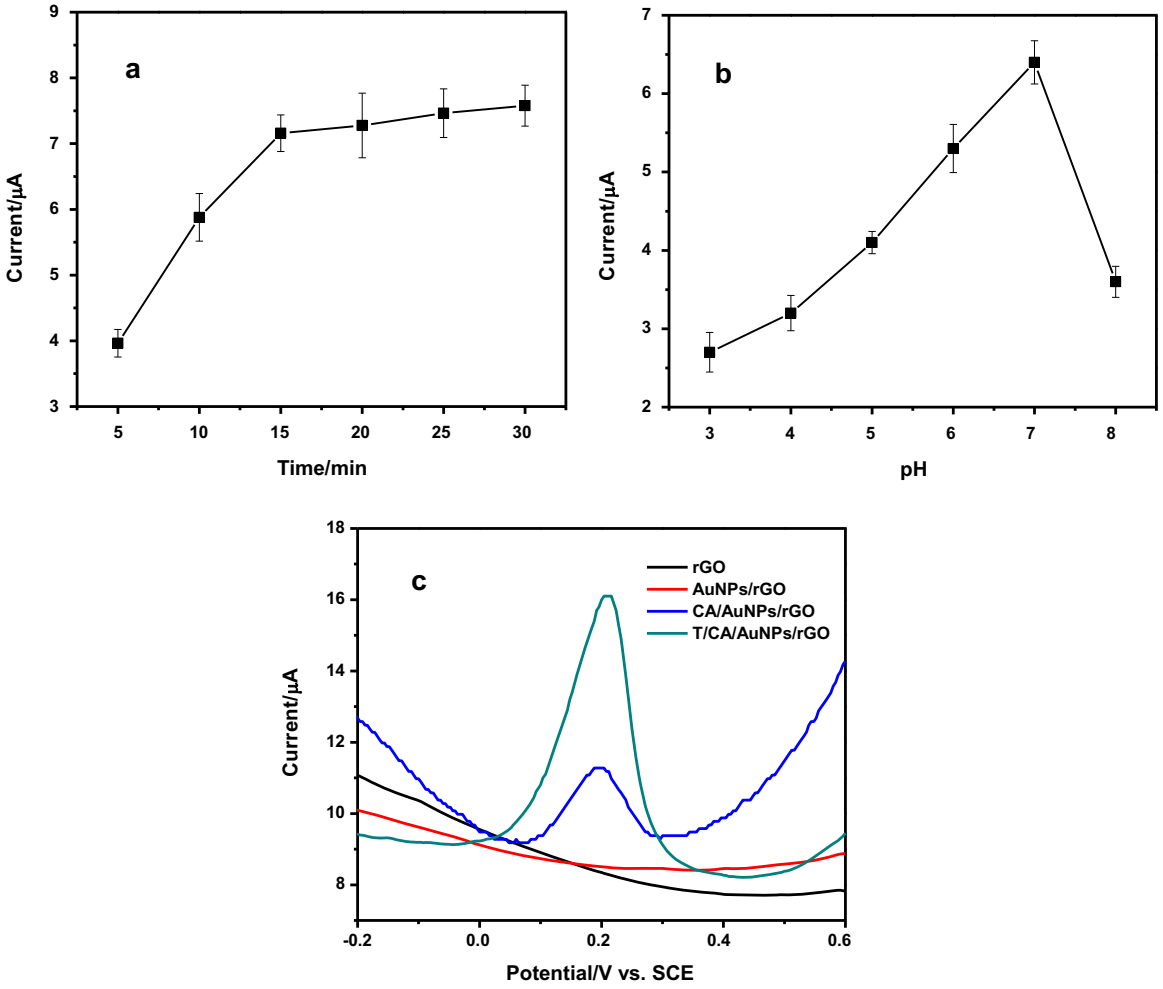


Fig. 3. Effects of incubation time (a) and pH (b) on the current responses of 1 μg/L Hg^{2+} on T/CA/AuNPs/rGO modified GCE electrode. Error bar represents the standard deviation of three repetitive experiments. (c) DPV curves of Hg^{2+} recorded at four different types of modified electrodes in 10 mM PBS (0.5 M NaCl, pH 7.0).

experiments (Liao et al., 2015).

3.3. *Analytical performances for Hg^{2+} detection*

In order to verify the effect of each component in the modified electrodes, electrochemical responses for four different types of electrodes were carried out by DPV. All the electrodes were immersed into 1.0 μg/L Hg^{2+} PBS at open circuit for 15 min, washed and reduced at a potential of −0.4 V for 120 s to analyze the DPV responses vs. a SCE (KCl sat'd) electrode. As shown in Fig. 3c, no appreciable electrochemical features are obtained at rGO and AuNPs/rGO modified GCE after accumulation and reduction. Owing to the complexing ability of CA to Hg^{2+} on the electrode surface, a small stripping peak at around 0.20 V is appeared at CA/

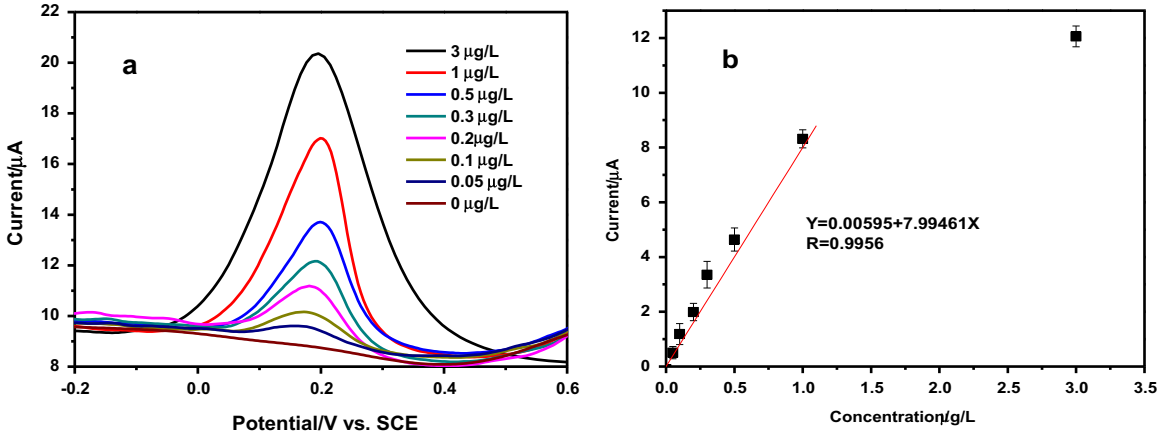


Fig. 4. (a) DPV curves for dissolution of Hg^{2+} captured by T/CA/AuNPs/rGO modified electrode in the range of 10 ng/L–1.0 μg/L Hg^{2+}, (b) calibration curve for T/CA/AuNPs/rGO modified electrode; error bars show the standard deviations of measurements taken from three independent experiments. Error bar represents the standard deviation of three repetitive experiments.

AuNP/rGO modified electrode (Pei et al., 2012). The peak at 0.20 V is observed because of electrochemical oxidation of Hg (Liao et al., 2015). The stripping voltammetry of the T/CA/AuNPs/rGO modified electrode shows a remarkably oxidative peak, and the stripping peak current is enhanced nearly 3.5-fold higher than that of CA/AuNP/rGO modified electrode, indicating that the surface-exposed T bases could effectively capture Hg^{2+}.

DPV is one of the most sensitive methods for heavy metal ions determination, due to its lower electrochemical detection limit (Liao et al., 2015). Under the same experimental conditions, the DPV responses for different concentrations of Hg^{2+} are illustrated in Fig. 4a. The peak current from DPV is proportional to the amount of Hg^{2+} in the range of 10 ng/L–1.0 μg/L, and the calibration curve (Fig. 4b) shows a linear increase in current with the Hg^{2+} concentration. The linear relative coefficient is found to be 0.9956 and the detection limit is 1.5 ng/L, demonstrating a high sensitivity of the T/CA/AuNPs/rGO modified electrode to Hg^{2+}. The high sensitivity is attributed to the strong affinity of T bases toward Hg^{2+}, as well as the electrochemical signal amplification by the AuNPs/rGO nanostructure. A comparison of the performance characteristics between the T/CA/AuNPs/rGO modified electrode with some reported chemically modified electrodes for electrochemical determination of Hg^{2+} is summarized in Table S1.

3.4. Selectivity and regeneration of the biosensor

The selectivity of the electrochemical Hg^{2+} biosensor was investigated in the presence of common heavy metal ion interferences, such as Zn^{2+}, Cd^{2+}, Pb^{2+}, Cu^{2+}, Ni^{2+}, and Co^{2+}. Fig. 5 displays the electrochemical responses obtained for the interfering metal ions one ten-fold more concentrated than Hg^{2+}. It is easily found that the value of the T/CA/AuNPs/rGO modified electrode in the presence of Hg^{2+} is greater than that in the presence of other metal ions. The stripping peak current of Hg^{2+} changes slightly in a mixing sample, indicating that these metal ions do not interfere with the determination of Hg^{2+}. The high selectivity of the sensing methods is due to the specific coordination between Hg^{2+} ions and T bases to form stable T–Hg^{2+}–T complexes. Therefore, the developed biosensor has excellent selective response to Hg^{2+} against other metal ions.

It is important to investigate the regeneration performance of the biosensor. After measurements, the regeneration of the modified electrodes was performed by soaking in 100 mM EDTA solution for 30 min. It is clear that almost all of the Hg^{2+} was removed from the surface of the modified electrode by EDTA-mediated regeneration process (Fig. S5). From the results, there is only 1–2% decrease in peak currents compared to use the freshly prepared T bases modified electrode at the first regeneration step. After 30 cycles by regeneration process, the regenerated electrode retained about 92% of its initial sensitivity, indicating that the modified electrode can be easily regenerated and reused for Hg^{2+} detection.

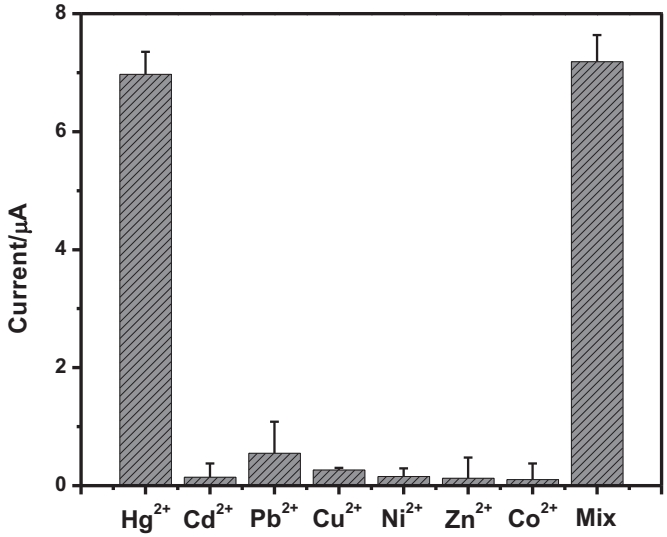


Fig. 5. Electrochemical responses for T/CA/AuNPs/rGO modified electrode to different metal ions, relative to the signal of Hg^{2+}. The concentration of Hg^{2+} was 1.0 μg/L and the others were 10 μg/L. Error bar represents the standard deviation of three repetitive experiments.

Table 1
Spike recovery efficiency of the thymidine functionalized biosensor for Hg^{2+} in the tap water ($n=3$).

Tap water	Spiked (μg/L)	Found (μg/L)	Recovery (%)
1	0.02	0.021	105
2	0.05	0.047	94
3	0.1	0.095	95

Recovery $=(C_{Found}-C_{Spiked})/C_{Spiked}\times 100$.

3.5. Real sample analysis

In order to assess the applicability of the T/CA/AuNPs/rGO modified electrode, the electrochemical performance of the modified electrode was tested in tap water. The samples were spiked with different concentrations of Hg^{2+} and the measurement results were shown in Table 1. From Table 1, the high recovery percentage demonstrates that the developed T/CA/AuNPs/rGO modified electrode has an excellent capability for accurate determination of Hg^{2+} in environmental water samples.

4. Conclusions

A thymine bases functionalized AuNPs/rGO GCE electrode was developed for the electrochemical determination of Hg^{2+}. GCE was modified with rGO and AuNPs by the electrochemical method. Thymine-1-acetic acid exhibiting high affinity to Hg^{2+} was covalently bound to AuNPs using CA as a linker. The modification of the biosensor was characterized by SEM, ATR-IR and EIS. Based on T–Hg^{2+}–T coordination chemistry, the biosensor shows greater preferential affinity to Hg^{2+}, while other coexisting ions have no interference. The results demonstrate that the T/CA/AuNPs/rGO modified electrode are highly sensitive to Hg^{2+} in the range of 10 ng/L–1.0 μg/L, and the detection limit is 1.5 ng/L. Moreover, the modified electrode has excellent high reusability, could be regenerated by simple washing in EDTA solution. The analytical application of the modified electrode is evaluated with good results for detection of Hg^{2+} through the electrochemical measurements.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in

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