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Label-free signal-on aptasensor for sensitive electrochemical detection of arsenite

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ABSTRACT

A signal-on aptasensor was fabricated for highly sensitive and selective electrochemical detection of arsenite with a label-free Ars-3 aptamer self-assembled on a screen-printed carbon electrode (SPCE) via Au–S bond. The Ars-3 aptamer could adsorb cationic polydiallyldimethylammonium (PDDA) via electrostatic interaction to repel other cationic species. In the presence of arsenite, the change of Ars-3 conformation due to the formation of Ars-3/arsenite complex led to less adsorption of PDDA, and the complex could adsorb more positively charged $[Ru(NH_3)_6]^{3+}$ as an electrochemically active indicator on the aptasensor surface, which produced a sensitive "turn-on" response. The target-induced structure switching could be used for sensitive detection of arsenite with a linear range from 0.2 nM to 100 nM and a detection limit down to 0.15 nM. Benefiting from Ars-3 aptamer, the proposed system exhibited excellent specificity against other heavy metal ions. The SPCE-based aptasensor exhibited the advantages of low cost and simple fabrication, providing potential application of arsenite detection in environment.

1. Introduction

Arsenic contamination is a pressing environmental issue in many regions and has brought about a series of health problems, including skin lesions, keratosis, heart disease and lung cancer (Cullen and Reimer, 1989; Li and Smart, 1996; Stojanovic et al., 1990; Su and Puls, 2001). The toxicity and mobility of As depend on its oxidation state, where the trivalent As(III) is more mobile and toxic than As(V) or organic arsenic compounds (Cullen and Reimer, 1989), because of its ability to form complexes with certain co-enzymes. Therefore, it is highly necessary to develop sensitive and cost-effective methods for detection of trace As(III) in the environment. Some techniques such as atomic absorption spectrometry (AAS) (Karthikeyan et al., 1999; Quináia and Rollemberg, 1997), atomic fluorescence spectrometry (Semenova et al., 2000; Yan et al., 2002), and inductively coupled plasma mass spectrometry (ICP-MS) (Feng et al., 1998; Goossens et al., 1993; Townsend et al., 2001) have been used for detection of As(III). However, these methods for arsenic detection require sophisticated equipment and long analysis time.

Electrochemical techniques have been demonstrated to provide possible means for rapid and portable detection (Ivandini et al., 2006; Majid et al., 2006; Simm et al., 2005). Anodic stripping voltammetry (ASV) for As(III) detection has been accomplished using the modified metal electrodes, such as gold (Forsberg et al., 1975; Gu et al., 2013; Hossain et al., 2008), platinum (Shin and Hong, 2010; Xu et al., 2008) and mercury (Ferreira and Barros, 2002) for many years. However, the ASV technique suffers the setback of severe interference caused by copper (Diu et al., 2009), and several factors influence the anodic stripping peak current of As(III), including electrode material, solution pH and Cl^- concentration in the electrolyte solution (Song and Swain, 2007). Thus this work tried to use arsenic-binding DNA aptamer (named Ars-3) to overcome these problems occurring in electrochemical detection of arsenite.

Aptamers, as single-strand RNA or DNA oligonucleotides can distinctly bind to a broad range of targets replacing antibodies and have attracted immense research attention in biosensing (Xu et al., 2005; Zhang et al., 2008). On the basis of its three-dimensional structure arising from a folded single-stranded nucleic acid (usually 20–60 bases), aptamers have been regarded as promising tools in environmental monitoring for real-time and on-site detection of heavy metals with high sensitivity and selectivity (Li et al., 2014; Wu et al., 2014). The Ars-3 containing 100 nucleotides possesses high affinity to As(III) with a dissociation constant (K_d) of 7.05 nM. Some Ars-3-based analytical methods have been reported for arsenic detection (Divsar et al., 2015; Song et al., 2016; Tang et al., 2014; Wu et al., 2012a; Wu et al., 2012c; Wu et al., 2013; Yang et al., 2015; Zhan et al., 2014). To develop an Ars-3-based electrochemical sensing method for As(III), this work designed a signal-on biosensing strategy by using a cationic polymer to repel the electrostatic adsorption of electrochemically active

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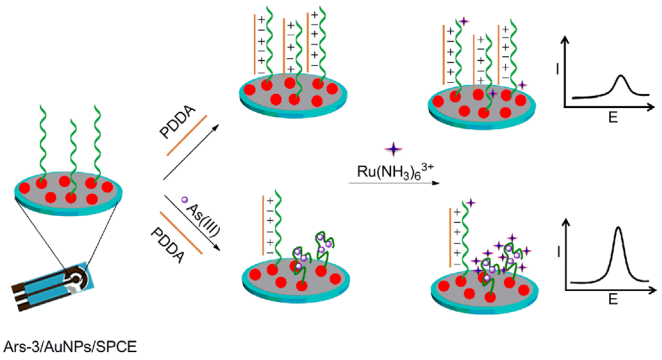

Scheme 1. Schematic illustration of the label-free electrochemical As(III) aptasensing strategy.

indicator on an aptasensor prepared with a home-made screen-printed carbon electrode (SPCE).

Recently, water-soluble cationic polymers have proven utility in a range of biosensor applications (Gaylord et al., 2002; Jeong et al., 2014; Preat et al., 2011; Wink et al., 1999; Xia et al., 2010). They are very useful for DNA sequence detection due to the electrostatic interaction between the positively charged cationic polymers and negatively charged phosphate backbone of DNA (Wu et al., 2012b; Xia et al., 2010). Herein, using cationic polydiallyldimethylammonium (PDDA) to neutralize the anionic charge of aptamer phosphate backbones, the amount of $[Ru(NH_3)_6]^{3+}$ (RuHex), served as an electrochemically active indicator, adsorbed on aptasensor surface was greatly decreased. In the present of As(III), the formation of Ars-3/arsenite complex led to the change of Ars-3 conformation, which decreased the adsorption of PDDA, and thus more positively charged $[Ru(NH_3)_6]^{3+}$ could adsorb on the aptasensor surface to produce a sensitive "turn-on" response (Scheme 1). The proposed label-free aptasensing strategy showed good selectivity and could detect arsenite down to 0.15 nM. Coupled with the low-cost and simple fabrication of the SPCE-based aptasensor, the excellent analytical performance indicated its promising application in sensitive detection of arsenite in the environment.

2. Materials and methods

2.1. Materials and reagents

Chloroauric acid ($HAuCl_4 \cdot 4H_2O$) and trisodium citrate were obtained from Shanghai Reagent Company (Shanghai, China). Tris (2-carboxyethyl)phosphinehydrochloride (TCEP), N-(2-hydroxyethyl) piperazine-N-2-ethanesulfonic acid (HEPES), 6-mercapto-1-hexanol (MCH), tris(hydroxymethyl)aminomethane (Tris), RuHex, chitosan ($\geq$85% deacetylation) and PDDA (MW < 100000, 35% in H_2O) were used as received from Sigma-Aldrich. Phosphate buffer solution (PBS, 10 mM) of various pHs were prepared by mixing the stock solutions of NaH_2PO_4 and Na_2HPO_4. Other reagents were of analytical grade and used as received. Ultrapure water obtained from a Millipore water purification system ($\geq$ 18 MΩ, Milli-Q, Millipore) was used in all assays. As(III) stock solution (0.10 M) was prepared from As_2O_3 (Gu et al., 2013): 0.9910 g As_2O_3 and 0.4000 g NaOH were dissolved in 10 mL ultrapure water, and diluted to 50.00 mL. Prior to use, the As(III) solution was diluted to different concentrations with 10 mM PBS (pH 7.4). The thiolated As-3 aptamer with a sequence 5′-HS-GGTAATACGACTCACTATAGGGAGATACCAGCTTATTCAATTTTAC AGAACAACCAACGTCGCTCCGGGTACTTCTTCATCGAGA-TAGTAAGTGCAATCT-3′ was purchased from Sangon Biological Engineering Technology & Services Co., Ltd. (Shanghai, China) and purified using high-performance liquid chromatography. Before use, the Ars-3 aptamer was dissolved in 50 mM HEPES buffer solution of pH 7.2.

2.2. Apparatus

All electrochemical experiments were recorded using CHI 660D electrochemical workstation (CH Instruments Inc., U.S.A.) with a portable homemade SPCE, which consisted of a carbon working electrode (2 mm diameter), a carbon auxiliary electrode and an Ag/AgCl reference electrode. The circular dichroism (CD) spectra were acquired in 1 mm path length cuvettes on a JASCO J-810 – 150S circular spectropolarimeter (Tokyo, Japan) equipped with a programmable temperature control unit in 15 min, of which the lamp was always kept under a stable stream of dry purified nitrogen (99.99%) during experiments.

2.3. Preparation of electrochemical As(III) aptasensor

Gold nanoparticles (AuNPs) with 13 nm diameter were prepared by reducing $HAuCl_4$ with trisodium citrate according to the previous protocol (Lu et al., 2012). The SPCE was firstly electrochemical activated by performing cyclic voltammetric scan (10 cycles) in 10 mM phosphate buffer (pH 7.2) with potential range from −0.3 to +0.6 V at a scan rate of 500 mV s^{-1}. After washing with ultrapure water and drying with nitrogen, 3 μL of 0.05 mg mL^{-1} chitosan was dropped on the SPCE surface and dried in air. Subsequently, 3 μL AuNPs was dropped on the surface, and allowed to dry at room temperature, washed with ultrapure water and dried with nitrogen. On the other hand, the thiolated Ars-3 (100 nM) was activated with10 μM TCEP for 1 h to reduce disulfide bonded oligos. 3 μL of Ars-3 was pipetted onto the pretreated electrode and then incubated overnight at room temperature in 100% humidity. After rinsing with 10 mM PBS (pH 7.4), the Ars-3 modified SPCE was passivated with 1 mM MCH solution for 2 h to remove nonspecific adsorption sites.

2.4. Electrochemical detection of As(III)

3 μL of As(III) solution was dropped on the aptasensor and incubated for 30 min. Then, PDDA (1%, v/v) was dropped on the surface and incubated for 1 h. After washing with ultrapure water, differential pulse voltammetric (DPV) detection was performed from 0 to −0.6 V with an amplitude of 25 mV and a step potential of 4 mV at a frequency of 15 Hz in 10 mM PBS (pH 7.4) containing 100 μM RuHex. The RuHex buffer was thoroughly purged with pure nitrogen for 15 min before the experiment.

3. Results and discussion

3.1. Interaction between As(III) and Ars-3 aptamer

Circular dichroism measurement is a powerful tool usually used to investigate some biomolecules such as amino acids, peptides, proteins and nucleic acids. The target-induced structure switching could be observed with the CD spectra of 5 μM Ars-3 aptamer in the absence (a) and presence (b) of 100 μM As(III) (Fig. 1). The Ars-3 aptamer had a negative peak at 240–260 nm and a positive peak at 270–290 nm (curve a), which was in accordance with previous report (Wu et al., 2012b). After the addition of As (III), the values of both negative and positive peaks decreased, but the location of the peaks did not change (curve b). This result indicated that As(III) could bind to some bases of the Ars-3 and change its conformation, which restrained the strong $\pi \rightarrow \pi^*$ transition of bases with deoxyribose, thus causing a decrease in

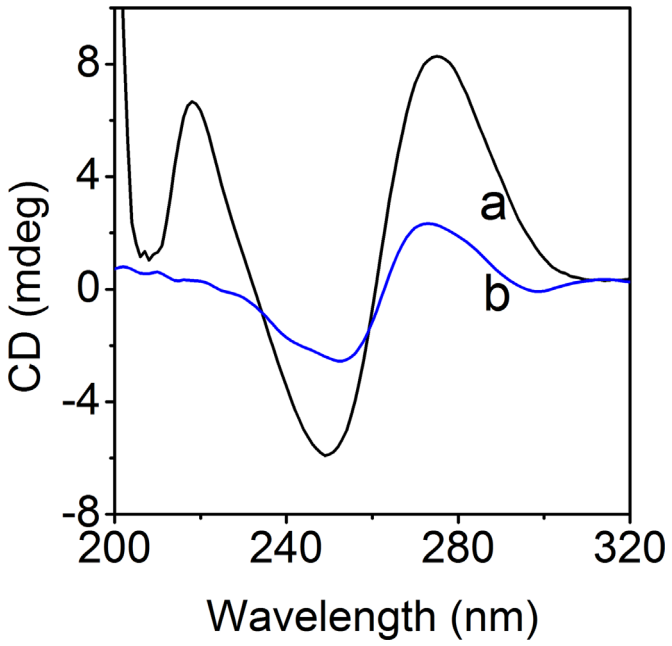

Fig. 1. CD spectra of 5 μM Ars-3 aptamer in the absence (a) and presence (b) of 100 μM As(III).

the CD peak (Wu et al., 2012c).

In order to assess the interaction of PDDA with the probe and its effect on the signal, DPV response of the PDDA modified electrode without aptamer was investigated data not shown. The modified electrode showed a large signal in the absence of As(III), while the presence of 100 nM As(III) did not affect the signal change, indicating only the change of Ars-3 conformation could alter the amount of the electrochemically active $[Ru(NH_3)_6]^{3+}$ indicator and lead to the change of signal.

3.2. Optimization of sensing conditions

In order to achieve high sensitivity for As(III) detection, the experimental parameters, including Ars-3 concentration for preparation of aptasensor, the amounts of PDDA and RuHex and reaction time, were optimized (Fig. 2). The effect of PDDA on the response was examined by varying its volume fraction of PDDA from 0.1% to 10%. In the presence of 20 nM As(III), the peak current decreased with the increasing PDDA concentration (Fig. 2A), while the background also decreased slowly and then plateaued at 1%. According to the maximum signal-to-noise ratio (S/N), 1% PDDA was selected for the following experiments. The concentration of RuHex was also optimized in the presence of 20 nM As(III). It was clear that the peak current increased with the increasing the RuHex concentration, whereas the background showed little changed from 5 to 100 μM and became larger from 100 to 500 μM (Fig. 2B). To obtain the high S/N, 100 μM RuHex was chosen as the optimum concentration. The concentration of Ars-3 could affect its density on sensor surface and thus the reaction efficiency. As shown in Fig. 2C, the peak current increased with the increasing Ars-3

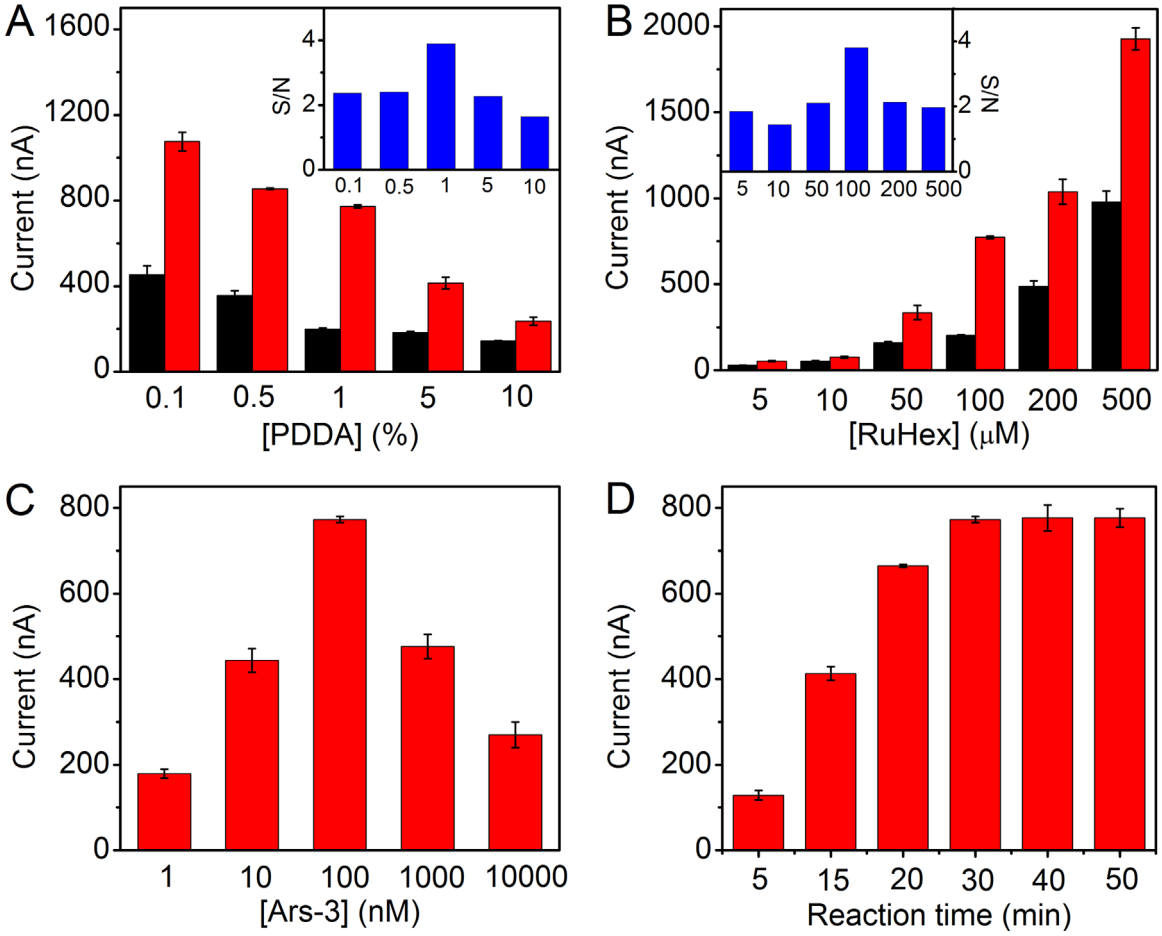

Fig. 2. Effects of (A) amount of PDDA, (B) concentration of RuHex, (C) Ars-3 concentration for aptasensor preparation, and (D) reaction time on DPV response of the proposed biosensor in the absence (in black) and presence (in red) of 20 nM As(III). Inset of (A) and (B): ratios of signal to noise at different amounts of PDDA and concentrations of RuHex, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

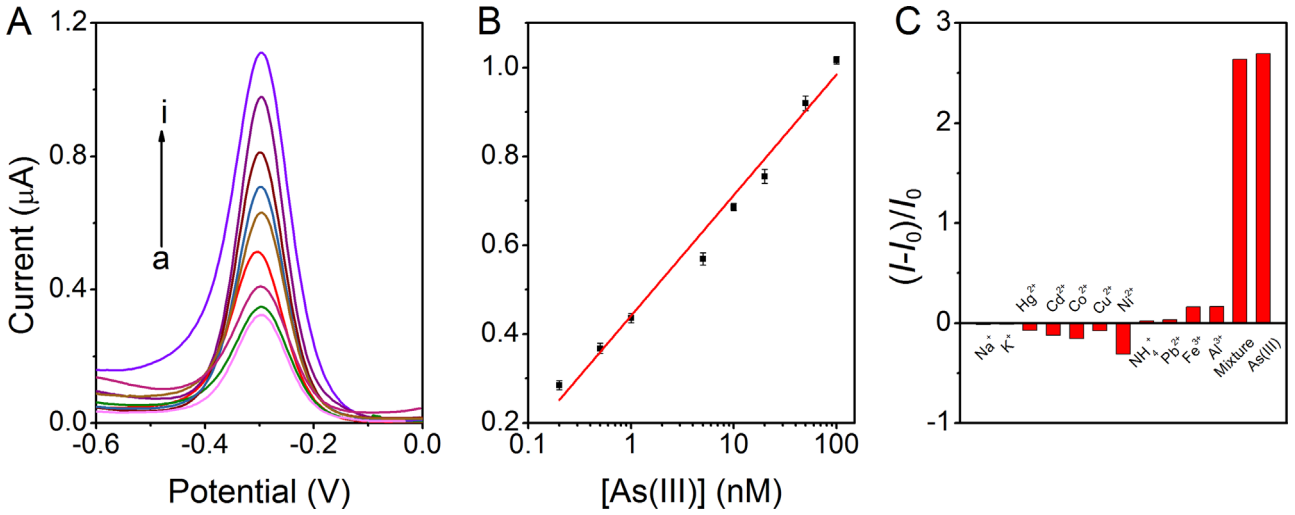

Fig. 3. (A) DPV responses of the aptasensor to As(III) at the concentrations of 0, 0.2, 0.5, 1, 5, 10, 20, 50 and 100 nM (from a to i) in PBS (pH 7.4), (B) plot of peak current *vs* logarithm value of As(III) concentration, and (C) DPV peak currents of the aptasensor to 100 nM Hg^{2+}, Cd^{2+}, Co^{2+}, Cu^{2+}, Ni^{2+}, Pb^{2+}, Fe^{3+}, Al^{3+}, Na^{+}, K^{+} and NH_4^+ and 20 nM As(III), respectively. Mixture represents a solution containing 20 nM As(III) and 100 nM of these interfering ions. I_0 and I are the peak current in the absence and presence of target, respectively.

concentration up to 100 nM at 20 nM As(III), then declined when further increased the concentration of Ars-3. Thus, 100 nM Ars-3 was selected for aptasensor preparation. As expected, the reaction time between Ars-3 and As(III) greatly influenced the response. The peak current increased gradually with an increasing reaction time and reached a plateau after the reaction time of 30 min (Fig. 2D). Therefore, 30 min was enough for the reaction in the detection of As(III).

3.3. Analytical performance of electrochemical aptasensor

Under optimal conditions, the reduction peak current of RuHex increased as the concentration of target As(III) increased (Fig. 3A). The Ars-3/arsenite complex formed on the electrode surface caused a steric effect for forming more Ars-3/As(III) complex structure at higher As(III) concentration. Thus the aptasensor showed a good linear relationship between the plot of peak current vs the logarithm value of As(III) concentration with a linear equation of I (μA)=0.441+0.272 log c (nM) in the range from 0.2 nM to 100 nM with a correlation coefficient of 0.9846 (Fig. 3B). Due to the difference among different batches of SPCEs, the calibration curve could be used for the same batch of SPCEs in practical application. The detection limit was calculated to be 0.15 nM (3σ, σ is the standard deviation for measurements of blank values), which was lower than the World Health Organization guideline of 10 ppb (Khan et al., 2005). In addition, the linear range and detection limit of the proposed method were much superior to stripping voltammetric and spectrometric detection methods (Table 1).

Table 1
Comparison of the performance among different As(III) detection methods.

Detection methods	Linear range (nM)	Detection limit (nM)	References
Electrochemistry	0.2–100	0.15	This work
Electrochemistry	2.68–4020	0.268	Li et al. (2012)
Electrochemistry	2.68–5025	0.536	Diu et al. (2009)
Electrochemistry	1.3–160	0.63	Huang and Chen (2013)
Electrochemistry	0–450.24	0.67	Liu and Wei (2008)
Electrochemistry	6.7–335	3.35	Majid et al. (2006)
Electrochemistry	120–15,000	3.75	Rahman et al. (2010)
Electrochemistry	50–200	12.06	Sakira et al. (2015)
Electrochemistry	134–1340	16.2	Prakash et al. (2012)
Colorimetry	347–2670	16.8	Zhan et al. (2014)
Colorimetry	67–4020	71.02	Wu et al. (2012b)
Colorimetry	668–9350	80.1	Divsar et al. (2015)
Colorimetry	134–2680	80.4	Wu et al. (2013)
SERS	1.34–1340	0.78	Yang et al. (2015)
SERS	3.86–308.7	1.34	Ye et al. (2014)
SERS	6.68–134	1.34	Song et al. (2016)
FRET	20–2000	6	Tang et al. (2015)
RRSS	1.34–1340	4.02	Wu et al. (2012c)
	1.34–2680	2.68	
RRSS	50.7–3080	25.4	Tang et al. (2014)

SERS: Surface enhanced Raman scattering; RRSS: resonance Rayleigh scattering spectral; FRET: Fluorescence resonance energy transfer.

3.4. Selectivity and stability of electrochemical aptasensor

The selectivity of the proposed aptasensor was evaluated by comparing the $(I\text{-}I_0)/I_0$ values toward the solutions containing either As(III) or other environmentally relevant metal ions, such as Hg^{2+}, Cd^{2+}, Co^{2+}, Cu^{2+}, Ni^{2+}, Pb^{2+}, Fe^{3+}, Al^{3+}, Na^{+}, K^{+} and NH_4^+. As shown in Fig. 3C, the aptasensor showed obvious response to the solution containing As(III), while negligible response was observed in the solutions containing these interference ions even at a 5-fold higher concentration than As(III) (100 nM *vs.* 20 nM). The response of the aptasensor to As(III) was hardly affected by the presence of interfering metal ions, indicating the proposed aptasensor possessed high selectivity for As(III) detection and potential application for analysis of complex samples.

The intraassay and interassay precision of the proposed aptasensor was examined by detecting 20 nM As(III) with the same aptasensor and different aptasensors, respectively. The relative standard deviation (RSD) for twenty measurements of As(III) with the aptasensor was 3.6%, while the RSD for six parallel measurements with six aptasensors was 7.2%, indicating good precision and acceptable fabrication reproducibility. No significant change in the magnitude of the peak height was noticed within the first 3 days, and only 5% decrease in the peak height was observed after storage by covering with glass beaker for 3 weeks at 4 °C in the refrigerator and then use at room temperature, demonstrating the long-term storage and operational stability.

Table 2
Determination of As(III) in water samples using the proposed method and AAS method.

Samples	Added (nM)	Found (nM)	RSD (%) ($n=3$)	Recovery (%)	AAS (nM)	Related error (%)
Tap water 1	20.0	19.9	1.3	99.5	22.8	−12.7
Tap water 2	100	96.2	4.7	96.2	93.6	2.8
Lake water 1	20.0	23.5	2.0	117.5	25.9	−9.3
Lake water 2	100	107.3	3.5	107.3	105	2.2

3.5. Determination of As(III) in real samples

The analytical reliability and application of the proposed aptasensor were investigated by detecting As(III) in natural water samples, including tap water and lake water. To perform the real water samples test, the lake water (from Xuanwu Lake in Nanjing, China) was filtered through a 0.2 μm membrane and boiled for 15 min prior to detection. No current response was observed in those water samples, which indicated that the concentration of As (III) was extremely low and could not be detected. Thus, different concentrations of As(III) were spiked into these samples for recovery evaluation. In addition, the results obtained by the proposed sensor were also comparable with those obtained by the AAS method (Table 2). The recoveries of 96.2%–118.5%, as well as the acceptable RSD and relative errors indicated good accuracy of the proposed method for As(III) detection, and this method could be applied in analysis of real samples.

4. Conclusions

A novel label-free signal-on electrochemical aptasensor is designed for simple, sensitive and selective electrochemical detection of As(III) by immobilizing Ars-3 aptamer on a SPCE and coupling with the adsorption of cationic polymer and RuHex as an electrochemically active indicator on aptasensor. The formation of Ars-3/As(III) complex decreases the adsorption of PDDA that can repel the cationic RuHex, thus strengthens the electrostatic interaction between aptasensor surface and RuHex to achieve the signal-on electrochemical detection of As(III). The proposed method can detect As(III) down to 0.15 nM with high selectivity against other metal ions. Combined with the disposable SPCE, this cost-effective As(III) detection method provides potential application in environmental analysis.

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