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Label free detection of lead using impedimetric sensor based on ordered mesoporous carbon–gold nanoparticles and DNAzyme catalytic beacons

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ABSTRACT

A novel label-free impedimetric sensing system based on DNAzyme and ordered mesoporous carbon–gold nanoparticle (OMC–GNPs) for the determination of Pb^{2+} concentration was developed in the present study. Firstly, gold nanoparticles deposited on the modified electrode surface were employed as a platform for the immobilization of thiolated probe DNA, and then hybridized with DNAzyme catalytic beacons. Subsequently, in the presence of Pb^{2+}, the DNAzyme could be activated to cleave the substrate strand into two DNA fragments, which causes differences in the electrical properties of the film. Randles equivalent circuit was employed to evaluate the electrochemical impedance spectroscopy (EIS) result. The charge transfer resistance (R_{CT}) value for the $[Fe(CN)_6]^{3-/4-}$ redox indicator was remarkably decline after hybridization with Pb^{2+}. The difference in R_{CT} values before and after hybridization with Pb^{2+} showed a linear relation with the concentration of the Pb^{2+} in a range of 5×10^{-10}–5×10^{-5} M, with a detection limit of 2×10^{-10} M ($S/N=3$). Furthermore, with the application of Pb^{2+} dependent 8-17DNAzyme, the proposed sensing system exhibited high selectivity without using any labeled probes. This biosensor demonstrated a promising potential for Pb^{2+} detection in real sample.

1. Introduction

Lead ions are important pollutants mainly arising from lead-based paints and contaminated water, soils and foodstuffs [1]. Lead causes adverse effects in human health, including renal malfunction, the inhibition of brain development and high blood pressure [2]. Thus, it is of great significance to monitor its level in the environment. Traditional quantitative methods, such as atomic absorption spectrometry (AAS) [3], and inductively coupled plasma mass spectroscopy (ICP/MS) [4], are rather complex laboratory techniques. Motivated by the desire for rapid and portable methods to quantify trace lead contamination, fluorescent [5], colorimetric [6], and electrochemical [7] methods utilizing organic fluorescent dyes, oligonucleotides, or lead(II)-dependent RNA cleaving DNAzymes have been developed in recent years, and achieving parts-per-billion detection limits.

DNAzymes, called catalytic DNA or deoxyribozymes elsewhere, are DNA molecules that can catalyze DNA sequences isolated via in vitro selection. Most of the reactions require specific metal ions as cofactor [1,8], which provides a novel platform for generating metal ion sensors [9]. Since Plaxco and coworkers reported a novel electrochemical sensor based on DNAzyme for rapid, simple, sensitive and selective Pb^{2+} detection in 2007 [1], various groups have used DNAzyme to detected metal ions recently [7,10]. The high selectivity against background interferences guarantees its application for real sample detection in which other intensity-based measurement methods were hampered [11]. Furthermore, an effective immobilization platform for thiolated probe DNA and DNAzymes on the modified electrode is a key technique for improving DNA sensing efficiencies. In our previous study, we used OMC–GNPs as the platform for electrochemical biosensors, which can obviously increase the sensitivity and lower the detection limit [12,13]. OMC has a special structure, which can be used as the building blocks in hybrid materials, and provides an excellent platform and microenvironment for immobilized biomolecules such as DNA, enzyme and protein. Meanwhile, the introduction of GNPs could extend the application of OMCs and strengthens its features, such as electron transfer, and catalytic and electrochemical activity [12,13].

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Electrochemical impedance spectroscopy (EIS) is a very attractive method in the analysis of interfacial property changes of modified electrodes with biorecognition on the electrode surfaces, such as conformational changes, DNA hybridization, or DNA damages. The capacitance and resistance changes such as Ohmic resistance (Rs) and Warburg impedance (Z_W), occurring at electrode surfaces was obtained by this technique with an equivalent circuit. In most cases of DNA-based biosensors, the target DNA must be labeled with a fluorophore [14], magnetic beads [15], or an enzyme [16] for its detection. However, DNA-based biosensors using EIS detection are label-free, leading to low cost, simplicity, and ease of miniaturization. Therefore, EIS has been increasingly applied in biosensor development for the detection of bimolecular interactions [17–20]. The published works of our and other groups have shown that EIS is a powerful tool for the detection of metal ions based on oligonucleotides [21,22].

This study demonstrates a label-free biosensor with OMC–GNPs as a transducer platform with DNA and 8-17DNAzyme for the ultrasensitive detection of lead(II) using EIS. The interfacial properties of the electrode, such as, electron transfer resistance and capacitance, were investigated in the presence of a redox probe, $[Fe(CN)_6]^{3-/4-}$. In the presence of Pb^{2+}, the trans-acting catalytic beacon cleaves the sessile of the substrate into two fragments (Scheme 1), resulting in the remarkable decrease of interfacial charge-transfer resistance (R_{CT}) for the negatively charged redox probe at the electrochemical biosensor due to the enhanced electron transfer by OMC–GNPs. Taking advantage of the R_{CT} change, Pb^{2+} can be detected at the concentration as low as 2×10^{-10} M. Meanwhile, the sensor maintained highly selectivity over other non-specific metal ions.

2. Experimental

2.1. Chemicals and reagents

Pluronic copolymer P123 (non-ionic triblock copolymer, $EO_{20}PO_{70}EO_{20}$) and Tris (hydroxymethyl) aminomethane were purchased from Sigma-Aldrich (USA). Tetraethoxysilane (TEOS), L-Lysine (L-Lys). Gold (III) chloride trihydrate ($HAuCl_4 \cdot 3H_2O$, 99.9%) was purchased from Dingguo Biotechnology Co., Ltd (Shanghai, China). Tetraethoxysilane (TEOS), acetate, sodium acetate, $K_3[Fe(CN)_6]$, $K_4[Fe(CN)_6]$, $Pb(ClO_4)_2$ and all other chemical reagents were of analytical grade and used as received. And all aqueous solutions were prepared using ultra-pure water (18 MΩ cm, Milli-Q, Millipore). 25 mM tris–acetate buffer (pH 7.4) including 0.2 M NaCl and phosphate buffer saline (PBS, 0.1 M KH_2PO_4 and 0.1 M Na_2HPO_4), were used in this work. The DNA target-specific probes used for hybridization in the experiment were synthesized by Sangon (Shanghai, China) and purified using high-performance liquid chromatography. The sequences of the oligonucleotides include:

5′-HS-$(CH_2)_6$-SS-$(CH_2)_6$-TTT-CATCTCTTC-TCCGAGCCGGTC-GAAA-TAGTGAGT-3′ (S1)

3′-GTAGAGAAGGrATATCACTCA-5′ (S2, a complementary substrate oligonucleotide of the DNAzyme which contains a single, scissile ribo–adenine)

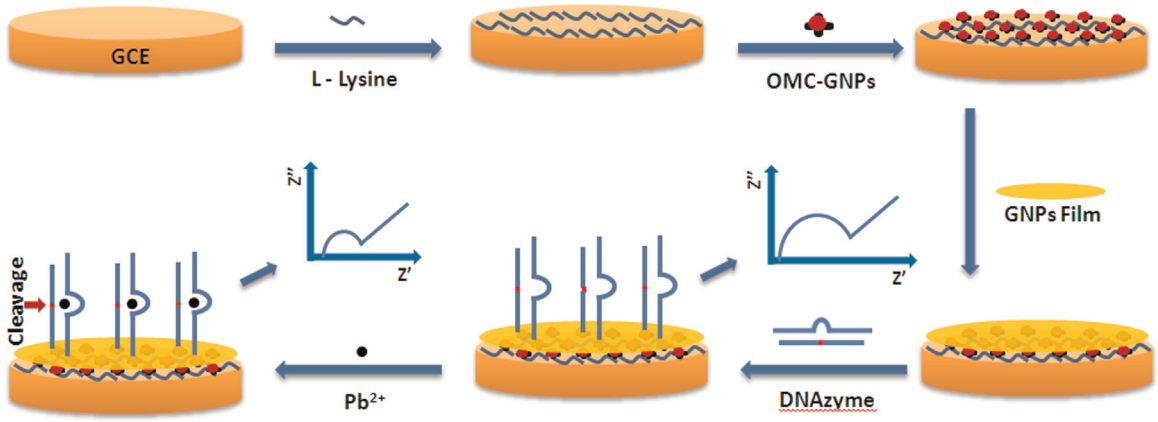
2.2. Apparatus

Electrochemical impedance spectroscopy (EIS) measurement and cyclic voltammetric (CV) measurements were carried out on CHI760D electrochemical workstation (Chenhua Instrument, Shanghai, China). Besides, in this work, the three-electrode system includes a saturated calomel electrode (SCE) as reference electrode, a Pt foil auxiliary electrode and a modified electrode as working electrode. All the work was conducted at room temperature (25 °C) unless otherwise mentioned. A model pHSJ-3 digital acidimeter (Shanghai Leici Factory, China) was used to measure the solution pH. A JEOL-1230 electron microscope (100 kV) was used to obtain Transmission electron microscopy (TEM) images. An ICP/MS (PQ-ECCELL, America) was used to measure Pb^{2+} concentrations in real samples. A vacuum freezing dryer, a mechanical vibrator and a sigma 4K15 laboratory centrifuge were used in the assay.

2.3. Preparation of OMC–GNPs, and fabrication of the biosensor

The OMC and OMC–GNPs were synthesized as described previously in our laboratory [13,14]. The bare glassy carbon electrode (GCE) used for the self-assembled monolayer was first polished in alumina slurry, rinsed with deionized water, and then etched for about 10 min in a "Piranha" solution (98% H_2SO_4:30% H_2O_2=3:1 (v/v)) to remove organic contaminants (*Caution: Piranha solution reacts violently with organic materials, thus should be handled with extreme care.*) [23]. Finally, we treated the electrode surface in H_2SO_4 (0.5 M) performing cyclic voltammetry scan (between 0 and 1.2 V, scan rate: 50 mV s^{-1}) until a reproducible circle was gained.

OMC–GNPs suspension was prepared by dispersing 1 mg purified OMC–GNPs into 2 mL N,N-dimethylformamide and the mixture was sonicated for 1 h to form a stable black suspension. The preparation of OMC–GNPs/ > L-Lys/GCE was carried out as follows (As shown in Scheme 1): initially, the L-Lys film modified electrode was obtained in L-Lys electrodeposition process (as seen in Fig. S-1). Then, a 5.0 μL of the OMC–GNPs dispersion was dipcoated onto L-Lys/GCE. The electrodes (OMC–GNPs/L-Lys/GCE) were dried



Scheme 1. The fabrication processes of the biosensor.

at room temperature (25 °C). Next, as seen in Fig. S-2, the GNPs film modified electrode was obtained by electrodeposition process in in 0.1% $HAuCl_4$ performing cyclic voltammetry between 0 and 1.6 V, then was washed successively with water. According to our previous study [12], the significant increase of the redox peak currents on increasing potential scan rate could result in the decrease of Au NPs immobilized on the electrode surface. Thus, we chose an eclectic scan rate of 40 mV/s in the following steps. Subsequently, the mixture solution of 10 μL S1 probe was dropped onto the electrode surface and kept at 4 °C for self-assembling through thiol–gold bonding for 10 h. Finally, the electrode was immersed in buffer (25 mM tris–acetate, 0.2 M NaCl, pH 7.4) for 10 min to reduce the nonspecific adsorption of S1.

2.4. The measurement procedure of electrochemical sensor

The hybridization and electrochemical detection of the biosensor are as follows. The modified electrode with S1 probes coated was immersed into 400 μL of 1 mM 6-mercapto-1-hexanol (MCH) solution for 1 h to improve the quality and stability, and blocking the non-specific binding of DNA to obtain a well aligned DNA monolayer [24]. Then, the electrode was soaked in the solution with S2, and incubated at room temperature for 1 h to yield the final lead–DNAzyme assembly on the surface. Subsequently, the electrode was immersed in buffer (25 mM tris–acetate, 0.2 M NaCl, pH 7.4) for 10 min to reduce the nonspecific adsorption of S2. The DNA surface was then allowed to react with various concentrations of target Pb^{2+} in the buffer (25 mM Tris–acetate, 0.2 M NaCl, pH 7.4) for 50 min in a 40 °C water bath to obtain the maximum cleavage of substrate strand on the modified electrode. Then the electrode was removed from the buffer, and allowed to cool down to room temperature within 1 h. A CHI760D electrochemical workstation was used, and all the measurements were carried out at room temperature with conventional three-electrode system. EIS was performed in 0.1 M PBS (pH 7.4) containing 5 mM $[Fe(CN)_6]^{3-/4-}$ (1:1) and 10 mM KCl in the frequency range from 0.1 Hz to 100 kHz with 5 mV as the amplitude at a polarization potential of 0.18 V. All the test data were repeated for three times with separate electrodes to gain statistically meaningful results. The impedance spectra for all the system were analyzed with the help of a modified Randles equivalent circuit, as shown in Fig. S-3A.

3. Results and discussion

3.1. DNAzyme-based electrochemical sensor and detection mechanism

Scheme 1 outlines the preparation process of the biosensor. Amino or thiol group-modified membrane is a common media to fix nanometer gold [12,25]. Herein, in the self-assembly process, L-Lysine could provide amino group and become the cross-linking agent between GCE and OMC–GNPs film. OMC–GNPs were used as carrier for loading DNA labels and accelerating electron-transfer. GNPs film was utilized for enhancing impedance signals by increasing the number of immobilized probe DNA molecules. The single strand oligonucleotide S1 with Au–S covalent bond acting as an anchor substrate on the composite platform was ready for the reconstitution of the S2 to form a stable structure. Once in the presence of Pb^{2+}, the trans-acting catalytic strand cleaved the sessile phosphodiester of the substrate into two fragments (as shown in Scheme 1 and Fig. 1 inset), leading to the change of interfacial charge-transfer resistance of the electrodes towards the $[Fe(CN)_6]^{4-/3-}$ redox couple.

As seen in Fig. 1, after hybridization with probes (S1+S2), the

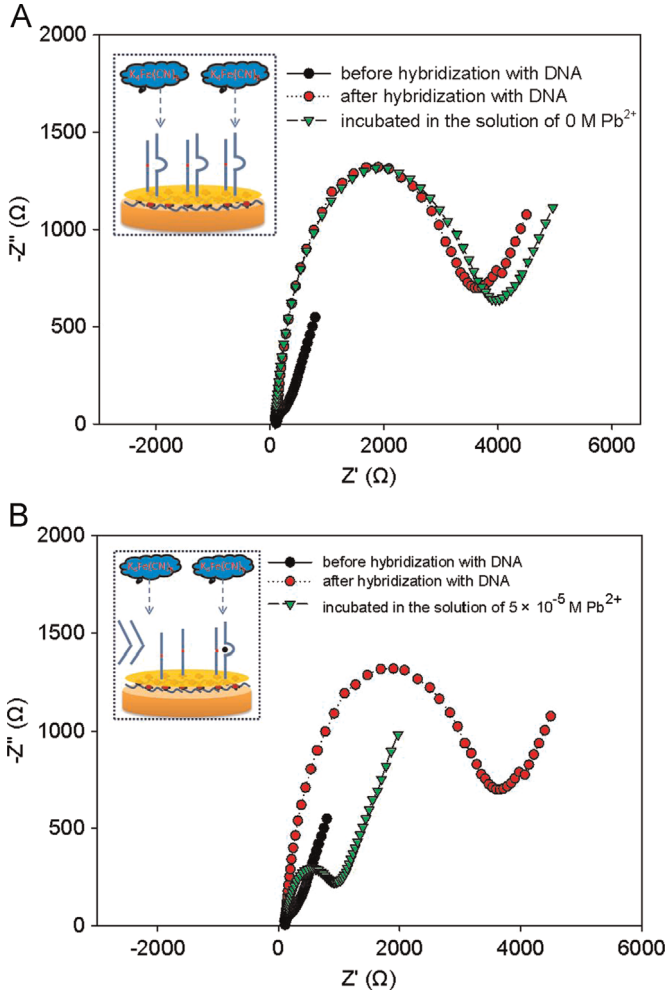


Fig. 1. Nyquist plots for the impedance measurement before and after hybridization with probes (S1+S2), and after incubation in the solution of (A) 0 M and (B) 5×10^{-5} M Pb^{2+} in PBS (0.1 M, pH 7.4) containing 5 mM $[Fe(CN)_6]^{3-/4-}$ (1:1) and 10 mM KCl, in the frequency range from 0.1 Hz to 100 kHz with 5 mV as the amplitude at a polarization potential of 0.18 V.

impedance increased, and in the presence of Pb^{2+} (5×10^{-5} M), the impedance shifted back. The reason might be ascribed to easier electron transfer between GCE surface and $[Fe(CN)_6]^{3-/4-}$ with Pb^{2+}. Thus the electron transfer of the whole system could be accelerated. In fact, the changes of the charge transfer resistance (ΔR_{CT}) of DNA films in the presence and absence of the metal ion were different and dependent on the concentration of the given metal ion. The result also coincided with the presumptive mechanism on the Pb^{2+} induced conformational change of the hybridization probes, thus enhanced the electron transfer of $[Fe(CN)_6]^{4-/3-}$ redox couple. The different charge-transfer resistances for the redox indicator ions were obtained from the various conformational characteristics and charge of DNA on the surface. On the basis of the results discussed above, the interaction between DNA and Pb^{2+} led to the decrease of R_{CT}, and ΔR_{CT} was a very important parameter to use for the detection of Pb^{2+}.

Besides, the interface could be modeled by an equivalent circuit by the software of EIS Spectrum Analyzer. The various elements, such as *Rs*, R_{CT}, Z_W and C_{dl} of the equivalent circuit, were obtained and the plot fitting and the fitting errors were less than 6.5%. As seen in Fig. S-4, the measured data and the fitting curve showed good agreement, which indicates this equivalent circuit (Fig. S-3A) is suitable and meaningful for this electrochemical system. Therefore, this equivalent circuit was used to fit the impedance

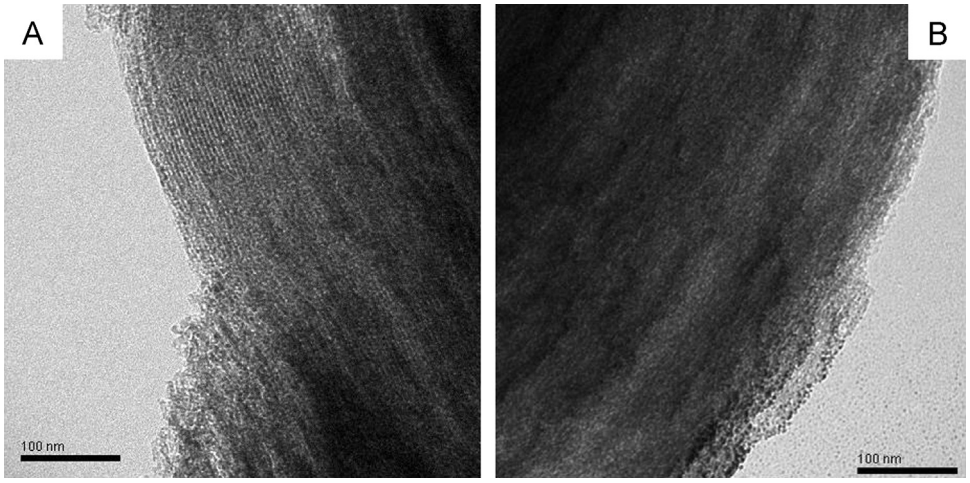


Fig. 2. TEM image of OMC and OMC–GNPs.

spectroscopy data and extract the values of the equivalent circuit elements. As for Fig. 1, the results of this analysis are listed in Table S-1. As seen in Table S-1, the R_{CT} value obtained before hybridizing DNA film was 102.4 Ω, and an obvious increase in the interfacial resistance, corresponding to a R_{CT} value of 2806 Ω, was observed when S1 and S2 were entrapped in the modified electrode. Such increased R_{CT} could be presumably due to changes in the film thickness for films of S1 and S2, which would increase the distance for electron transfer through the film and hence increase R_{CT}. Importantly, the trans-acting catalytic strand cleaved the sessile of the substrate into two fragments in the presence of Pb^{2+}, and the charge-transfer resistance R_{CT} was significantly reduced. The reason may be that the S2 would introduce significant disorder into the solution, and the redox probe may penetrate the film to a larger extent, giving rise to a lower R_{CT}.

3.2. Characterization of OMC, OMC–GNPs and electrode assembly process

The TEM images of OMC and OMC–GNPs are shown in Fig. 2. The OMC clearly exhibits highly ordered carbon nanowires (Fig. 2A). Compared with Fig. 2A, the well-dispersed GNPs (Fig. 2B) are observed on outer surface of OMC. It could be noted that the ordered structure is not obviously affected by the presence of GNPs, and GNPs are uniformly dispersed on the OMC.

Cyclic voltammetric (CV) was used to test the performance of the modified electrode, and carried out in PBS with pH 7.4 containing 5 mM $[Fe(CN)_6]^{3-/4-}$ (1:1) and 10 mM KCl. As shown in Fig. S-5, the immobilization of L-Lys on the GCE resulting in a significant decrease in peak current of the redox probe. After modification with OMC–GNPs and GNPs, the peak current increased obviously and the peak potential difference reached the minimum. Correspondingly, electrochemical impedance spectroscopy (EIS) of $[Fe(CN)_6]^{3-/4-}$ can provide information about the impedance changes of the electrode surface during the modification process. An almost straight line was observed for the assembly of OMC–GNPs and GNPs on the modified GCE (Fig. S-5B), indicating that the introduction of OMC–GNPs and GNPs could improve the electron transfer kinetic to a large extent. Moreover, it can clearly be seen that the interfacial resistance was increased obviously when L-Lys was used to modified electrode (Fig. S-5B). The reason might be that the low conductivity of L-Lys could slow down the redox reaction of $[Fe(CN)_6]^{3-/4-}$, resulting in the increased interfacial resistance. Although the L-Lys film did not increase electrical conductivity and the rate of electron transfer on the electrode surface, it could make the OMC–GNPs film and L-Lys film fixed more tightly through carboxyl–amino bonding. This assembly process may extend the using life and stability of the impedimetric biosensor. Furthermore, the electron transfer ability reflected by changes in the impedance of the modified electrode was in accordance with the current response reflected by cyclic voltammetry (Fig. S-5A).

3.3. Analytical properties of the impedimetric DNA biosensor

Under the optimized experimental conditions (Fig. S-6), the modified electrode in 0.1 M PBS (pH 7.4) containing 5 mM $[Fe(CN)_6]^{3-/4-}$ (1:1) and 10 mM KCl after the electrodes were incubated with different concentrations of Pb^{2+}, and obtained with the Nyquist plots. As seen in Fig. 3A, upon decreasing the concentration of Pb^{2+} from 5×10^{-5} M to 5×10^{-10} M, less trans-acting catalytic strands cleaved the sessile of the substrate into two fragments, which led to the decrease in ΔR_{CT} (as shown in Table S-2, Supporting information). The change in the R_{CT} was linear with the logarithm of the concentration of Pb^{2+} within a concentration range from 5×10^{-10} M to 5×10^{-5} M. The linear regression equation was $Y=(-373.7486\pm8.6726)X+(3778.9752\pm60.8136)$ (Y is the ΔR_{CT} (Ω), X is the common logarithmic value of the target concentration (M)) with a correlation coefficient $R^2=0.9973$. The detection limit (LOD) of this sensor was estimated to be 2×10^{-10} M (based on $S/N=3$). As seen in Table 1, this novel impedimetric biosensor showed a relatively low detection limit for Pb^{2+} detection and a relatively wide dynamic working range comparable to some of the other methods.

3.4. Precision, specificity and stability of the developed DNA sensor

The repeatability of the same biosensor was examined by detecting 5×10^{-9} M Pb^{2+} in 0.1 M PBS (pH 7.4) containing 5 mM $[Fe(CN)_6]^{3-/4-}$ (1:1) and 10 mM KCl using EIS technique (As shown in Fig. 4). The value of the relative standard deviation (R.S.D.) was calculated to 4.3% for three measurement repetition. The result indicated a good repeatability of this biosensor and no need for a complicated treatment of the electrode before the measurements.

As seen in Fig. S-7, five different GCEs constructed by the same steps independently were used to investigate the reproducibility of this sensor. The RSD was 4.9% for the ΔR_{CT} to 5×10^{-9} M Pb^{2+}, indicating that the fabrication procedure was reliable and the modified GCE had good reproducibility. The long-term stability of the biosensor was explored. It was investigated through the response to 5×10^{-9} M Pb^{2+} in 0.1 M PBS (pH 7.4) containing 5 mM

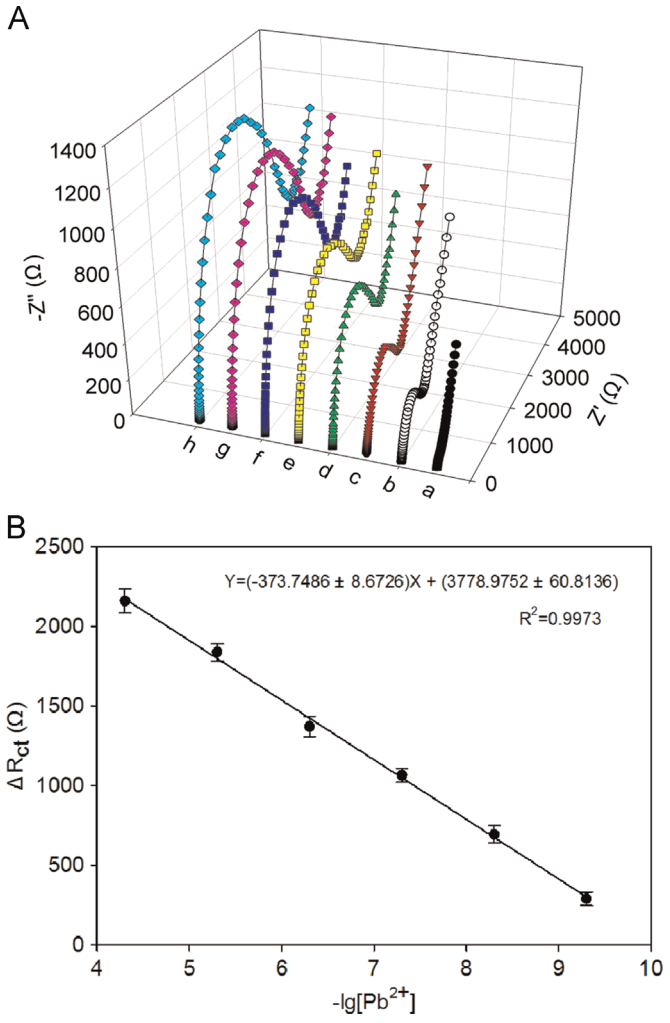


Fig. 3. (A) Series of Nyquist plots before hybridization (a), and after hybridization with DNA (S1+S2) (h), and followed by incubation in 5×10^{-5} M (b), 5×10^{-6} M (c), 5×10^{-7} M (d), 5×10^{-8} M (e), 5×10^{-9} M (f), and 5×10^{-10} M (g) Pb^{2+}. (B) The plot of ΔR_{CT} vs. Pb^{2+} concentration ranging from 5×10^{-10} M–5×10^{-5} M. Error bars indicate standard deviations from three replicative tests.

$[Fe(CN)_6]^{3-/4-}$ (1:1) and 10 mM KCl for 1 month. When not in use, electrode was stored at 4 °C in a refrigerator, and the current response was periodically measured. In this work, the experiment was carried out per 10 days beyond this period. After 1 month, the biosensor retained about 87% of its original ΔR_{CT} (Fig. S-8). The result indicated that this biosensor has relatively good stability, and the reason might be that the DNAzyme-based sensor is

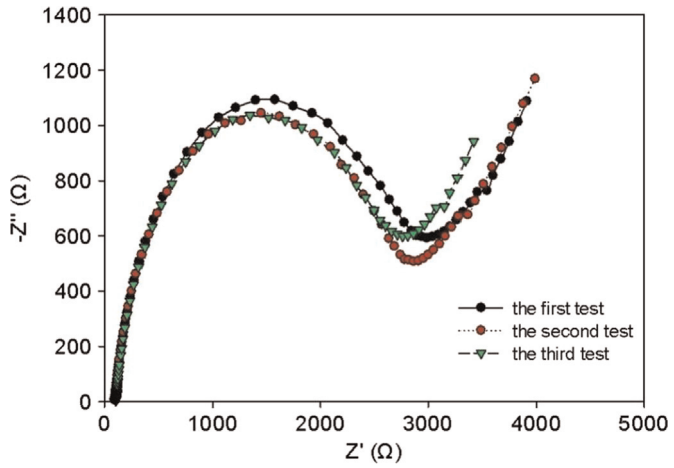


Fig. 4. The repeatability of the same biosensor for 5×10^{-9} M Pb^{2+} detection (different line represents different testing sample with the same biosensor).

sensitively and specifically responsive to its target ion, and the film (OMC–GNPs and GNPs) could provide a biocompatible microenvironment.

Besides, methods related to the sensing of metal ions by the DNAzyme-based sensor concerns the specificity of the system. Thus selectivity of this detection method was tested using impedimetric Pb^{2+} sensor in 0.1 M PBS (pH 7.4) containing 5 mM $[Fe(CN)_6]^{3-/4-}$ (1:1) and 10 mM KCl. Under the optimal experimental conditions, 5×10^{-9} M of Pb^{2+}, 1×10^{-7} M of Mn^{2+}, Ca^{2+}, Zn^{2+}, Co^{2+}, Hg^{2+}, Cu^{2+}, Cd^{2+} and K^{+}, their mixture containing 5×10^{-9} M of Pb^{2+}, and their mixture without Pb^{2+} were respectively measured. As seen in Fig. 5, negligible signal response was observed upon the addition of other tested ions, and on the contrary, significant response of ΔR_{CT} was observed for Pb^{2+}. Hence, the results showed excellent selectivity toward Pb^{2+} over other ions due to a specificity of DNAzyme for Pb^{2+} ion. Secondly, 5×10^{-9} M Pb^{2+} and other ions were mixed to form a mixture solution as a sample for testing the anti-jamming capability of this sensor (Fig. 5). Its ΔR_{CT} was obviously higher than other samples without Pb^{2+}. However, its ΔR_{CT} was similar as that of the sample containing 5×10^{-9} M of Pb^{2+}. These results clearly indicated that the approach is not only insensitive to other ions but also selective toward Pb^{2+} in their presence. As noted above, the present sensor had excellent selectivity and anti-jamming capability.

3.5. Analysis of real samples

To evaluate the practicality of the present method, the environmental water samples (tap water, lake water from Taozi Lake

Table 1
Comparison with other published Pb^{2+} detection sensor.

Method	Materials	Linear range (mol L^{-1})	LOD (mol L^{-1})	References
Electrochemiluminescence/DNAzyme	Si@CNCs	3×10^{-11}–1×10^{-6}	10^{-11}	[26]
Fluorescence detection/DNAzyme	Microarray	10^{-9}–10^{-6}	10^{-9}	[27]
Fluorescence detection/DNAzyme	Magnetic beads	10^{-9}–10^{-7}	6×10^{-10}	[28]
Electrochemical detection/DNAzyme	Magnetic beads	10^{-11}–10^{-6}	7.8×10^{-12}	[29]
SPR/DNAzyme	GNPs	10^{-15}–10^{-11}	5×10^{-16}	[30]
Electrochemical detection/DNAzyme	3DOM Au–Pd, MB@SWCNTs	10^{-17}–10^{-4}	10^{-19}	[31]
Electrochemical detection/G-rich DNA	Multi-walled carbon nanotubes/GNPs	5×10^{-11}–1×10^{-14}	4.3×10^{-15}	[32]
Fluorescence detection/DNAzyme	Graphene	–	3×10^{-10}	[33]
Electrochemical detection/G-rich DNA	Gold electrode	5×10^{-10}–5×10^{-5}	–	[34]
Electrochemical detection/DNAzyme	Gold electrode	5×10^{-7}–1×10^{-5}	3×10^{-7}	[1]
Electrochemical detection/DNAzyme	OMC–GNPs	5×10^{-10}–5×10^{-5}	2×10^{-10}	This work

Abbreviations: Si@CNCs, Carbon nanocrystals capped silica nanoparticles; SPR, Surface plasmon resonance; 3DOM Au–Pd, Three dimensional ordered macroporous (3DOM) Au–Pd Bimetallic; MB@SWCNTs, Methylene blue-single walled carbon nanotubes.

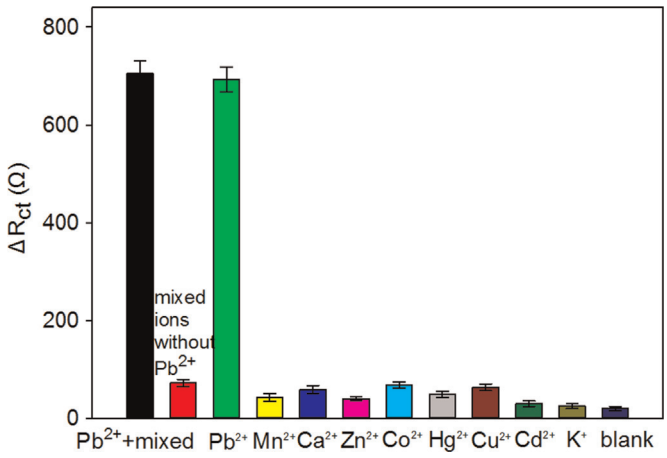


Fig. 5. Selectivity and interference study in the analysis of Pb^{2+} by this biosensor. The data are averages of three replicate measurements. Error bars indicate standard deviations.

and fresh river water from Xiangjiang River) were used in the study. The samples from lake water and river water were collected from different sites along the lake or river banks. The biosensor was employed for the determination of Pb^{2+} ions. The lake water and river water samples were filtered by a 0.45 μm membrane (purchased from Millipore) and then centrifuged for 15 min at 12,000 rpm, and tap water was tested without any processing before experiment. The pH values of tap water, lake water and river water samples were around 7.6, 7.5 and 7.8, respectively, and all samples were adjusted to pH 7.4 using Tris–acetate. The concentration of total Pb^{2+} in the sample of River water 1 was detected to be 17.35 nM (measured by proposed sensor) or 18.21 nM (measured by ICP/MS), which varied slightly in the other two river water samples. None of Pb^{2+} ions could be detected in both tap water and lake water samples (measured by ICP/MS and proposed sensor). 5 or 50 nM of Pb^{2+} was added into some samples using Pb^{2+} stock solutions, which were tested using the proposed sensor and ICP/MS. The results are summarized in Table 1 and show good agreement, indicating the potential of the sensor as a simple and reliable analytical method of Pb^{2+} ions in environmental samples.

4. Conclusion

In summary, an unlabeled immobilized DNA-based sensor was reported to determine Pb^{2+} concentration through use of the difference in charge-transfer resistance (ΔR_{CT}) before and after DNA interactions with Pb^{2+}, which were monitored by electrochemical impedance spectroscopy (EIS). ΔR_{CT} is sufficiently sensitive to detect Pb^{2+} as low as 2×10^{-10} M, and the linear range is from 5×10^{-10} M to 5×10^{-5} M. Moreover, because of the signal amplification on the OMC–GNPs and GNPs platform and the merits of high specificity of the 8-17DNAzymes, the sensor maintained high selectivity over other nonspecific metal ions. Considering the good performance of this electrochemical Pb^{2+} sensor, it is expected to obtain great success in environmental monitoring. Furthermore, by controlling the target probes with various metal ions-specific DNAzymes (such as Cu^{2+}, Mg^{2+} and Hg^{2+}), the assay can be easily extended for other metal ions, and thus represents a versatile detection method.

Associated content

Supporting information

More information is detailed about the optimization of the variables of experimental conditions, the repeatability and reproducibility of the biosensor, electrodeposition of L-Lys, analysis the Pb^{2+} in real samples. This material is available free of charge via the Internet at http://pubs.acs.org.

Acknowledgments

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2015.06.063.

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