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Electrochemical biosensor for Ni^{2+} detection based on a DNAzyme-CdSe nanocomposite

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ABSTRACT

The detection and speciation analysis of metal-ion is very important for environmental monitoring. A novel electrochemical biosensor for Nickel(II) detection based on a DNAzyme-CdSe nanocomposite was developed. We firstly hybridized with capture probe (DNA1) and sequentially with DNA (DNA2) on the gold electrode. Then CdSe QDs were incorporated the specific recognition of DNA2 by covalent assembling. Upon addition of nickel ion into the above system, the substrate strand of the immobilized DNAzyme was catalytically cleaved by target Ni^{2+}, resulting in disassociation of the shorter DNA fragments containing CdSe QDs. The remaining CdSe QDs on the electrode surface detected by differential pulse anodic stripping voltammetry (DPASV). Under optimal conditions, the as-prepared sensor exhibited high sensitivity and fast response to Ni^{2+} with the linear range from 20 nM to 0.2 mM and a low detection limit of 6.67 nM. The prepared biosensor also shows good stability and good reproducibility and high selectivity toward target Ni^{2+} against other metal ions because of highly specific Ni^{2+}-dependent DNAzyme. Thus, our strategy has a good potential in the environment surveys.

1. Introduction

In recent years, electrochemical sensors have been widely used for detecting the concentration of heavy metal ions that are harmful to humans. Knowing the concentration of metal ions is very important, and some sensors have been used to detect Hg^{2+} (Li et al., 2014; Shi et al., 2014), Cu^{2+} (Park et al., 2010), Zn^{2+} (Qian et al., 2014, 2015), Fe^{2+} (Wei et al., 2011), Mn^{2+} (Gou et al., 2011), Pb^{2+} (Cui et al., 2015; Tang et al., 2014a) and Ce^{3+} (Huang et al., 2014). Nickel is a biologically important metal ion. Nickel not only stimulates certain enzymes but is also an important active factor for several enzymes that participate in various microbial pathways, including carbon monoxide dehydrogenase (CODH) and methyl-coenzyme M reductase (MCR) (Yan and Catherine, 2011; Cheng et al., 2006). The bioaccumulation of nickel causes skin allergies, lung fibrosis, acute pneumonitis, and neoplastic transformations, and poisons the kidneys and cardiovascular system (Patrick et al., 1987). Hence, Ni^{2+}detection has considerable environmental and biological significance.

DNAzymes, as a DNA-based biocatalysts, have attracted great research interest because of excellent activity properties, high stability of the enzyme and the diversification of signal generation (Li et al., 2010; Wang et al., 2009). DNAzymes have high catalytic ability and binding activity for a specific metal ions, which could been utilized as novel and ideal platforms in the detection of metal ions based on fluorescence (Wang et al., 2008), colorimetry (Li et al., 2009) or electrochemistry (Lu et al., 2015). The biggest advantages of this type of sensor are that it does not require expert knowledge to design a metal-binding site. Hence, it is very suitable for the monitoring of metal ions in the environment because of their biocompatible, water-soluble, and low toxicity. Furthermore, with the remarkable development of nanotechnology, nanomaterials have been extensively used to enhance the sensitivity of electrochemical biosensor. Combining the specific recognition toward target metal ion and catalytic reaction of DNAzyme with the high sensitivity of electrochemical, a DNAzyme-based electrochemical strategy is designed for the first time to detect nickel ion (Ni^{2+}).

In this work, a novel electrochemical probe based on DNAzyme-CdSe nanocomposite for detection of Ni^{2+} was developed. The probe consists of a CdSe QDs, enzyme strand (DNA1) with a thiol group, and its substrate strand (DNA2) with an amino group (NH_2). The substrate strands would be cleaved in the presence of the Ni^{2+} target, resulting in disassociation of the shorter DNA fragments containing CdSe QDs. The remaining CdSe quantum dot on the electrode surface detected by differential pulse anodic stripping voltammetry. The linear range of the proposed biosensor

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is from 20 nM to 0.2 mM, with a detection limit of 6.67 nM.

2. Material and methods

2.1. Reagents

Bovine serum albumin (BSA) was provided by Sigma-Aldrich Co. Ltd. (USA). 1-Ethyl-3-(3-dimethyl aminopropyl) carbodiimide (EDC), N-hydroxy succinimide (NHS), DNA and Tris (hydroxymethyl) aminomethane (Tris, $C_4H_{11}NO_3$, ≥ 99.9%) were provided by Shanghai Sangon Biotech. Co. Ltd. (Shanghai, China). Sodium borohydride ($NaBH_4$, 98%), cadmium chloride ($CdCl_2 \cdot 2.5H_2O$, 99.0%) were purchased from Sinopharm. Chemical Reagent Co. Ltd. Selenium powder (Se), thioglycolic acid (TGA, 98%) and some nitrates were obtained from Aladdin Reagent Co. Ltd. (Shanghai, China). All reagents were used without further purification. Ultrapure fresh water was obtained from an AK Exceed-E-V lab pure water system and was used throughout this work (electrical resistivity ≥ 18 MΩ).

The DNA constructs were synthesized by Shanghai Sangon Biotechnology Co. Ltd. (Shanghai, China). The sequences of two oligomers are given as follows:

DNA-1: C*A*CGTCCATCTCTGCAGTCGGGTAGTTAAACCGACCTTCAGACATAGTGAGTAGCAAAAAAAAAA*A*A-SH
DNA-2: GT*G*CAGGTAGAGAAGG/RA/TATCACTC*A*-NH_2

2.2. Apparatus

The morphology and structure were characterized by transmission electron microscopy (TEM; JEM-2100, JEOL, Japan). All electrochemical measurements were performed using a CHI 660D electrochemical workstation (Shanghai Chenhua Instrument Co. Ltd., China) with a conventional three-electrode system comprising modified Au electrode or glassy carbon electrode as the working electrode, a platinum wire as the auxiliary electrode, and a reference electrode (Ag/AgCl). The cyclic voltammetry (CV) analysis were did at 100 mV/s scan rate range from −0.2 V to 0.6 V in the electrolyte containing 5 mM of $Fe(CN)_6^{3-/4-}$ and 0.1 M of KCl. Electrochemical impedance spectra (EIS) were measured with a Thales electrochemical workstation (Zahner-elektrik GmbH & Co. KG, Germany), using the three-electrode system comprising a platinum wire electrode as the auxiliary electrode, a saturated calomel as the reference and a glassy carbon electrode as the working electrode. The frequency range in EIS experiments is between 0.01 Hz and 100 kHz, with a signal amplitude of 5 mV.

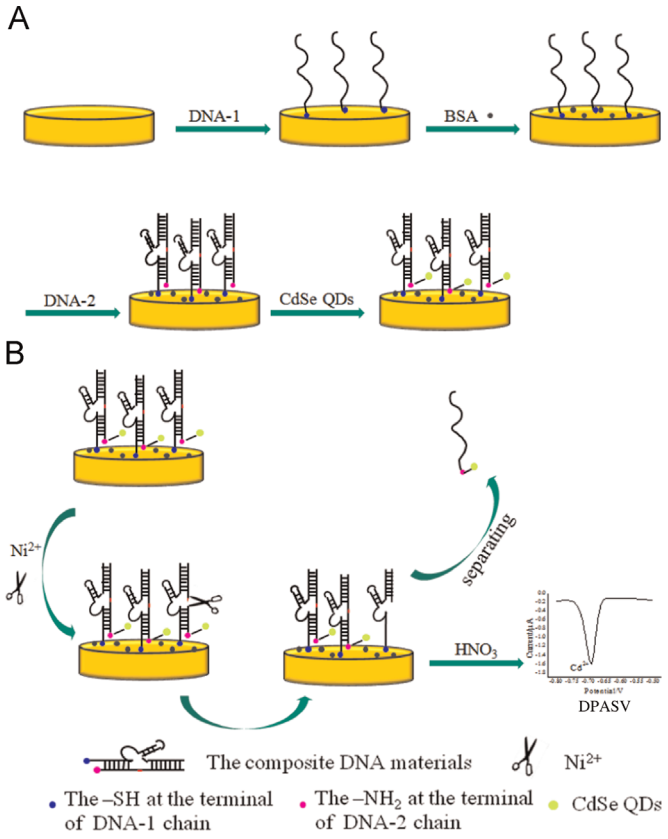
2.3. Preparation of CdSe composite

CdSe QDs were synthesized using a previously reported process with slight modifications (Li et al., 2012). 50 mL of 0.1 M $CdCl_2 \cdot 2.5H_2O$ and 60 mL diluents of 0.84 mL 90.0% TGA were added into a flask. The mixture solution was adjusted to approximately pH 10 by 0.1 M NaOH, and then12 mL of 0.2 M NaHSe was added to the mixture. The solution was heated and refluxed for 4 h, resulting in the successful synthesis of the transparent CdSe QDs.

2.4. Fabrication of electrochemical biosensor

2.4.1. Pretreatment of gold electrode

The gold electrode (d=4 mm) was first carefully polished to a mirror finish using alumina powder and then sonicated in ethanol and ultrapure water in turn. Subsequently, 10 μL of the mixture (98% H_2SO_4:30% H_2O_2=7:3, V/V) was dropped onto the Au electrode to clean the surface of the electrode. The gold electrode was scanned in 0.5 M H_2SO_4 until a reproducible cyclic voltammogram (CV) was obtained. Finally, the electrode was allowed to dry in the presence of N_2.



Scheme 1. Schematic representation of (A) preparation procedure of electrochemical biosensor and (B) the stepwise experiment principle.

2.4.2. Fabrication of electrochemical biosensor

The preparation steps are shown in Scheme 1. Firstly, The end of DNA1 is functionalized with a thiol group (SH) for immobilization onto gold electrode. After hybridization with capture probe (DNA1) and sequentially with DNA (DNA2), CdSe QDs were incorporated the specific recognition of DNA2 by covalent assembling. Upon addition of nickel ion into the above system, the substrate strand of the immobilized DNAzyme was catalytically cleaved by target Ni^{2+}, resulting in disassociation of the shorter DNA fragments containing CdSe QDs. Then the remaining CdSe QDs on the electrode surface was detected by DPASV. Finally, the DPASV response signals correlated with concentration of the Ni^{2+}.

The Au electrode was first modified with 20 μL of 5 μM DNA1 and incubated overnight at 37 °C. Blocking reagent (BSA) was added to the surface of the Au electrode to reduce nonspecific adsorption, and then the modified Au electrode was hybridized with DNA2 (5 μM) for 1 h at 37 °C. The Au electrode was finally incubated with the liquor mixture of CdSe QDs and EDC (20 mg/mL) -NHS (10 mg/mL) for 1.5 h at 37 °C. The prepared electrode was stored at 4 °C until used. To cleave Ni^{2+}-dependent DNAzyme, the resulting Au electrode was dipped into $Ni(NO_3)_2$ solution at a certain concentration for 60 min. Finally, the as-prepared electrode was rinsed three times with deionized water before it was used in electrochemical measurements.

2.4.3. Electrochemical analysis

The detection of Cd was determined by differential pulse anodic stripping voltammetry (DPASV). The resulting electrode was

rinsed with deionized water and immersed into HNO_3 (200 μL, 0.1 M) for 2 h to dissolve the residual CdSe QDs. The dissolved solution was added into a glass cell containing 5 mL acetate buffer (0.2 M, pH 5.2) spiked with 40 ppm mercury(II). The anodic stripping voltametric detection involved pretreatment at +0.6 V for 2 min, and electrodeposition at −1.1 V for 6 min, and stripping from −0.9 to −0.2 V under N_2 atmosphere using a differential pulse voltammetry, with 4 mV potential steps, 25 Hz frequency and 25 mV amplitude.

The optimization of detection conditions was carried out range from 0 V to 0.6 V by differential pulse voltammetry (DPV).

3. Results and discussion

3.1. Characterization of CdSe QDs

The TEM image (Fig.1A) shows that the average size of CdSe QDs is about 5 nm. Meanwhile the high-resolution transmission electron microscopy (HRTEM) image (Fig. 1B) reveals the prepared CdSe QDs is quite uniform and good morphology.

3.2. Feasibility of sensor

The process of electrochemical biosensor was studied by cyclic voltammetry (CV) in the electrolyte containing 5 mM of $Fe(CN)_6^{3-/4-}$ and 0.1 M of KCl (Fig. 2 A). After the DNA1 was modified on the Au electrode surface, the CV signal was dramatically decreased due to the weak conductivity of DNA1, which hinders the electron transfer. After BSA was added, the CV signal was further decreased because the BSA further hinders the electron transfer. When DNA2 was hybridized with DNA1 on the surface of the modified Au electrode, the CV signal decreased to some extent, indicating successful hybridization. Modifying the CdSe QDs at the terminal of DNA2 further decreased the CV signal, which may be due to the repulsive electrostatic interactions between the anionic $Fe(CN)_6^{3-/4-}$ and the negatively charged CdSe QDs (Tang et al., 2014b). This phenomenon suggests that the captured CdSe QDs were successfully immobilized at the terminal of DNA2.

The Electrochemical impedance spectroscopy (EIS) measurements were implemented to monitor the impedance changes during the hybridization process, and the results were consistent with those of the CV test. The typical electrochemical interface can be represented as an electrical circuit as shown in the inset of Fig. 2B. The components in the equivalent circuit consist of the solution resistance R_s, charge-transfer resistance R_{ct}, constant phase element (CPE) and Warburg impedance Z_w. The diameter of the semicircle reveals the R_{ct} in the Nyquist plot of impedance spectroscopy. The bare gold electrode revealed a small semicircle domain, which implies the lowest electron-transfer resistance (curve a in Fig. 2B). Compared with curve a in Fig. 2B, the semicircle diameter in curve b is increased due to the introduction of DNA1. Curve c shows the results of adding BSA to the surface of the modified Au electrode. The semicircle diameter is largely increased, which shows that the addition of BSA further prevented the transformation of electrons to the electrode surface. Furthermore, EIS of the hybridization between the two DNA chains shows a large increase in diameter (curve d in Fig. 2B), suggesting that the DNA2 formed an additional barrier. After modifying the CdSe QDs at the terminal of DNA2, the diameter remains unchanged tolerably (curve e in Fig. 2B), suggesting that the CdSe QDs successfully modified to the surface of electrode.

3.3. Optimization of detection conditions

To efficiently apply the electrochemical system to Ni^{2+} detection, several experimental parameters including pH value and incubation time of the hybridization and the time of Ni^{2+} cleaving reaction were optimized. Prior to testing the sensor, the pH value were optimized for the performance of hybridization process. The DPV response signals were investigated with different pH value. As shown in Fig. 3A, the DPV response increased from 4.0 to 7.4 and then decreased. The maximum current intensity was observed at pH 7.4. The influence of pH value on the peak current may be explained as follows. The pH value affects hybridization efficiency. Since the optimal pH value for the biological systems is about 7.4, the detection is performed in pH 7.4.

In addition, the incubation time were also optimized for the performance of hybridization. As shown in Fig. 3B, the intensity of the DPV response signals increased as the incubation time increased. The DPV response signal did not change at incubation time longer than 60 min, which was attributed to the limitation of the saturated hybridization site. Thus, 60 min was selected as the optimal incubation time.

The detection sensitivity depended on the cleaving reaction of DNA1 and Ni^{2+} on the electrode surface. Thus, the cleaving reaction time is important in this method. The effect of the cleaving reaction time in the presence of 200 μM of Ni^{2+} was optimized. As shown in Fig. 3C, the intensity of the DPV response signal decreased rapidly with prolonged reaction time. The DPV peak current was stable when the cleaving reaction was maintained at approximately 60 min, which was attributed to the limitation of the saturated cleaving site. Therefore, 60 min was selected as the reaction duration for the cleaving reaction process.

Fig. 3D shows the DPV response – concentration curves of the electrochemical biosensor with different concentrations of Ni^{2+} in Tris–HCl buffer solution. When the Ni^{2+} was put into the solution, the DNA was cleaved. As the concentration of Ni^{2+} increased, the DPV response signal increased accordingly. The linear calibration equation was $I = -1.014\log C + 35.64$ (I is the DPV response signal (μA) for cleaving the DNA, and C is the concentration (nM) of Ni^{2+}). The correlation coefficient (R) was 0.999 (Fig. 3D).

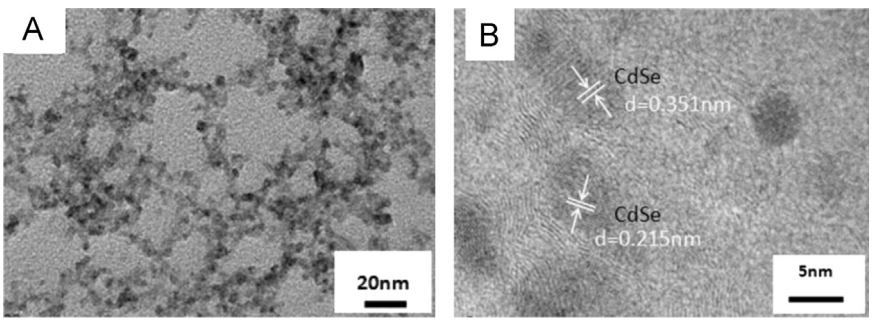


Fig. 1. TEM images of (A) CdSe QDs and HRTEM images of (B) CdSe QDs.

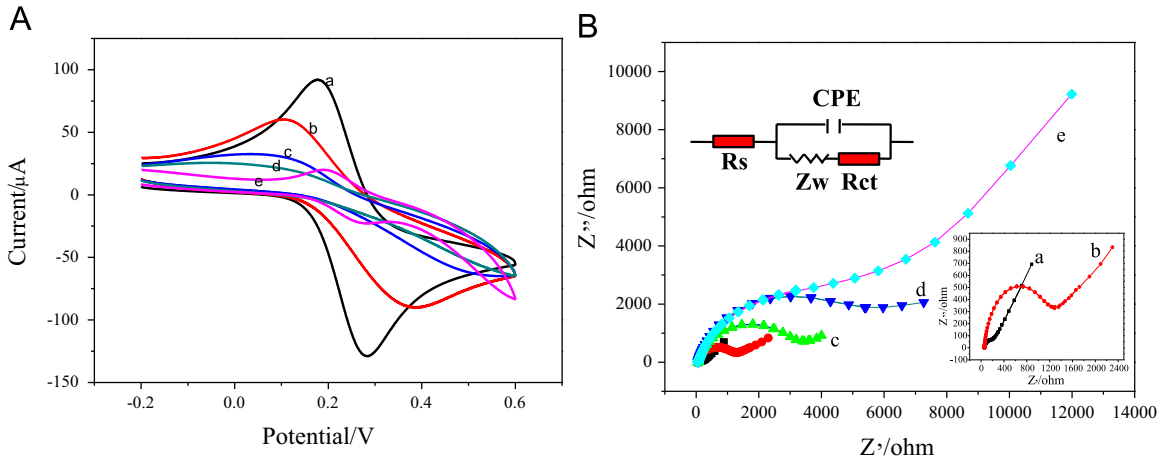


Fig. 2. (A) CVs of gold electrodes modified using different materials in 5 mmol L^{-1} of $Fe(CN)_6^{3-/4-}$ and 0.1 mol L^{-1} of KCl: (a) bare Au electrode; (b) DNA1/Au electrode; (c) BSA/DNA1/Au electrode; (d) DNA2/BSA/DNA1/Au electrode; (e) CdSe/DNA2/BSA/DNA1/Au electrode. (B)EIS of the electrode at different stages: (a) bare Au electrode; (b) after immobilization of DNA1; (c) after obturation with BSA; (d) after hybridization reaction with DNA2; (e) after loading the CdSe QDs. The frequency range in EIS experiments is between 0.01 Hz and 100 kHz, with a signal amplitude of 5 mV.

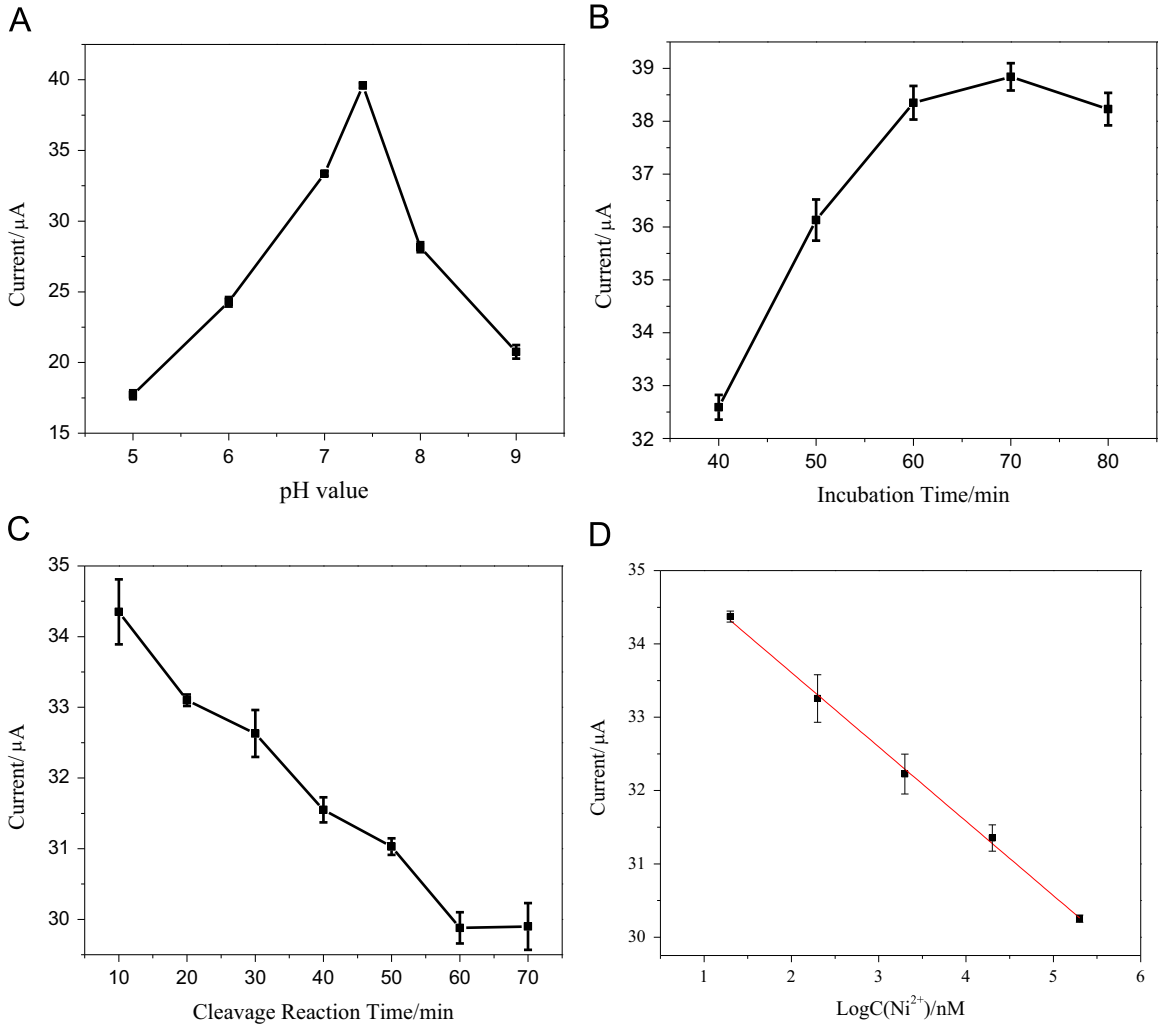


Fig. 3. The effects of (A) pH value, (B) the incubation time, (C) the cleaving reaction time on DPV response signals, and (D) the linear relationship of Ni^{2+} to cleave the DNA.

3.4. Electrochemical detection of Ni^{2+}

The DPASV response at different concentrations of Ni^{2+} are shown in Fig. 4A. As the concentration of Ni^{2+} increased, the DPASV response of Cd^{2+} decreased accordingly. The corresponding calibration plot of peak currents versus the logarithm of the Ni^{2+} concentration was linear in the range from 20 nM to 200 μM, which is suitable for quantitative analysis of low levels of metal ions. The correlation equation was $I = 0.143\log C - 2.359$ (I is the peak current (μA) of resulting CdSe on

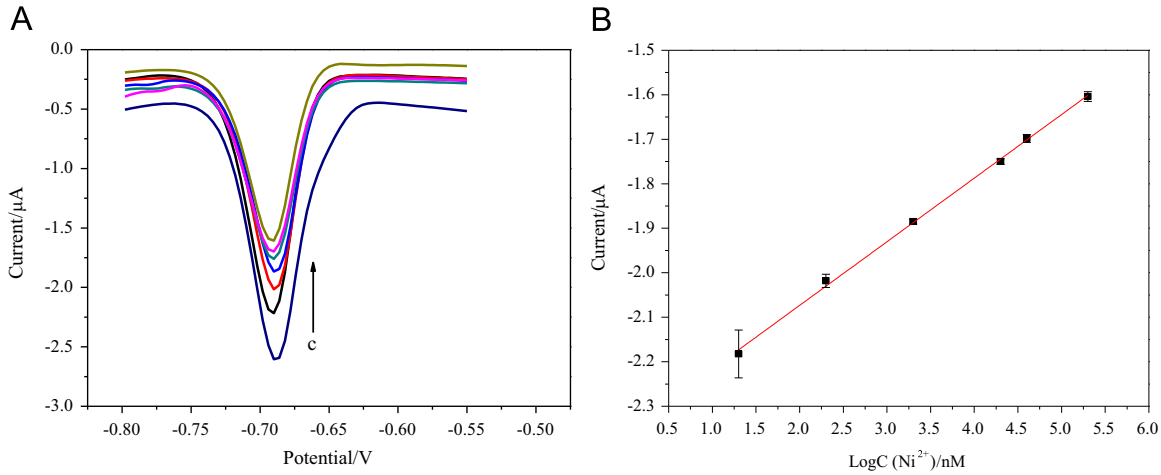


Fig. 4. (A) DPASV response for Cd^{2+} with different concentration; (B) calibration curve for Ni^{2+} concentration.

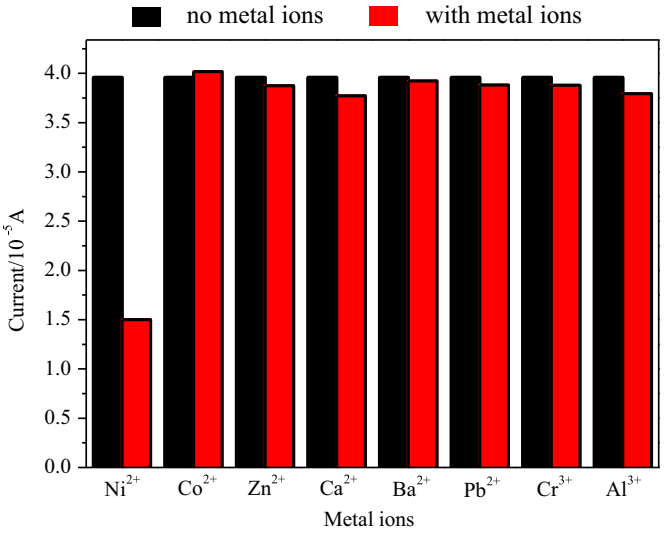


Fig. 5. Selectivity investigation of the proposed sensor.

the electrode surface, and *C* is the concentration (nM) of Ni^{2+}), and *R* was 0.999 (Fig. 4). When the concentration of Ni^{2+} increased, more the shorter DNA fragments containing CdSe QDs was cleaved and disassociated into the electrolyte. As a result, the peak current of the remaining CdSe quantum dot on the electrode surface was decreased. This phenomenon was observed for another electrochemical biosensor (Qian et al., 2014).

Furthermore, the analytical performance of the proposed biosensor was compared with that of reported sensors. The detectable concentration range was much wider than those of 10–50 μM (Ghosh et al., 2014) for PL detection and 1.18 nM–73.4 μM for optical method (Alizadeh et al., 2014). Meanwhile, an LOD of 6.67 nM was much lower than the 0.1 μM of colorimetric method (Diecenia et al., 2014). The proposed biosensor shows a wide linear range and low detection limit for the detection of Ni^{2+}.

3.4.1. *Selectivity and reproducibility*

The selectivity of the sensor was investigated by adding the same concentration of other metal ions, such as Co^{2+}, Zn^{2+}, Cd^{2+}, Ba^{2+}, Pb^{2+}, Cr^{3+} and Al^{3+}, to the electrolyte, respectively. Compared with the current intensity of Ni^{2+}, no obvious DPV response signal was observed (Fig. 5). This result indicated that the biosensor has a excellent selectivity for Ni^{2+}, and that it can be used as a sensitive and selective probe for Ni^{2+} determination. The reproducibility of the sensor was estimated five different electrodes. The relative standard deviation (RSD) was 4.03%, which indicates the sensor has good electrode-to-electrode reproducibility. After the proposed sensor was stored at 4 °C for one month, the electrochemical strength did not show any apparent change, which suggests that the sensor has good stability.

4. Conclusions

In this work, we developed a novel electrochemical biosensor based on a DNAzyme-CdSe nanocomposite. On the basis of the catalytic properties of the DNAzyme, the electrochemical peak intensity has been regulated upon the concentrations of Ni^{2+}. By integrating the specific recognition of DNAzyme toward targets with the elcectrochemical sensing strategy was achieved with a wide linear response range, a low detection limit, and high anti-interferential capability. Notably, based on the proposed DNAzyme nanocomposite modified electrochemical biosensor, a series of sensors made up of DNAzyme can be easily fabricated.

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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.bios.2015.09.014.

References

Alizadeh, K., Rezaei, B., Khazaeli, E., 2014. Sensor. Actuators B Chem. 193, 267–272.
Cheng, B., Charles, C.R., Bruce, W.W., 2006. Plant Physiol. 140, 433.
Cui, L., Wu, J., Ju, H.X., 2015. Biosens. Bioelectron. 63, 276–286.
Diecenia, P.D., Mario, R., Gabriel, R.O., 2014. Sensor. Actuators B Chem. 207, 511–517.
Ghosh, K., Mohan, V., Kumar, P., Ng, S.W., Tiekink, E.R.T., 2014. Inorg. Chim. Acta 416, 76–84.
Gou, C., Wu, H., Jiang, S., Yi, C., Luo, J., Liu, X., 2011. Chem. Lett. 40, 1082–1084.
Huang, P.J.J., Lin, J., Cao, J., Vazin, M., 2014. Anal. Chem. 86, 1816–1821.
Li, Y.F., Liu, L.L., Fang, X.L., Bao, J.C., Han, M., Dai, Z.H., 2012. Electrochimica Acta. 65, 1–6.

Li, D., Song, S.P., Fan, C.H., 2010. Acc. Chem. Res. 43, 631–641.
Li, H.B., Xue, Y., Wang, W., 2014. Biosens. Bioelectron. 54, 317–322.
Li, T., Dong, S.J., Wang, E., 2009. Anal. Chem. 81, 2144–2149.
Lu, L., Jing, S.C., Zhong, F.G., Yu, Z., Jing, L.L., 2015. Biosens. Bioelectron. 63, 14–20.
Park, M., Seo, S., Lee, S.J., Jung, J.H., 2010. Analyst 135, 2802–2805.
Patrick, H.B., Ross, M.W., Earle, E.C., 1987. Plant Physiol. 85, 801–803.
Qian, Y., Wang, C.Y., Gao, F.L., 2014. Anal. Chim. Acta 845, 1–6.
Qian, Y., Wang, C.Y., Gao, F.L., 2015. Biosens. Bioelectron. 63, 425–431.
Shi, L., Chu, Z.Y., Liu, Y., Jin, W.Q., Chen, X.J., 2014. Biosens. Bioelectron. 54, 165–170.
Tang, S.R., Lu, W., Gu, F., Tong, P., Yan, Z.M., Zhang, L., 2014a. Electrochim. Acta 134, 1–7.
Tang, S.R., Tong, P., Lu, W., Chen, J.F., Yan, Z.M., 2014b. Biosens. Bioelectron. 59, 1–5.
Wang, H., Kim, Y., Liu, H., Zhu, Z., Bamrungsap, S., Tan, W., 2009. J. Am. Chem. Soc. 131, 8221–8226.
Wang, Z.D., Lee, J.H., Lu, Y., 2008. Chem. Commun., 6005–6007.
Wei, D., Suna, Y., Yina, J., Weia, G., Dua, Y., 2011. Sensor. Actuators B Chem. 160, 1316–1321.
Yan, K., Catherine, L.D., 2011. Curr. Opin. Chem. Biol. 15, 276–283.