Chitosan-Stabilized Gold Nano Composite Modified Glassy Carbon Electrode for Electrochemical Sensing Trace Hg$^{2+}$ in Practice

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A modified nano gold-based sensor, being stabilized on the substrate of polymer chitosan (chitosan-AuNPs), was developed for analyzing trace Hg$^{2+}$ by cyclic voltammetry, which expected possessing excellent hydrophilic, high dispersion, little aggregation and nice biological compatibility. By virtue of the predominant electro-chemical property of AuNPs combining with the strong chelating to Hg$^{2+}$ of chitosan main chain, the proposed glassy carbon electrode modified by chitosan-AuNPs could exclusively sense Hg$^{2+}$ using cyclic voltammetry by a mixed diffusion and adsorption-controlled process, accompanying with a good linear range of 1.0–2200 μg/L and a detection limit as low as 1.3 × 10$^{-7}$ μg/L. What’s more, it was successfully applied for detecting Hg$^{2+}$ in environmental water samples and some fruit juices with excellent recovery between 96.2 and 105.8% and good R.S.D. < 4.1%. The work offers a simple way to stabilize gold nano-based electro-chemical sensors for practical applications environment-friendly.

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Nowadays, the situation of environmental contamination is getting more and more serious, especially from heavy metals with the rapid development of modern industry, agriculture and mineral industry.$^{1−6}$ Among them, Hg$^{2+}$ is one of the most toxic, which could cause the dysfunction of proteins or enzymes to result in a variety of diseases in brain, kidney, central nervous system, due to its strong affinity to S-containing substrates.$^{7−10}$ Accordingly, great efforts have been devoted to design and develop a series of analytic methods for Hg$^{2+}$ detection during the past years, such as atomic spectrometry,$^{11−13}$ mass spectrometry,$^{14,15}$ liquid chromatography,$^{16−18}$ fluorescent spectrophotometry,$^{19−24}$ colorimetric analysis$^{25−27}$ and electrochemical analysis$^{28−31}$ with excellent performances and efficiency. However, most of them are achieved at the expenses of expensive instrumentation, cumbersome laboratory procedures or poor analytical accuracy, which could not be applied straightforward and conveniently to meet the limit of 3.0 × 10$^{-8}$ mol·L$^{-1}$ defined by the World Health Organization (WHO) in drinking water.$^{32}$

Recently, gold nanoparticles (AuNPs) have attracted considerable attention in electrochemical fields by virtue of the excellent catalytic activity and high stability.$^{33,34}$ Especially their good conductivity could improve the electron transfer to amplify electrochemical detection signals.$^{35−40}$ For example, Raj, et al.,$^{41}$ developed AuNP-based electrode for the amperometric detection of ultra-trace amount of toxic Cr(VI) with highly sensitivity (30 ± 0.2 nA/ppb) and low detection limit of 0.1 ppb, which was far below the guideline value set by World Health Organization (WHO). Mashhadizadeh, et al.,$^{42}$ presented a screen-printed electrode modified by 3-Mercapto propionic acid self-assembled on gold nano-particles for electrochemical determination of digoxin. By using signal-on strategy, the detection limit of digoxin was improved largely to 0.050 pM with a linear dynamic response range of 0.1 pM to 1.0 μM. Especially, Jena, et al.,$^{43}$ described an effective electrochemical method for simultaneous detection of As(III), Hg(II), and Cu(II) using a gold nanoelectrode ensembles, which were successfully applied for the detection of ultratrace As(III), Hg(II), and Cu(II) in the real samples. AuNPs and their derivatives will be one of the most promising electrochemical sensors for Hg$^{2+}$ detection with excellent performances.$^{44−48}$ Although AuNPs could be formed onto these electrodes by the electrochemical deposition easily,$^{49}$ here we aimed to prepare chitosan-AuNPs composite and then modify them on glassy carbon electrode (GCE) by a simple coating method, which needed no additional adhesion agent.

Given the complementary electro-chemical characteristics of AuNPs and the excellent biological compatibility of chitosan, AuNPs being stabilized on the substrate of polymer chitosan (chitosan-AuNPs) would be developed by one-pot reaction, which was further modified on glassy carbon electrode (GCE) to act as a sensitive electrochemical platform for the detection of toxic Hg$^{2+}$, meeting the threshold set WHO. The combination of conducting gold nanoparticles with biological compatible chitosan would expect providing a potential electrochemical candidate for environment-friendly sensing trace Hg$^{2+}$ in real fruit juice and aqueous samples.

Experimental

Reagents and apparatus.—Gold (II) chloride trihydrate, chitosan and all the other chemicals were of analytical grade, which were all purchased from Shanghai Chemical Reagent Company and used directly without any purification. Water applied in the work was doubly deionized.

Electrochemical measurements and impedance spectroscopy were carried out with CHI660 electrochemical analyser (Shanghai Chenhua Co., China). A traditional three-electrode system was employed in the measurements, with a chitosan-AuNPs modified glassy carbon electrode (chitosan-AuNPs/GCE) as the working electrode, an Hg|$\text{Hg}_2\text{Cl}_2$ electrode as the reference electrode and a platinum wire as the auxiliary electrode. The transmission electron micrograph (TEM) images were obtained on a JEM-2100PLUS$^{80}$-200kV transmission electron microscope. FTIR spectra of HNMAP with KBr disc was recorded using a Nicolet NEXUS 870 FTIR spectrophotometer at room temperature from 4000-500 cm$^{-1}$. UV-vis spectra were recorded on a Lambda 35 UV/vis spectrometer using a 1-cm square quartz cell. pH was measured using a PHS-25 pH meter.

Preparation of chitosan-AuNPs.—Into a 200 mL round-bottom flask, 0.75 g (0.05 mol) chitosan, 70.0 mL H$_2$O and 1.4 mL glacial acetic acid were dropped and swelled at 55°C for 5.0 h. To the mixture, 6.0 mL 10.0 g/L gold (III) chloride trihydrate solution was added and kept stirring for 45 min. After the temperature was increased to 85°C, 1.5 mL 0.1 mol/L ascorbic acid was added into and the temperature kept at 85°C for another 20 min. Then the resultant mixture was cooled down to room temperature, which was further centrifuged and washed with enough H$_2$O to get dark red AuNP-chitosan.

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Fabrication of the modified electrode.—Before modification, the bare glassy carbon electrode was sequentially polished with 1.0 μm, 0.3 μm and 0.05 μm alumina slurry, successively sonicated in H₂O, ethanol and H₂O for 5 min and then dried in air.

Chitosan-AuNPs dispersion in H₂O was centrifuged for 10 min at 3,000 rpm with the supernatant discarded and sonicated for 20 min to get a homogeneous dispersion. 10.0 μL chitosan-AuNPs dispersion was dropped onto the surface of GCE. Then the proposed chitosan-AuNPs/GCE was obtained after drying at room temperature.

Electrochemical measurements.—The electrochemical measurements were all performed in an electrochemical cell at room temperature. Cyclic voltammograms (CVs) were recorded in 0.001 M phosphate buffer solution (pH 7.0) at a scan rate of 100 mV s⁻¹ using KCl as supporting electrolyte. Electrochemical impedance spectroscopy (EIS) was performed in 5 μM HgNO₃ mixture at the open-circuit potential (Eocp) in 1.0 mol/L KCl solution containing 3.0 μmol/L Hg²⁺ with the frequencies ranging from 1.0 × 10⁵ to 1.0 Hz respectively.

Results and Discussion

Characterization of chitosan-AuNPs.—The as-prepared chitosan-AuNPs were characterized by TEM, FTIR and UV-vis spectroscopy as shown in Figure 1. From the TEM image (Figure 1a), we could find that the resultant AuNPs are uniformly distributed onto chitosan substrate with an average diameter of ca. 15.0 nm. The reason for the uniform distribution of AuNPs onto chitosan substrate maybe results from the strong coordination interaction between the blank d-orbits in AuNPs and the lone-pair electrons in O atoms or N atoms on chitosan substrate, as what we expected. For UV-vis spectrum, there is an obvious absorption peak at 521 nm, accompanied with two shoulder ones at 252 nm and 283 nm (Figure 1b). The peak at 521 nm originates from the surface plasmon of AuNPs and the ones at 252 nm and 283 nm result from the n→π* transition of OH, NH and C=O bonds of chitosan substrate, well hinting that the as-prepared composite composes of both AuNP and chitosan.

Further from FTIR spectra (Figure 1c), the characterized peaks of pure chitosan centered at ca. 3427, 1621 and 1074 cm⁻¹ could be observed, ascribed to the stretching vibration of N(O)-H, C-N and C-O bonds, respectively. While for the resulting chitosan-AuNPs, all the three peaks shift to longer wavenumbers owing to the fact that N and O atoms of N(O)-H, C-N and C-O bonds in chitosan chains chelate to the electron-deficient blank d-orbits in AuNPs, i.e., from 3427 to 3435 cm⁻¹ for N(O)-H bond, 1621 to 1644 cm⁻¹ for C-N bond and 1074 to 1089 cm⁻¹ for C-O bond. The FTIR peaks shifted to high-wavenumber further confirm that AuNPs grafted onto chitosan via strong electrostatic interaction as what we expected.

Electrochemical behavior of chitosan-AuNPs/GCE.—The electrochemical behaviors of bare GCE, Chitosan/GCE, AuNPs/GCE and Chitosan-AuNPs/GCE in the presence of Hg²⁺ were investigated by CVs firstly. As shown in Figure 2a, upon addition of Hg²⁺, both CV curves of bare GCE and Chitosan/GCE do not display any redox peak between 0 and −0.3 V. AuNPs/GCE shows only a weak oxidation peak, whereas GCE modified with chitosan-AuNPs displays an observable one at −0.175 V vs Hg/Hg₂Cl₂, indicating that Chitosan-AuNPs/GCE possesses outstanding sensitivity toward mercury and the present electrode could be utilized to detect Hg²⁺. Also, the content of chitosan in chitosan-AuNP composites was optimized as shown in Figure 2b. From it we could find that when the mass fraction of chitosan is 15.0%, the peak current of Chitosan-AuNPs/GCE reaches the maximum.

Electrochemical impedance spectroscopy (EIS) has also been utilized for investigating the interfacial electron transfer properties of bare GCE, Chitosan/GCE, AuNPs/GCE and Chitosan-AuNPs/GCE (Figure 2c). As shown in Figure 2c, the profiles of EIS curves for all tested electrodes are quite similar, i.e., high-frequency semicircles and a straight line at low frequency region. The corresponding electrical resistance was also listed in Figure 2c. From Figure 2c, we could find that the resistance of Chitosan-AuNPs/GCE was the lowest, indicating that the present composite electrode possesses superior sensitivity toward mercury.
Figure 3. a) The effect of pH on the cyclic voltammograms over pH 6.0∼10.0 containing 3.0 μmol/L Hg²⁺; b) The effect of scan rate on the cyclic voltammograms and c) the dependence of peak current on square root of the scan rate in 0.01 M phosphate buffer system (pH 7.0) containing 3.0 μmol/L Hg²⁺; d) Chronoamperometric response at chitosan-AuNPs/GCE in 0.01 M phosphate buffer system (pH 7.0) containing different cHg²⁺, i.e., 0, 80, 150 and 200 μmol/L.

Effect of pH and supporting electrolyte.—It’s well known that the redox potential of analyte is quite sensitive to pH. To ensure its practical application for electrochemically sensing Hg²⁺ with excellent repeatability and stability, the effect of pH on the cyclic voltammogram of Chitosan-AuNPs/GCE electrode was first investigated over pH 6.0∼10.0, owing to the fact that chitosan substrate could dissolve in low pH. As shown in Figure 3a, the peak current intensity increases significantly with pH increasing from 6.0 to 7.0 and reaches the maximum at pH 7.0. Afterward, the peak currents begin to decrease gradually with further increasing in pH, which could be attributed to the fact that Hg²⁺ would become unstable in high pH values. Hence, pH 7.0, a normal physiological pH value, is selected in all the experiments.

Further, the effect of some common supporting electrolytes, such as NaCl, Na₃PO₄, NaOH, NaNO₃ and Na₃Fe(CN)₆ on the peak current intensity was also investigated. It was found that the peak shape, position and intensity depend strongly on the electrolyte solution used. The maximum peak current value was obtained in the medium containing Na₃PO₄, and so Na₃PO₄ buffer system was selected in the work.

Effect of scan rate.—The influence of scan rate on the redox reaction of Hg²⁺ at chitosan-AuNPs/GCE was recorded using cyclic voltammetry with the scan rate ranging from 50 mV s⁻¹ to 500 mV s⁻¹. As shown in Figure 3b, the peak current intensity increases linearly with the scan rates from 100 mV s⁻¹ to 450 mV s⁻¹ and keeps nearly unchangeable to 500 mV s⁻¹. The fitted linear regression equation is I(μA) = −12.5959 + 2.3706 v¹/² (mVs⁻¹)¹/² with R² = 0.9919 (Figure 3c), hinting that the electrochemical reaction of Hg²⁺ on chitosan-AuNPs/GCE is controlled by the diffusion-controlled process.\footnote{ Further study is needed to understand the diffusion-controlled mechanism.}
Accordingly, the chronoamperometry curves were conducted for calculating the diffusion coefficient of Hg$^{2+}$ at chitosan-AuNPs/GCE in 0.01 M phosphate buffer system (pH 7.0), with different c$_{Hg^{2+}}$, i.e., 0, 80, 150 and 200 μmol/L as shown in Figure 3d. From Figure 3c and Cottrell’s equation as below,$^{51}$ the diffusion coefficient is calculated to be 2.16 × 10$^{-6}$ cm$^2$s$^{-1}$, suggesting the high detection rate for sensing Hg$^{2+}$.

\[ I = nFAD^{1/2}c^{1/2}t^{1/2} \]

where \( n \) is electron transfer number in the electrode reaction, \( F \) is Faraday constant, \( A \) is the electrode surface area, \( D \) is the diffusion coefficient (cm$^2$s$^{-1}$), \( c \) is the bulk concentration (mol cm$^{-3}$) and \( t \) is the reaction time.

**Effect of accumulation potential and accumulation time.**—The effect of the incubation time$^{52}$ and successive scans (number of cycles)$^{53}$ was explored for the electro-chemical response of Hg$^{2+}$ on Chitosan-AuNPs/GCE (Figures 4a and 4b). From Figure 4a, we could find that the peak current intensity increases slightly with incubation time changing from 0 s to 180 s, which could be attributed to the adsorption and accumulation of Hg$^{2+}$ at the electrode surface, hinting that the reaction process of chitosan-AuNPs/GCE could be controlled by adsorption controlled process to some extent too. Accordingly, the reaction process can be controlled by mixed diffusion and adsorption-controlled process. With further increase in the immersion time, the current intensity keeps nearly unchangeable, owing to Hg$^{2+}$ saturation on the surface of chitosan-AuNPs/GCE. It can also find that the peak current increases with successive scans (number of cycles) from 1st to 5th, and then keeps almost unchangeable from 5th to 8th cycle (Figure 4b), suggesting that the electro-chemical response of chitosan-AuNPs/GCE to Hg$^{2+}$ is stable and reliable. The reason may be attributed to no further sites for mercury on the surface of chitosan-AuNPs/GCE after 5th cycle, because the entire electrode surface has been already covered. Accordingly, a 180 s immersion time and the 5th cycle are selected for Hg$^{2+}$ qualification in the work.

**Interference studies.**—For Hg$^{2+}$ determination in environmental and biological samples, some coexist metal ions will make some potential interferences. Thus, interference experiments with some common metal ions in the environments, i.e., Ag$^+$, Ba$^{2+}$, Cd$^{2+}$, Cu$^{2+}$, Fe$^{2+}$, Fe$^{3+}$, Mg$^{2+}$, Na$^+$, Pb$^{2+}$ and Zn$^{2+}$ with equal equivalent to Hg$^{2+}$, were carried out to illustrate the electro-chemical selectivity of chitosan-AuNPs/GCE. From Figure 4c, we could find that no significant variation in peak current intensity of chitosan-AuNPs/GCE by comparison with or without other coexisted interfering ions. All the changes in peak current intensities are less than 5% relative to that in the presence of Hg$^{2+}$ only. The above results suggest that all the coexisted metal ions above have no impact on the electro-chemical detection of Hg$^{2+}$ using chitosan-AuNPs/GCE.

**Analytical parameters and samples detection.**—To discover the rule for chitosan-AuNPs/GCE as electrochemical sensing platform to sense Hg$^{2+}$, a series of cyclic voltammetry titration experiments were carried out with different c$_{Hg^{2+}}$ ranging from 0 mol/L to 300 × 10$^{-8}$ mol/L (Figure 5).

Figure 4. The relationship between the peak current intensity and a) the incubation time or b) successive scans (number of cycles) of Chitosan-AuNPs/GCE in 0.01 M phosphate buffer system (pH 7.0) containing 3.0 μmol/L Hg$^{2+}$; c) The relative peak currents in the presence of various metal ions (3.0 μM) without (black) and with (red) Hg$^{2+}$ in 0.01 M phosphate buffer system (pH 7.0).

Figure 5. a) The cyclic voltammetry titration curves of Hg$^{2+}$ with different c$_{Hg^{2+}}$ ranging from 0 mol/L to 300 × 10$^{-7}$ mol/L; 0, 1, 5, 15, 30, 50, 70, 100, 130, 150, 180, 200, 220, 250, 300 × 10$^{-8}$ mol/L; b) Linear plotted relationship of redox peak current intensities versus c$_{Hg^{2+}}$ in 0.01 M phosphate buffer system (pH 7.0).
The linear regression equation is 

$$I = 0.037c \times 10^{-8} \text{ mol L}^{-1} + 4.6197 \left( R^2 = 0.9820 \right).$$

According to the definition of detection limit, three times of average deviation of peak current intensity ($I$) in blank samples without Hg$^{2+}$ divided by the slope of the standard curve, the detection limit for Hg$^{2+}$ is as low as $1.3 \times 10^{-8}$ mol/L (S/N = 5), far below the limit of $3.0 \times 10^{-8}$ mol/L for drinking water defined by WHO.

To illustrate its potential application, the proposed chitosan-AuNPs/GCE was applied to directly detect Hg$^{2+}$ in some fruit juice and environmental water samples, i.e., from the Yi River, underground water and tap water in campus and 3 fruit-vegetable samples. For the recovery study, a fixed concentration of Hg$^{2+}$ of 100.0 $\times 10^{-8}$ mol/L was added into and the total Hg$^{2+}$ content was calculated using the present method. As shown in Table I, all the recoveries for Hg$^{2+}$ are between 96.2 and 105.8% with R.S.D. $\leq 4.1\%$, which are all quite consistent with the analytic results reported before by colorimetric methods. The excellent reproducibility for sensing Hg$^{2+}$ may be attributed to the stable AuNPs units in chitosan chains as well as the chelating effect between chitosan chains and AuNPs.

### Conclusions

In conclusion, a kind of chitosan-stabilized AuNP composite and its modified glassy carbon electrode (chitosan-AuNPs/GCE) were constructed and developed for electrochemical detection of Hg$^{2+}$ by a mixed diffusion and adsorption-controlled process. Under the optimized conditions, chitosan-AuNPs/GCE could exclusively sense Hg$^{2+}$ linearly within the range of 1.0 to 220.0 $\times 10^{-8}$ mol/L and a detection limit as low as $1.3 \times 10^{-8}$ mol/L (S/N = 5), due to the synergistic effects between the excellent electrical conductivity of AuNPs and the high chelating ability of chitosan substrate. More importantly, when the proposed sensor was directly applied for detecting Hg$^{2+}$ in some environmental water and fruit juice samples, all the results were quite satisfactory. These attractive electrochemical performances made the present sensing system promising for practical purposes environment-friendly.

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