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Short communication

Amplified electrochemical sensing of lead ion based on DNA-mediated self-assembly-catalyzed polymerization

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ABSTRACT

Lead ion (Pb^{2+}, a heavy metal ion existed every aspect of life) is one of the most important hazards for environment and human health. Herein we design an *in-situ* amplified electronic monitoring system for sensitive determination of Pb^{2+} on Pb^{2+}-specific DNAzyme-modified sensing interface. The assay consists of target-triggered cleavage of Pb^{2+}-specific DNAzyme, initiator strand-induced DNA hybridization chain reaction, formation of hemin/G-quadruplex -based DNAzyme and its automatically catalyzed polymerization of aniline monomer along the double-stranded DNA. Experimental results indicated that the catalytic currents of the as-produced polyaniline were linearly dependent on target Pb^{2+} concentrations from 0.05 to 50 nM with a detection limit (LOD) of 32 pM. Such a synergistic effect of hybridization chain reaction with DNAzyme-catalyzed polymerization provided a universal platform for sensitive screening of target Pb^{2+}, thereby holding great promise for application in practice.

1. Introduction

Human living environment and health are facing severe threat from heavy metal pollution in the present-day society (Islam et al., 2014; Chen et al., 2014a, 2014b), and thus, their ultrasensitive and quantitative detection is in great demand. The research on lead ion (Pb^{2+}, as a typical heavy-metal pollutant) has attracted of particular interest. Various methods and strategies have been developed for quantitative screening of Pb^{2+} based on different signal-transduction principles, *e.g.*, atomic absorption spectroscopy (Liu et al., 2014), inductively coupled plasma atomic emission spectroscopy (Lin et al., 2014), and (electrochemi-)luminescence (Dong et al., 2014; Wang et al., 2012). One of the interesting methods is the catalytic nucleic acid to Pb^{2+}, that is, Pb^{2+}-specific RNA-cleaving DNAzyme (Xiao et al., 2007). DNAzyme is catalytic DNA sequences isolated *via in vitro* selection (Wang et al., 2014a, 2014b). Cofactor-dependent DNAzyme can be often generated from this approach by adding varying cofactors and cofactor concentrations during the selection. Pb^{2+}-specific DNAzyme has been used in various sensors including fluorescent (Li et al., 2014), colorimetric (Liu and Lu, 2004) and electrochemical biosensors (Zhang et al., 2013a, 2013b).

DNA-mediated self-assembly, an important part of modern nanotechnology, has attracted widespread interest because of its double helix structure and the base pairing. DNA is one of the most promising chemical moieties to direct the self-assembly process. Recently, different self-assembled DNA nanostructures have been constructed and employed in biosensors, such as DNA tetrahedral probe (Chen et al., 2014a, 2014b) and hybridization chain reaction (HCR) (Yang et al., 2014). Moreover, DNA self-assembly process was also reported for signal amplification of electrochemical DNA sensors. During the process, high sensitivity was achieved by using the labeling indicators (*e.g.*, ferrocene and methylene blue) in the DNA concatamers. Our group had developed silver nanocluster-based rolling circle amplification strategy (Zhang et al. 2013a, 2013b) and immune-HCR assay strategy (Zhang et al., 2012) for the amplification of the detectable signal. Unfortunately, the amount of the labeled indicators on each molecule was always limited due to the limitation of available active sits. In this regards, our expectation is to utilize the DNA-mediated self-assembly for the online synthesis of electrocatalytic indicators, *e.g.*, hemin/G-quadruplex-based complex.

G-quadruplex with a unique higher-order structure is common in biologically important chromosomal features including telomeres, gene promoters, and IgG switch regions. Hemin-based G-quadruplex complex is an important discovery due to its peroxidase-like activity. Sharon et al. (2014) reported an ultrasensitive and highly selective colorimetric method for analyzing of telomerase activity through the telomeric hemin/G-quadruplex-controlled aggregation of gold nanoparticles in the presence of

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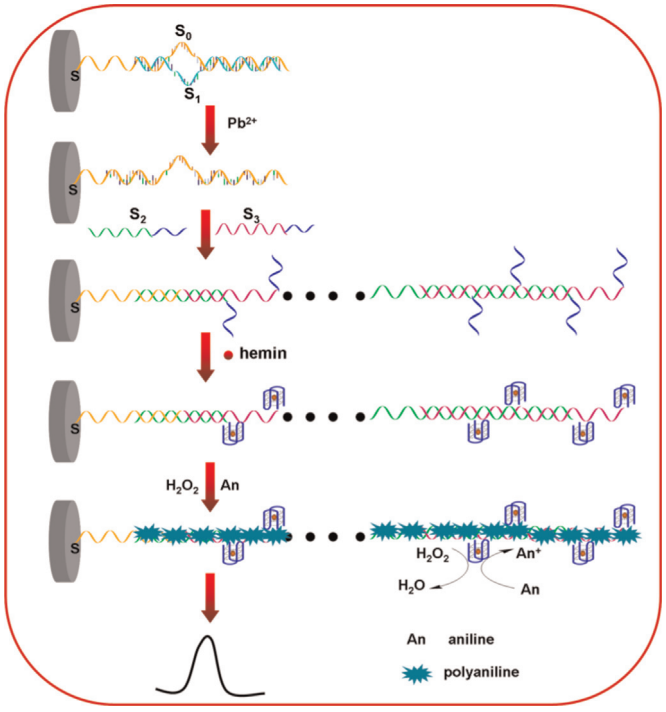
cysteine. Wang et al. (2013) developed self-assembled catalytic DNA nanostructures for the synthesis of para-directed polyaniline. Polyaniline (PAn), a useful conductive polymer, has been extensively studied in the fields of electrocatalysis and electrochemical sensors due to its excellent conductivity and redox property (Arora et al., 2007; Ruecha et al., 2014; Wang et al., 2014a, 2014b). It was reported that peroxidases, such as horseradish peroxidase (HRP), effectively catalyze the polymerization of aniline in the presence of H_2O_2 under very mild conditions (Fan et al., 2007). Also, hemin/G-quadruplex could control the form of PAn along with double-stranded DNA because of the electrostatic interaction between the protonated aniline molecules and phosphates in the DNA.

Herein, we report on the proof-of-concept of a novel and powerful electrochemical biosensor for the detection of Pb^{2+} by coupling with DNA-mediated self-assembly and hemin/G-quadruplex DNAzyme as the signal-amplified strategy. The assay is carried out as follows: (i) cleavage of Pb^{2+}-specific DNAzyme on the electrode, (ii) DNA self-assembly based on hybridization chain reaction on the electrode, (iii) formation of hemin/G-quadruplex complex, (iv) polyaniline generation through the enzyme-catalyzed polymerization and (v) electrochemical measurement. The signal derives from the electroactive polyaniline. The aim of this study is to probe trace Pb^{2+} with high sensitivity through the online amplification strategy of detectable signal.



Scheme 1. Schematic illustration and electrochemical measurement of target Pb^{2+}-triggered DNA self-assembly-catalyzed polymerization of monomer aniline on HCR-based double-helix DNA accompanying formation of hemin/G-quadruplex complex.

2. Experimental

2.1. Material and reagent

Hemin was purchased from Tokyo Chem. Inc. (Japan). All the oligonucleotides used in this work were obtained from Sangon Biotech. Co. Ltd. (Shanghai, China). The sequences of oligonucleotides were listed as follows:

Catalytic strand (S_0): 5′-HS-TTTCATCTCTTCTCCGAGCCGGTC-GAAATAGTGAGT-3′
Substrate strand (S_1): 5′-ACTCACTATrAGGAAGAGATG -3′
Hybridization strand (S_2): 5′-TGGGTTGGGCGGGATGGGA-CAGCAGCTGACTCACTATT -3′
Hybridization strand (S_3): 5′-CAGCTGCTGTAA-TAGTGAGTGGGTAGGGCGGGTTGGGT -3′

Pb^{2+}-dependent DNAzyme comprising of catalytic strand (S_0) and substrate strand (S_1) was obtained by using the SELEX process as the reference (Fu et al., 2013). DNA stock solution was obtained by dissolving the oligonucleotides in Tris–HCl buffer (pH 7.4). Each oligonucleotide was heated to 90 °C for 5 min and slowly cooled to room temperature before use. All other reagents were of analytical grade. Ultrapure water from a Milli-Q water purification system (18.2 MΩ cm^{-1}, Millipore) was used in all runs. 0.1 M acetic acid-buffered saline (ABS) solutions with various pH values were prepared by mixing 0.1 M HAc and 0.1 M NaAc, and 0.1 M KCl was added as the supporting electrolyte.

2.2. Fabrication of Pb^{2+}-specific DNAzyme-based sensor

A gold electrode (3 mm in diameter) was polished repeatedly with 0.3 and 0.05 μm alumina slurry, followed by successive sonication in bidistilled water and ethanol for 5 min and dried in air. Before modification, the gold electrode was continuously scanned within the potential range from −0.3 to 1.5 V in freshly prepared deoxygenated 0.5 M H_2SO_4 until a voltammogram characteristic of the clean gold electrode was established. After rinsing with distilled water and absolute ethanol, the cleaned gold electrode was immersed into a 0.2 M Tris–HCl buffer (pH 7.4) containing 2.0 OD S_0 and 8.0 mM Tris–(2-carboxyethyl) phosphine hydrochloride (TCEP) (Note: The aim of using TCEP was to reduce the disulfide-bonded oligonucleotides) for 16 h. After rinsing with distilled water, the modified gold electrode was incubated with 1.0 mM 6-mercaptohexanol in 10 mM Tris–acetate buffer, pH 7.4, for 60 min. Following that, the S_0-modified electrode was dipped again into 2.0 OD S_1. To make S_0 hybridize with S_1, the electrode was initially incubated in an oven at 65 °C for 10 min, and then further carried out at room temperature for 12 h. Finally, the as-prepared Pb^{2+} sensor was stored at 4 °C when not in use.

2.3. Electrochemical measurement

Scheme 1 gives the measurement process of DNA self-assembly-based signal amplification strategy for Pb^{2+} detection. Initially, the Pb^{2+} sensor reacted with target Pb^{2+} ion for 60 min at room temperature. During this process, Pb^{2+} ion specifically cleaved the S_1 (rA), and the newly exposed 3′ end of S_0 acted as the similar primer to re-hybridize with the S_2. Afterward, 10 μL of the newly prepared buffer (50 mM, pH 7.5 Tris–HCl buffer) containing 1.0 μM S_2 and 1.0 μM S_3 was dropped on the surface of the resulting electrode and incubated for 60 min to complete the long-range DNA self-assembly. Following that, the modified electrode was suspended into 0.2 mM hemin solution and reacted for 30 min at room temperature to form hemin/G-quadruplex complex. To carry out the deposition of polyaniline (PAn) on the double-helix DNA by the formed hemin/G-quadruplex complex, the resulting electrode was immersed into a 0.1 M HAc-NaAc (pH 4.3) containing 1.0 mM aniline and 5.0 mM H_2O_2 for 15-min accumulation time. All electrochemical measurements were carried out on a CHI 620D Electrochemical Workstation (Shanghai, China) with a conventional three-electrode system including a modified gold working electrode, a platinum auxiliary electrode, and an Ag/AgCl reference electrode. The current was recorded in pH 4.3 ABS containing

1.0 mM aniline and 5.0 mM H_2O_2 by using square wave voltammetry (SWV) from 50 mV to 450 mV (Parameters: potential step: 4 mV, frequency: 25 Hz, amplitude: 25 mV). Analyses were always made in triplicate.

3. Results and discussion

3.1. Feasibility study

The designed strategy of the DNAzyme and DNA self-assembly-based sensing system is depicted in Scheme 1. Firstly, the S_0 labeled with a sulfydryl at the 5′ end is incubated with gold electrode, and then hybridized with S_1 to form a Pb^{2+}-specific DNAzyme containing a large loop. In the presence of Pb^{2+}, the DNAzyme is activated and cleaved the S_1 at the RNA site into two parts, releasing the oligonuleotide fragment immobilized on the electrode. In addition, two auxiliary DNA strands (S_2 and S_3) were designed for the concatamer reaction. The S_0 can propagate a chain reaction of hybridization events between alternating S_2 and S_3 in sequence to form a nicked double-helix. Upon addition of hemin, the hemin-binding aptamer can be bound to form the hemin/G-quadruplex-based DNAzyme. Hence, a large number of hemin/G-quadruplex complexes are cascaded through the double-helix DNA. Meanwhile, the hemin/G-quadruplex complex, as an HRP-mimicking DNAzyme, is an efficient catalyst for the polymerization of anilines in the presence of H_2O_2. Importantly, the double-helix DNA, on which the aniline monomers preferentially align and promote a para-directed reaction, could be used as the template of the fabrication of polyaniline nanowires.

To realize our design, we firstly investigate the electrochemical characteristics of the as-prepared DNAzyme sensor before and after reaction with lead ion by using electrochemical impedance spectroscopy (EIS) (Fig. 1A). As seen from curve '*a*', a small resistance was obtained at bare gold electrode, while the DNAzyme-modified electrode exhibited a high resistance (curve '*b*'). The reason might be attributed to the repulsion between the negatively charged phosphate skeleton of probe DNA and $Fe(CN)_6^{3-/4-}$ ions. Surprisingly, when the immobilized DNAzyme reacted with Pb^{2+} ion, a larger resistance was achieved (curve '*c*'). This is most likely as a consequence of the fact that when the DNAzyme was cleaved by target Pb^{2+}, the conformation of probe S_0 was changed, which enhanced the steric repulsion of the surface-bound DNA, thus acting as the dominant barrier against access of the redox to the surface. The results preliminarily revealed that the designed DNAzyme sensor could be used for determination of lead ion.

As mentioned above, the electrochemical signal mainly derived from polyaniline. As shown in Fig. 1B, one pair of well-defined redox peaks at +200 and +250 mV was observed (curve '*b*'), ascribing to the first redox process of polyaniline. To further prove the formation of polyaniline, atomic force microscope (AFM) images of the surface morphology during the assembling process are listed in Fig. 1C–D. Fig. 1C shows a relatively clean and smooth surface of gold substrate before polymerization. The nanoparticles can be seen clearly when the polyaniline was formed on the gold substrate (Fig. 1D), thus giving a clear impression on the morphology of the surface.

3.2. Comparison of different sensing strategies

To investigate the signal amplification of the HCR assay during the electrochemical measurements, the as-prepared biosensors

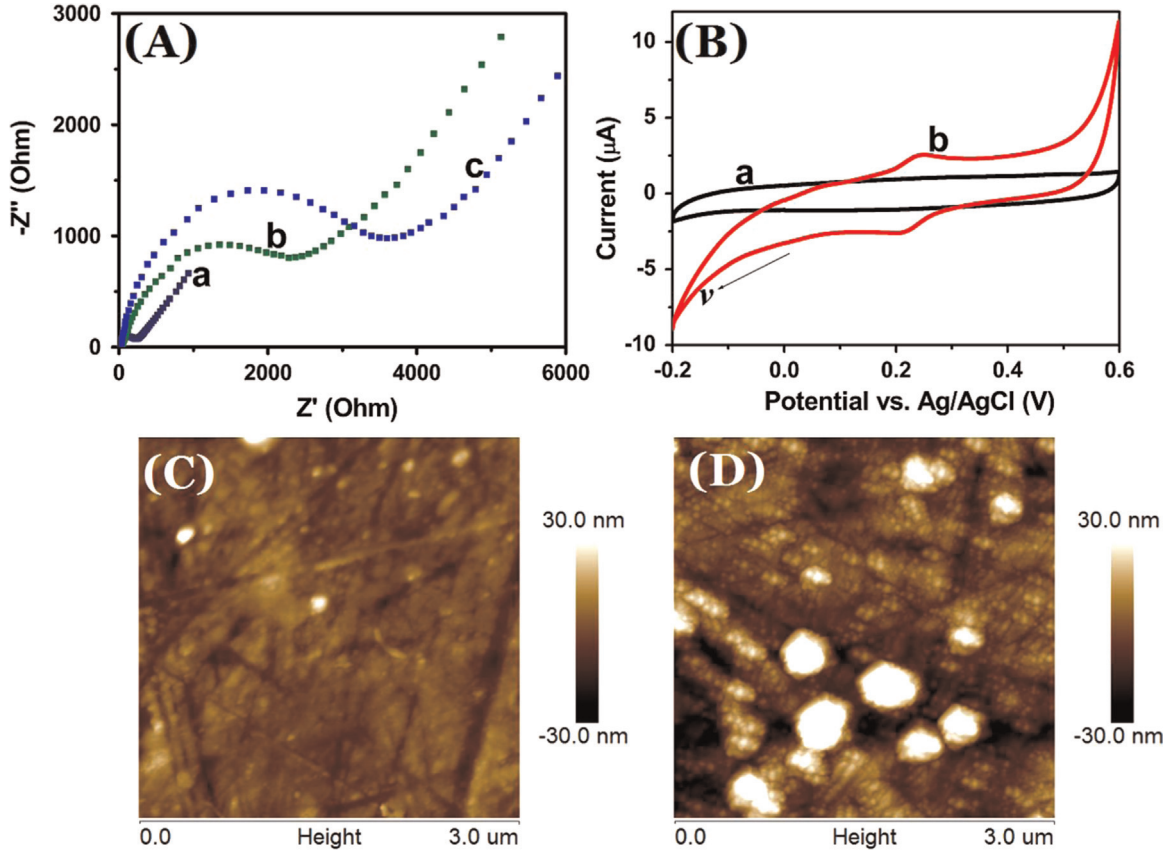


Fig. 1. (A) SWV responses of S_0-modified electrode after incubation with different-time S_2+S_3 ('*n*'), hemin and aniline (The letters '*a*–*e*' represent 2, 4, 6, 8 and 10 times, respectively); (B–D) the effects of (B) cleavage time of Pb^{2+} toward Pb^{2+}-specific DNAzyme, (C) hybridization time of S_2+S_3 and (D) binding time with hemin; and (E) calibration plots and (F) specificity of the Pb^{2+} sensor (Inset: the corresponding SWV curves in Fig. 2E). Error bar represents the standard deviation ($n=3$).

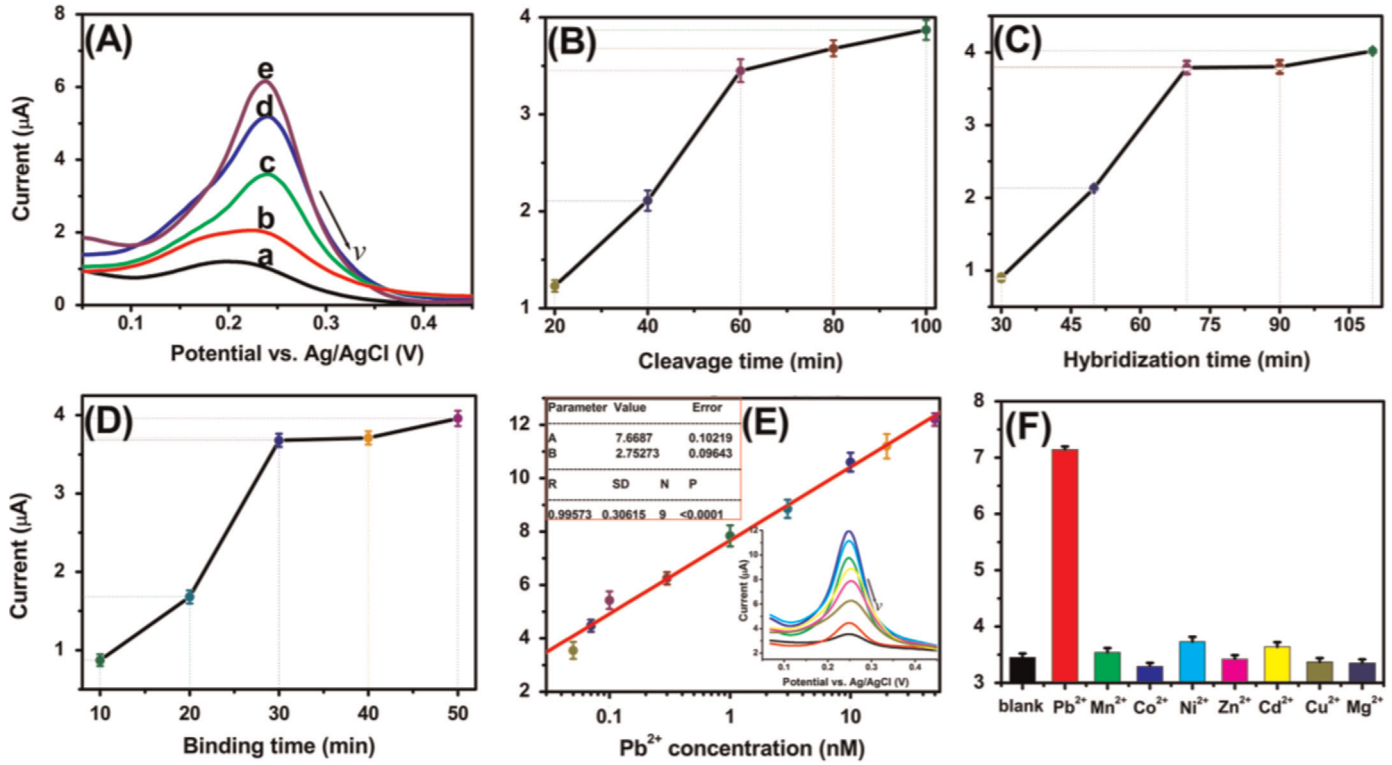


Fig. 2. (A) SWV responses of S_0-modified electrode after incubation with different-time S_2+S_3 ('*n*'), hemin and aniline (The letters '*a*–*e*' represent 2, 4, 6, 8 and 10 times, respectively); (B–D) the effects of (B) cleavage time of Pb^{2+} toward Pb^{2+}-specific DNAzyme, (C) hybridization time of S_2+S_3 and (D) binding time with hemin; and (E) calibration plots and (F) specificity of the Pb^{2+} sensor (Inset: the corresponding SWV curves in E). Error bar represents the standard deviation ($n=3$).

were utilized for determination of 1.0 nM Pb^{2+} under the different conditions (Fig. 2A). As shown in Fig. 2A, the peak current increased with the increasing hybridization times '*n*'. The increasing peak currents mainly derived from the amount increment of the hemin/G-quadruplex complex with the progress of hybridization reaction, which could enhance formation of polyaniline.

To ensure an optimal analytical performance, the experimental conditions should be investigated. As seen from Fig. 2B, the peak currents increased with the increasing incubation time, and tended to level off after 60 min. A longer incubation time did not obviously change the electrochemical signal. Hence, 60 min was chosen for the cleavage of DNAzyme in this work. In addition, the hybridization time of S_0 with S_2/S_3 on the electrode also affected the electrochemical signal. As seen from Fig. 2C, the maximum peak current was reached at 60 min. So, 60 min for the hybridization reaction between S_0 and S_2+S_3 was selected through this experiment. And it could be conclude from the Fig. 2D that 30 min was affirmed for the hemin/G-quardruplex binding.

3.3. Analytical performance

Under optimal conditions, the sensitivity and working range of the electrochemical biosensor was studied by assaying routine samples with different Pb^{2+} standards based on the developed protocol in pH 4.3 ABS containing 1.0 mM aniline and 5.0 mM H_2O_2 by using SWV. Typically, the peak currents increased with the increasing Pb^{2+}, and displayed a linear relationship between the SWV peak currents and the logarithm of Pb^{2+} concentrations in the range from 0.05 to 50 nM (Fig. 2E). The detection limit (LOD) was 32 pM at the $3s_B$ criterion (*i.e.*, $3\sigma_{blank}$/slope, where σ_{blank} refers to the standard deviation of blank samples, $n=11$).

The intra-assay and inter-assay precisions of the biosensor were examined. The relative standard deviation (RSD) for target Pb^{2+} with the same-batch biosensor was 8.3% ($n=20$), while the RSD with various-batch biosensors was 8.9% ($n=20$), indicating good precision of the bioassay and acceptable fabrication reproducibility of the biosensors. When the biosensor was not in use, it was stored at 4 °C. An 89.3% of initial response was retained after one week.

To investigate the selectivity and specificity of the biosensor, contrast experiments were performed (Fig. 2F). The biosensors were incubated with 100 nM interfering substances, *e.g.*, Zn^{2+}, Cu^{2+}, Cd^{2+}, Mn^{2+}, Mg^{2+}, Co^{2+} and Ni^{2+}, respectively. Compared with the signal of target Pb^{2+} (1.0 nM), the signals of interfering substance were obviously low although the concentrations of the interfering substrates were higher than that of Pb^{2+}. These results indicated a good selectivity and specificity.

Finally, the method was applied for determination of Pb^{2+} in water obtained from Minjiang River. Six samples were prepared by spiking Pb^{2+} standards into the water filtered through filter paper, and then diluted 100-μL water sample to 100-fold with detection solution. The recoveries were 105–123%, which all exceed 100% due to the inherent presence of Pb^{2+} in the river. Therefore, the methodology was feasible for screening of target Pb^{2+} in practical samples.

4. Conclusion

In summary, we have demonstrated the potential of an on-line amplified electronic monitoring platform for target Pb^{2+} by coupling with Pb^{2+}-specific DNAzyme and hemin/G-quadruplex complex-induced catalytic polymerization. By taking advantage of the high catalytic efficiency of hemin/G-quadruplex complex, the developed sensor exhibits a high sensitivity toward Pb^{2+}. Moreover, the sensing system was used for the determination of Pb^{2+} in river water samples with satisfying results. Nevertheless, only one disadvantages of the methodology is the long-time reaction

for DNA-mediated self-assembly-catalyzed polymerization. To fulfill the potential application in future, the inescapable work should focus on the improvement of the assay time.

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