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ORIGINAL ARTICLE

An ionic liquid supported CeO_2 nanoparticles–carbon nanotubes composite-enhanced electrochemical DNA-based sensor for the detection of Pb^{2+}

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Abstract An electrochemical sensor incorporating a signal enhancement for the determination of lead (II) ions (Pb^{2+}) was designed on the basis of the thrombin-binding aptamer (TBA) as a molecular recognition element and ionic liquid supported cerium oxide (CeO_2) nanoparticles–carbon nanotubes composite modification. The composite comprises nanoparticles CeO_2, multi-wall carbon nanotubes (MWNTs) and hydrophobic room temperature ionic liquid (RTIL) 1-ethyl-3-methylimidazolium tetrafluoroborate ($EMIMBF_4$). The electrochemical sensors were fabricated by immersing the CeO_2–MWNTs–$EMIMBF_4$ modified glassy carbon electrode (GCE) into the solution of TBA probe. In the presence of Pb^{2+}, the TBA probe could form stable G-quartet structure by the specific binding interactions between Pb^{2+} and TBA. The TBA-bound Pb^{2+} can be electrochemically reduced, which provides a readout signal for quantitative detection of Pb^{2+}. The reduction peak current is linearly related to the concentration of Pb^{2+} from 1.0×10^{-8} M to 1.0×10^{-5} M with a detection limit of 5×10^{-9} M. This work demonstrates that the CeO_2–MWNTs–$EMIMBF_4$ nanocomposite modified GCE provides a promising platform for immobilizing the TBA probe and enhancing the sensitivity of the DNA-based sensors.

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1. Introduction

Detection and quantification of metal ions and organic molecules found in biological systems and in the environment remains an active area of research, as these molecules are either quite beneficial or toxic to human health. Lead is one of the most toxic metallic pollutants, for example, lead can cause renal malfunction and inhibit brain development [1–3]. Although conventional detection methods, including atomic absorption spectrometry [4], inductively coupled plasma (ICP)-mass spectrometry [5], anodic stripping voltammetry [6], and atomic

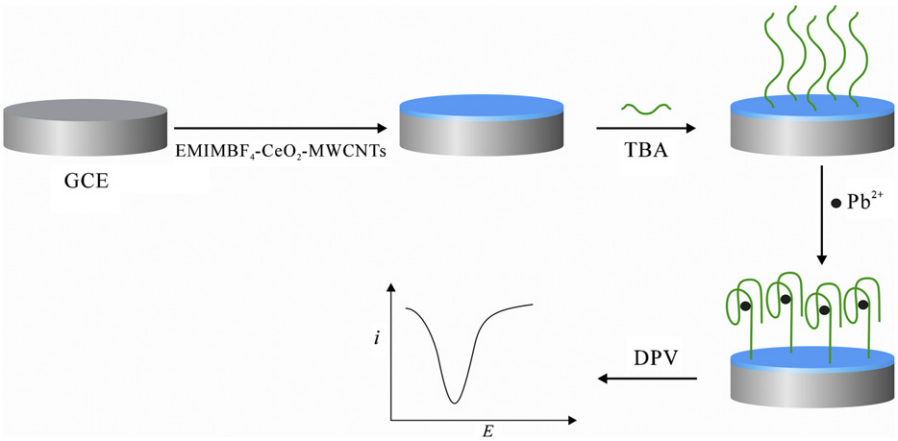

Figure 1 Schematic description of the electrochemical DNA-based sensor for the detection of Pb^{2+} ion.

fluorescence spectrometry [7], provide high sensitivity, they are not suitable for on-site detection because of their sophisticated equipments and sample treatments. In recent years, much optical and electrochemical techniques have been developed for the selective detection of Pb^{2+} based on small molecules [8], DNAzymes [9–13], oligonucleotides [14], polymers [15], and functional nanoparticles [16]. For example, Li and Lu [9] and Liu and Lu [10] developed a fluorescence resonance energy transfer (FRET)-based DNAzyme system for Pb^{2+} sensing. The sensor was made of FRET between fluorophore and quencher labeled on the DNAzyme (17E) and its substrate, respectively. In the presence of Pb^{2+}, the 17E catalyzes hydrolytic cleavage of substrate and it turned on the fluorescence for sensing. Xiao et al. [11] developed an electrochemical DNAzyme-based Pb^{2+} biosensor fabricated by thiol-assembling a methylene blue-tagged thiol containing DNAzyme with a detection limit of 0.3 μM. However, many of these systems have limited practical use because of, for example, poor aqueous solubility, cross-sensitivity toward other metal ions, matrix interference, high cost (e.g., enzymes), complicated processing, the use of unstable molecules (e.g., RNA), or poor sensitivity [14]. Recently, Liu et al. [14] reported a technique for the highly selective and sensitive detection of Pb^{2+} using a thrombin-binding aptamer (TBA) probe labeled with the donor carboxyfluorescein and the quencher 4-([4-(dimethylamino) phenyl] azo) benzoic acid at its 5′ and 3′ termini. Owing to the high sensitivity, inherent simplicity, low cost, and excellent compatibility to miniaturization technology for electrochemical techniques, the development of electrochemical sensors for Pb^{2+} on the basis of TBA as a molecular recognition might hold great potential for decentralized studies and on-site monitoring.

Room temperature ionic liquid (RTIL), composed of organic cations and various anions, represents a kind of novel nonaqueous but polar solvent [17]. It exhibits many unique advantages such as high chemical and thermal stabilities, negligible vapor pressure, high ionic conductivity, wide electrochemical windows, low toxicity, and ability to dissolve a wide range of organic and inorganic compounds [18]. The attractive properties of RTIL make them promising candidates for electrochemical DNA-based sensor [19–21]. Increasing attention has been paid to the modified electrodes with ionic liquid and nanomaterial composite in hopes of combining their unique properties. In recent years, that inorganic oxide nanoparticles are utilized to be the immobilizing carriers of ssDNA probe is becoming the focus of research due to unique properties derived from their low dimensionality and possible quantum-confinement effects [22–24]. Among the inorganic oxide nanoparticles, cerium oxide (CeO_2) has been exploited as a promising material for biosensing owing to its unusual properties including large surface area, excellent biocompatibility, nontoxicity, high chemical stability, and strong adsorption ability (high isoelectric point ~9.2) [25,26]. It may be noted that positively charged surface of CeO_2 nanoparticles could be utilized for binding of negatively charged biomolecules. Recently, a novel nanocomposite membrane, comprising nanosized shuttle-shaped CeO_2, single-wall carbon nanotubes (SWNTs) and hydrophobic RTIL, was developed on the glassy carbon electrode for electrochemical sensing of the immobilization and hybridization of DNA [27]. However, to our best knowledge, the application of ionic liquid supported CeO_2 nanoparticles–multi-wall carbon nanotubes (MWNTs) composite for the determination of Pb^{2+} has not been reported on the basis of the TBA as a molecular recognition element.

The aim of this work was to improve the sensitivity of electrochemical DNA-based sensor for the detection of Pb^{2+} utilizing TBA as a molecular recognition element and ionic liquid supported CeO_2 nanoparticles–MWNTs composite modified gold electrode. The schematic diagram of the electrochemical DNA-based sensor for the detection of Pb^{2+} ion is shown in Fig. 1. In this paper, an electrochemical sensor for the determination of Pb^{2+} was designed and the electrochemical characteristics of the electrochemical sensor fabricated were investigated. To our best knowledge, it is new example of electrochemical sensor for the determination of Pb^{2+} based on signal enhancement.

2. Experimental

2.1. Reagents and apparatus

MWNTs were obtained from Shenzhen Nanotech Port Co. Ltd. (Shenzhen, China). CeO_2 nanoparticle was obtained from Beijing Nachen Science and Technology Ltd. Company (Beijing, China). 1-Ethyl-3-methylimidazolium tetrafluoroborate ($EMIMBF_4$) was obtained from Lanzhou Institute of Chemical Physics of the Chinese Academy of Sciences. Pb^{2+} stock solution was prepared by dissolving $Pb(NO_3)_2$ with Millipore Milli-Q water. Thrombin-binding aptamers were synthesized by Shenggong Bioengineering

Ltd. Company (Shanghai, China). A 21-mer target aptamer probe adopted from literature [28] was used to bind with thrombin: 5′-CAC TGT GGT TGG TGT GGT TGG-$(CH_2)_6$-NH_2-3′. $MgCl_2$, $CaCl_2$, Hg $(NO_3)_2$, $CuCl_2$, $ZnCl_2$, and all other reagents were of analytical grade. A 0.10 M phosphate buffer solution (PBS, pH 7.40, 0.10 M NaCl+10 mM NaH_2PO_4/Na_2HPO_4) was used as hybridization buffer, washing solution and electrolyte. All reagents were of analytical grade. Millipore Milli-Q water (18 MΩ cm) was used throughout.

The experimental set-up for electrochemical measurement was the same as the previous paper [29].

2.2. *Preparation of the CeO_2–MWNTs–$EMIMBF_4$/GCE*

The purified MWCNTs were prepared as reported in the literature [29–31]. 10 mg of purified MWNTs were dispersed in 10 mL of dimethylformamide (DMF) with the aid of ultrasonication for 2 h to give a 1.0 mg/mL homogeneous black suspension. Then 0.2 mg of CeO_2 nanoparticles were added to the MWNTs suspension and the resulted suspension was ultrasonicated for 2 h. After that, $EMIMBF_4$ (final concentration (v/v) 50%) was dispersed in the CeO_2–MWNTs composite with the aid of ultrasonication [32]. Finally, a uniform CeO_2–MWNTs–$EMIMBF_4$ nanocomposite suspension was obtained. Before modification, the GCE was pretreated as previously described [33]. 5 μL of the CeO_2–MWNTs–$EMIMBF_4$ suspension was dropped on the GCE and let it dry at room temperature for 2 h, thus a uniform membrane coated electrode (CeO_2–MWNTs–$EMIMBF_4$/GCE) was obtained. The CeO_2/GCE, CeO_2–$EMIMBF_4$/GCE and CeO_2–MWNTs/GCE were fabricated through similar procedure.

2.3. *DNA probe immobilization*

Immobilization of TBAs was performed by immersing the CeO_2–MWNTs–$EMIMBF_4$/GCE into 2.0 mL PBS (pH 7.0) solution containing 5.0×10^{-6} M TBAs probe for 4 h at room temperature, followed by washing the electrode with 0.10 M PBS and then rinsing it with ultrapure water to remove the unimmobilized TBA probe, and this TBA probe-captured electrode was denoted as TBA/CeO_2–MWNTs–$EMIMBF_4$/GCE.

2.4. *Electrochemical measurement*

A sensor fabricated was immersed into different concentrations of Pb^{2+} solution for 60 min at 37 °C, followed by thoroughly washing with 0.10 M PBS to remove unbound Pb^{2+}. After that, the electrode was transferred into electrochemical cell. The electrochemical reduction of Pb^{2+} to Pb^{+} was recorded by a cathodic scan of DPV. The DPV measurement was performed in the potential range from −0.5 to 0 V in 0.10 M PBS (pH 7.4, containing 0.1 M $NaNO_3$) with pulse amplitude of 50 ms and pulse width of 50 mV.

3. Results and discussion

3.1. *Electrochemical characteristics of the CeO_2–MWNTs–$EMIMBF_4$ nanocomposite membrane*

CV and EIS were used for monitoring the process of the fabrication of electrochemical DNA-based sensor for the detection of Pb^{2+} in each step. As can be seen from Fig. 2, the CeO_2–MWNTs/GCE (Fig. 2, curve b) and the CeO_2–MWNTs–$EMIMBF_4$/GCE (Fig. 2, curve c) had larger CV current than bare GCE (Fig. 2, curve a), which ascribed to the fact that modification of the electrode with CeO_2–MWNTs–$EMIMBF_4$ could significantly enhance the effective electrode surface area and ionic conductivity. A current decrease (Fig. 2, curve d) appeared after exposing to TBA probe. It is well-known that an immobilized TBA probe as an electron-transfer blocking layer can hinder the diffusion of ferricyanide toward the electrode surface [34]. After incubation with 1.0×10^{-7} M Pb^{2+}, the formation of the Pb^{2+}/TBA/CeO_2–MWNTs–$EMIMBF_4$/GCE contributed to a significant increase in redox current (Fig. 2, curve e). This is probably attributed to the fact that the positive charge of Pb^{2+} serves to reduce the repulsion of $[Fe(CN)_6]^{3-/4-}$ to the surface, promoting the interfacial electron transfer between the redox anions in solution and the electrode [35]. The results showed that the TBA probe is immobilized on the CeO_2–MWNTs–$EMIMBF_4$/GCE and the interaction between the electrochemical DNA-based sensor and Pb^{2+} arises.

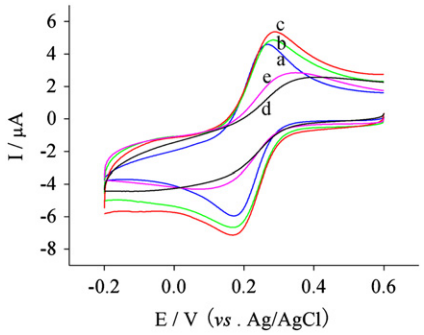

Figure 2 CV of obtained in 1 mL PBS (0.1 M, pH 7.40) containing 1 mM $Fe(CN)_6^{3-}$/$Fe(CN)_6^{4-}$ and 0.1 M KCl at a scan rate of 50 mV/s at (a) bare GCE, (b) CeO_2–MWCNTs/GCE, (c) CeO_2–MWNTs–$EMIMBF_4$/GCE, (d) TBA/CeO_2–MWNTs–$EMIMBF_4$/GCE, and (e) TBA/CeO_2–MWNTs–$EMIMBF_4$/GCE incubate in Pb^{2+} solution for 60 min.

Fig. 3 shows Nyquist plots of impedance spectra obtained at different electrodes. The change in semicircle diameter is a result in the change in the interfacial resistance R_{et} to electron transfer from their modified electrode to ferricyanide in solution. The electrochemical response was a nearly straight line (curve a), which is characteristic for a limiting step of the electrochemical process at a bare GCE. After CeO_2–MWNTs–$EMIMBF_4$ nanocomposite was modified (curve b), the electrochemical response was a nearly straight line. After TBA probe was immobilized onto the surface of CeO_2–MWNTs–$EMIMBF_4$/GCE, the R_{et} markedly increased to 96,370 Ω (curve c). This is attributed to the fact that the redox couple of $K_3[Fe(CN)_6]$/$K_4[Fe(CN)_6]$ is suffered electrostatic repulsive forces from the immobilized TBA probe. In the presence of Pb^{2+}, the R_{et} significantly decreased to 44,040 Ω (curve d). This is probably attributed to the fact that the conformation variation enhances the steric and coulombic force between adjacent DNA sequences [36], resulting in a decrease of R_{et}. The results extracted from EIS measurements (Fig. 3) are in good agreement with the results obtained from CV (Fig. 2).

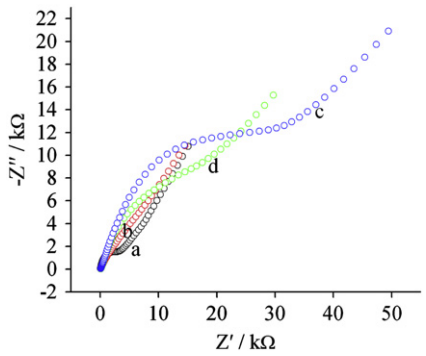

Figure 3 Nyquist plots of impedance spectra obtained in 1 mL PBS (0.1 M, pH 7.40) containing 1 mM $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$ and 0.1 M KCl at (a) bare GCE, (b) CeO_2–MWNTs–$EMIMBF_4$/GCE, (c) TBA/CeO_2–MWNTs–$EMIMBF_4$/GCE, and (d) TBA/CeO_2–MWNTs–$EMIMBF_4$/GCE incubate in Pb^{2+} solution for 60 min. The biased potential was 0.172 V. The frequency was 0.1–100 kHz and the amplitude was 5.0 mV.

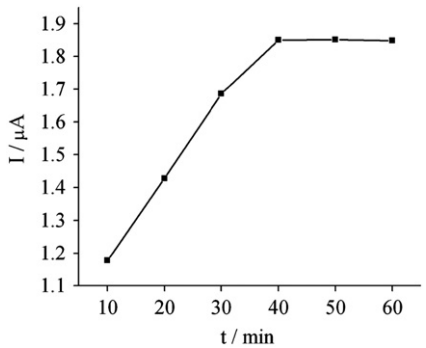

Figure 4 Dependence of peak current of the DNA-based sensor on incubation time in PBS (0.1 M, pH 7.40) solution containing 1.0×10^{-7} M Pb^{2+} by the CeO_2–MWNTs–$EMIMBF_4$ nanocomposite amplified strategy. DPV parameters: pulse amplitude, 50 ms; pulse width, 50 mV.

3.2. *Optimization of incubation time*

It was found that a different incubation time of Pb^{2+} caused a visible difference in the increase of peak current. Therefore, the dependence of Pb^{2+} incubation time on the increase of the peak current was studied to determine the optimum incubation time of Pb^{2+}. As shown in Fig. 4, the reduction peak current increased immediately when 1.0×10^{-7} M Pb^{2+} was introduced and then tended to reach the maximum after more than 40 min. To ensure that sufficient amount of Pb^{2+} can be gathered on the electrode, 60 min was chosen as the Pb^{2+} incubation time. This incubation time is longer than that required in homogenous fluorescent methods (15 min) [14], indicating that the reaction of the surface-confined TBA probe with Pb^{2+} is much slower than that of the TBA probe with Pb^{2+} in solution. The incubation time of this electrochemical sensor for a real sample should be reduced.

3.3. *Performance of the electrochemical sensor*

The quantitative behavior of the electrochemical sensor fabricated was assessed under optimized analytical condition.

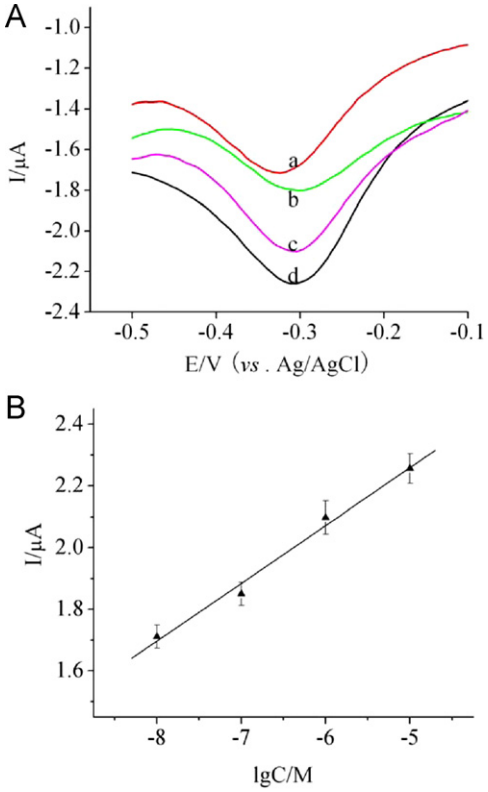

Figure 5 (A) DPV of the electrochemical sensor for the detection of different concentrations of Pb^{2+} by the CeO_2–MWNTs–$EMIMBF_4$ nanocomposite amplified strategy: (a) 1.0×10^{-8} M, (b) 1.0×10^{-7} M, (c) 1.0×10^{-6} M, and (d) 1.0×10^{-5} M. (B) The linear relationship between the peak current and the concentration of Pb^{2+}. The DPV conditions are the same as Fig. 4.

Fig. 5 shows the DPV profiles of the sensor at different concentrations of Pb^{2+}. The reduction peaks of Pb^{2+} appeared at ~0.3 V by DPV detection. From Fig. 5A, it can be seen that the peak current increases with an increase of the concentration of Pb^{2+}. The peak current had a linear relationship with the concentration of Pb^{2+} in the range from 1.0×10^{-8} to 1.0×10^{-5} M (Fig. 5B). The linear regression equation was $I = 3.202 + 0.1882 \lg C$, (unit of C is M) and the correlation coefficient was 0.9943. The detection limit was 5.0×10^{-9} M Pb^{2+} ($S/N = 3$). The detection limit is considerably lower than 72 nM, the EPA-defined maximal contamination level for Pb^{2+} in drinking water. The relative standard derivation for 5.0×10^{-7} M Pb^{2+} was 3.6% ($n = 7$). The storage stability of the electrochemical DNA-based sensors fabricated was also checked. The results showed that the current response of the electrochemical DNA-based sensor to 1.0×10^{-7} M Pb^{2+} decreased about 4.5% after the sensor was stored in air at room temperature for 7 days. This suggests that the sensor has good stability. A satisfactory detection sensitivity, reproducibility and stability of the proposed sensor are, therefore, verified.

The electrochemical DNA-based Pb^{2+} sensor was also highly selective. Fig. 6 depicts the electrochemical response of Pb^{2+} in a mixture of five different interference metal ions. As shown in Fig. 6, the sensor hardly exhibited substantial responses to a mixture of another five different metal ions (1 mM of Ca^{2+}, Mg^{2+}, Hg^{2+}, Cu^{2+}, and Zn^{2+}) (curve a). The

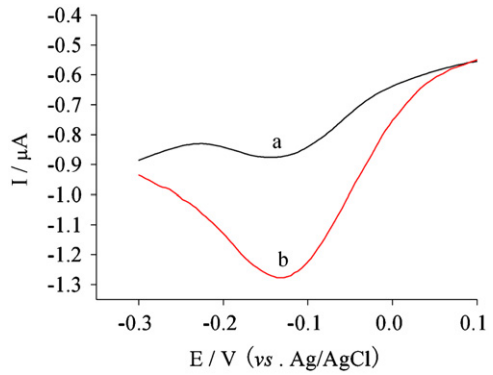

Figure 6 DPV corresponding to detection of (a) 0 and (b) 1.0×10^{-7} M of Pb^{2+} in a mixture of metal ions containing Ca^{2+}, Mg^{2+}, Hg^{2+}, Cu^{2+}, and Zn^{2+} (1 mM, each). The DPV conditions are the same as Fig. 4.

Table 1 The concentration of Pb^{2+} in water samples detected using the proposed electrochemical DNA-based sensor.

Samples	Added Pb^{2+} concentration (M)	Concentration found (M)	Recovery (%)	RSD (%, $n=3$)
1	1.0×10^{-8}	9.52×10^{-7}	95.2	4.2
2	1.0×10^{-7}	9.68×10^{-6}	96.8	3.8
3	1.0×10^{-6}	9.76×10^{-5}	97.6	3.2

cross sensitivity of the sensor in a mixture of five different metal ions containing 1.0×10^{-7} M Pb^{2+} was also examined (curve b). The signal obtained from the mixture was slight lower than that obtained from pure Pb^{2+} solution. These results indicate that the electrochemical DNA-based sensor for the detection of Pb^{2+} exhibits high specificity. The result was in agreement with that the unique Pb^{2+} conformation formed by the T-base of DNA sequence, which is quite specific to Pb^{2+} [14,37].

The practical application of the designed sensor was evaluated by determination of the recovery of spiked Pb^{2+} in tap water samples. The analytical results are shown in Table 1. The analytical results show the acceptable relative standard deviation and quantitative recoveries, implying that the proposed sensor was applicable for practical Pb^{2+} detection.

4. Conclusions

An electrochemical DNA-based sensor for the detection of Pb^{2+} was developed based on the TBA as a molecular recognition element and ionic liquid supported CeO_2 nanoparticles–carbon nanotubes composite modification. The developed sensor exhibited a high sensitivity and selectivity. It demonstrated that the sensitivity of electrochemical DNA-based sensor could be greatly improved by using the ionic liquid supported nanoparticles–carbon nanotubes composite modified the electrode. Also because of the high sensitivity, low cost, miniaturization, and simple operation of electrochemical methods, we expect that the CeO_2–MWNTs–$EMIMBF_4$ nanocomposite amplified electrochemical DNA-based sensor could be effective in detecting Pb^{2+} in the actual environment, and also provide a reference value for the detection of other heavy metals ions.

Acknowledgments

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