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A Novel Electrochemical Biosensor Based on DNA for Rapid and Selective Detection of Cadmium

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A sensitive platform for the rapid detection of cadmium basing on single-stranded DNA modified Au electrode (ssDNA/Au) was presented. The ssDNA/Au biosensor screened 6 kinds of chemicals including cadmium salts, sodium salts, copper salts, magnesium salts, plumbum salts and zinc salts. The results demonstrated that the detection signal of the ssDNA/Au biosensor for cadmium was related to the concentration. Linear responses were gotten in the range from 1 to 20 ng L^{-1} with a detection limit (S/N=3) of 0.3 ng L^{-1} for cadmium under the optimized conditions. It also showed that the ssDNA/Au biosensor could successfully differentiate cadmium salts from the interferent (magnesium salts and zinc salts, etc.). The interaction mechanism between ssDNA and cadmium ions was deduced from the electrochemical method and ultraviolet-visible spectrophotometer. The ssDNA/Au biosensor proved to be a potential alternative tool for the rapid detection of cadmium ions, and can be used for rapid detecting of cadmium in real water samples, evaluation of food safety and the status of environmental contamination.

Keywords: Electrochemical biosensor; ssDNA; rapid dectection; cadmium ions

1. INTRODUCTION

Environmental heavy metals pollution is one of the greatest concerns for the public nowadays [1, 2]. Especially, cadmium as a highly toxic and dangerous environmental polluant has been produced several major pollution incidents recently [3, 4]. For example, environmental cadmium contamination led to an epidemic of bone disease (itai-itai disease) in japan in the late 1960s [5], 20 tons of cadmium had been discharged into the river in southern of china in 2012 which could affect up to 4 million people. Therefore, it is urgent to develop a new method which can detect cadmium in a rapid and sensitive way to ensure remediation in time. Currently, cadmium are usually determined by atomic

absorption spectroscopy [6] and inductively coupled plasma mass spectrometry [7, 8]. These methods can reach good precision, but they have complicated steps, time-consuming and costly instruments. It could not be widely used for detecting cadmium on site.

Biosensors are ideal tools for cadmium pollutants rapid detection as they are portable and sensitive [9-11]. Among these biosensors, the electrochemical DNA (E-DNA) biosensor has emerged as an attractive screening tool for its simplicity, sensitivity, high-throughput and reusability [12, 13]. The ssDNA/Au biosensor has been intensively studied for its significant advantages, such as rapid and inexpensive screening of pollutants. Wang's group constructed a DNA biosensor for detecting hydrazine pollutant by monitoring the signal changes of the surface-confined double-stranded (ds) DNA [14]. Others also used E-DNA biosensor for detecting toxic pollutants, such as arsenic trioxide [15, 16]. All the mentioned, the redox peak current of guanine bases was identified as detection signal for monitoring toxic pollutants. While the detection signals of DNA biosensor were unobvious and only nanomolar per liter of detection limits have been obtained, because only few guanine was immobilized onto the surface of electrode. Otherwise, the high redox potential of guanine bases (typically 1.0~1.2 V) were easy to co-existing other compound.

In this research, a new method was developed for rapidly detecting of environmental cadmium ions. This strategy used DNA with negative charge to attract cadmium ions and magnify the signals, and reduce the interferences for the low redox potential of MB (methylene blue) (typically -0.25~0 V). The interaction mechanism between ssDNA and cadmium ions was deduced from the electrochemical method and ultraviolet-visible spectrophotometer. The ssDNA/Au biosensor was developed for detecting cadmium from co-existing interferent (such as zinc ions, sodium ions, etc). The results indicated that the ssDNA/Au biosensor exhibited superior sensitivity and limit of detection. These also showed that the ssDNA/Au biosensor was a potential instrument for rapid screening of toxic cadmium pollutants.

2. EXPERIMENTAL SECTION

2.1. Materials and Solutions.

Cadmium chloride and other chemicals used in this work were of analytical grade (purchased from Sigma, USA). The thiol-terminated DNA (the DNA sequence is rich in A (adenine) and T (thymine)) 5'-SH$(CH_2)_6$-TAGCAAGAATAGAATAACAAGAATAAGAATAAGC-MB-3' were prepared by Takara Biotechnology Co. Ltd. (Dalian, China). The DNA stock solutions were mixed with 50 mmol L^{-1} phosphate buffer solution (PBS pH7.0) containing 100 mmol L^{-1} NaCl, and kept in the refrigerator (-20°C). Unless otherwise mentioned, PBS (50 mM pH 7.0) was prepared for the electrolyte in all electrochemical experiments.

2.2. Apparatus.

Cyclic voltammetry (CV) was monitored by an Au electrode (diameter, 2 mm) using a CHI 660 Electrochemical Workstation (CHI Instruments Inc., China). A three-electrode system consisted of a single-stranded DNA modified Au electrode (ssDNA/Au) as the working electrode, an Ag/AgCl as the

reference electrode, and a platinum wire as the auxiliary electrode. UV-Vis spectrum was monitored by UV-Vis spectrometer (Jena, Germany).

2.3. Preparation and Characterization of ssDNA/Au.

The surface of Au electrode was polished by slurries of alumina (1, 0.3, and 0.05μm diameter in turn) and washed ultrasonically by ethanol and Milli-Q water three times, respectively. Later, the Au electrode was electrochemically cleaned in 1 mol L^{-1} H_2SO_4 by potential scanning between -0.3 and 1.55 V until a reproducible cyclic voltammogram got. Thiolated DNA solution (6μL 3 μmol L^{-1}) was titrated onto the surface of cleaned Au electrode for 6 hours at room temperature to get the ssDNA/Au biosensor. Subsequently, the ssDNA/Au biosensor was washed by abundant of 50 mmol L^{-1} PBS to remove the weakly adsorbed ssDNA.

The ssDNA/Au was characterized by electrochemical scanning in potassium ferricyanide solution ($[Fe(CN)_6]^{3-}$.) to monitor the immobilization process. The ssDNA/Au was immersed into 2 mmol L^{-1} potassium ferricyanide solution. Later, CV was carried out on the ssDNA/Au from 0.6 to -0.1 V at 100 mV s^{-1}.

2.4. Detection of Cadmium Pollutants with ssDNA/Au.

Cadmium ions were accumulated onto the surface of ssDNA/Au by immersing the electrode into 8 mL PBS (pH 7.0) for 15 minutes. Subsequently, cyclic voltammetric scans (range from 0.2 to -0.6 V at 100 mV s^{-1}) was gotten by the ssDNA/Au biosensor in PBS solution. 5 kinds of co-existing chemicals were also detected by ssDNA/Au biosensor at 10 μg L^{-1}, including zinc ions, sodium ions, plumbum ions, copper ions and magnesium ions.

2.5. The Interaction Mechanism between ssDNA and Cadmium Characterization by UV-Vis

UV-Vis spectra (from 230 to 290 nm) of PBS, ssDNA and cadmium solutions were monitored in a quartz cuvette, respectively. Firstly, 10 mL 50 mmol L^{-1} PBS was scanned by the UV-Vis spectrometer. Then 10 μL ssDNA (10 μmol L^{-1}) was added into the PBS solution. Finally, 100 μL cadmium solution (10 μmol L^{-1}) was added into previous solution to get the UV-Vis spectra.

3. RESULTS AND DISCUSSION

3.1. ssDNA Immobilization onto the Surface of Au Electrode.

Figure 1 showed the CV signals of bare Au electrode in 1 mol L^{-1} H_2SO_4 from -0.3 to 1.55 V. It indicated that the electrode was cleaned further by electrochemical etching in 1 mol L^{-1} H_2SO_4. The immobilization efficiency was correlated to the sensitivity of the ssDNA/Au biosensor.

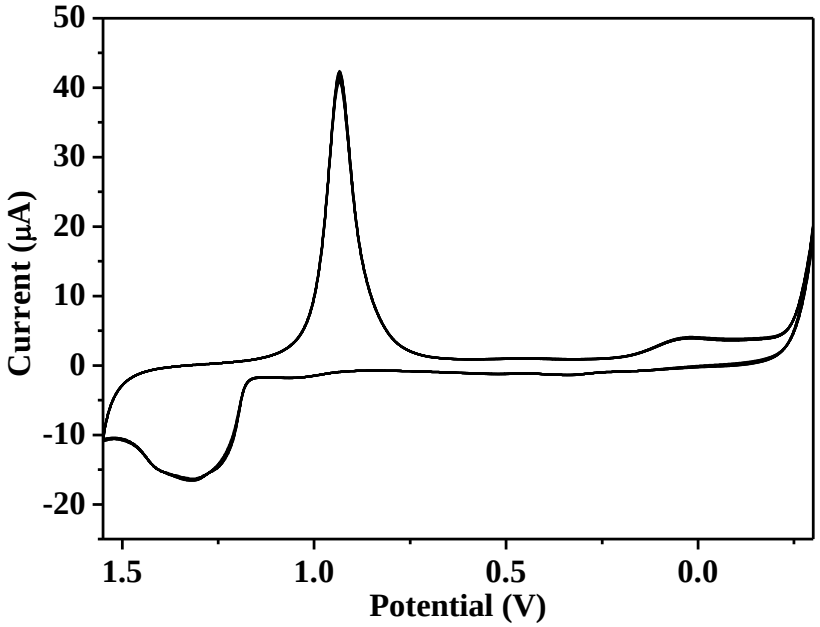


Figure 1. Cyclic voltammograms cycling (20 times) of bare Au electrode in 1 mol L^{-1} H_2SO_4 from -0.3 to 1.55 V.

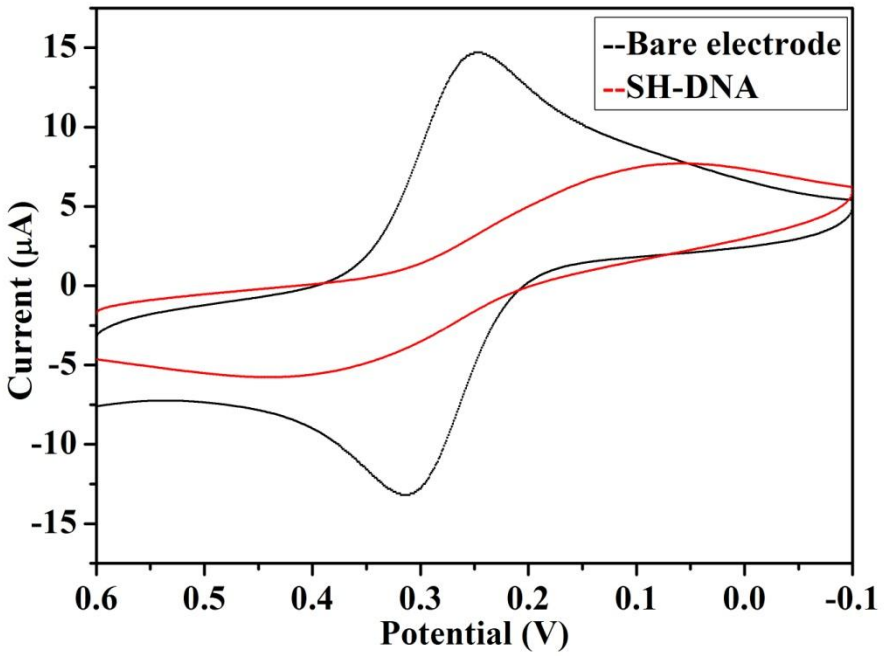


Figure 2. Cyclic voltammograms of bare gold electrode and ssDNA/Au in 2mmol L^{-1} $[Fe(CN)_6]^{3-}$. Scan rate, 100mV s^{-1}.

In this research, the immobilization process was detected in potassium ferricyanide solution by electrochemical method. Figure 2 displayed cyclic voltammetric signals of $[Fe(CN)_6]^{4-}/[Fe(CN)_6]^{3-}$ on the bare electrode and the ssDNA/Au electrode in 2 mmol L^{-1} potassium ferricyanide. A pair of

obvious redox peak were obtained by the bare Au electrode, which should be attributed to the $[Fe(CN)_6]^{4-}/[Fe(CN)_6]^{3-}$ transformation. The 69 mV peak separation between anodic and cathodic peak indicated the electrochemically reversible one electron redox process [17]. Due to the electrostatic between negatively charged DNA and $[Fe(CN)_6]^{3-}$, potassium ferricyanide molecules could not bind to the ssDNA/Au electrode. As to thiol-terminated ssDNA, $[Fe(CN)_6]^{4-}/[Fe(CN)_6]^{3-}$ could not be oxidation-reduction on the ssDNA/Au electrode. So the CV signal of $[Fe(CN)_6]^{4-}/[Fe(CN)_6]^{3-}$ decreased after the thiol-terminated ssDNA covered onto the bare Au electrode.

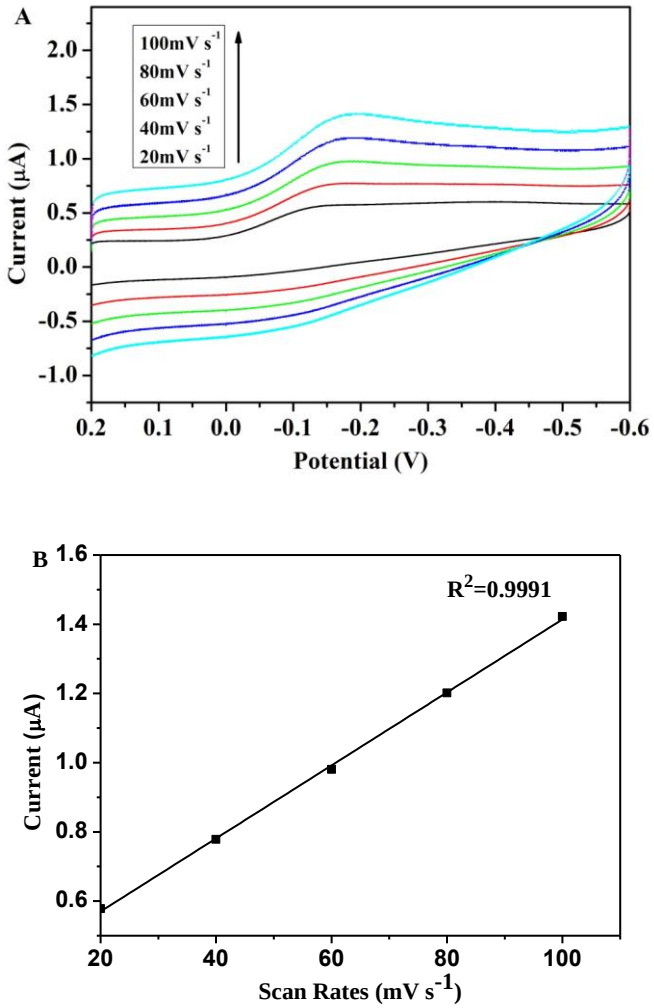


Figure 3. A: Cyclic voltammogram of ssDNA/Au after incubation of in 50 mmol L^{-1} PBS buffer (pH 7.0). Scan rate from inner to outer: 20, 40, 60, 80, 100 mV s^{-1}. B: Plot of reduction peak current versus scan rate.

The ssDNA immoblization process was further monitored the signal of MB which terminated the 3'-ssDNA by cyclic voltammetric. MB (a kind of organic dye) belongs to the phenothiazine family which is a redox indicator with the formal potential (about -0.2 V versus SCE) in neutral solution [18]. Figure 3 was the CVs of the ssDNA/Au biosensor with different scan rates. The MB peak increased linearly with the scan rates range from 20 to 100 mV s^{-1}. This indicated that the electron transfer

reaction from Au electrode to MB was a surface-controlled electrochemical process. It also revealed that the electron transfer through the ssDNA was significantly efficient [19].

3.2 Electrochemical Detection of Cadmium by ssDNA/Au.

Cadmium is a kind of heavy metal, which is specifically listed in the European Restriction of Hazardous Substance. Some studies linked exposure to cadmium with lung and prostate cancer [20]. Both double-stranded DNA (dsDNA) and single-stranded DNA (ssDNA) can be used for detecting cadmium. Due to the double helix structure of dsDNA, dsDNA is more rigid and stable than ssDNA. Meanwhile, ssDNA may be more sensitive to the change of outer environment than dsDNA. For these reasons, the ssDNA rich in adenine and thymine might be very sensitive to the cadmium. It was selected to detect different concentrations of cadmium. The MB molecules as redox signal molecules magnified the interaction between cadmium and ssDNA. It must be pointed that the low reduction potential of MB (about -0.2 V) minimized interferents. Besides, the compact-packed self-assembly monolay of ssDNA on the surface of electrode could prevent potential interferent from getting close to the surface of electrode.

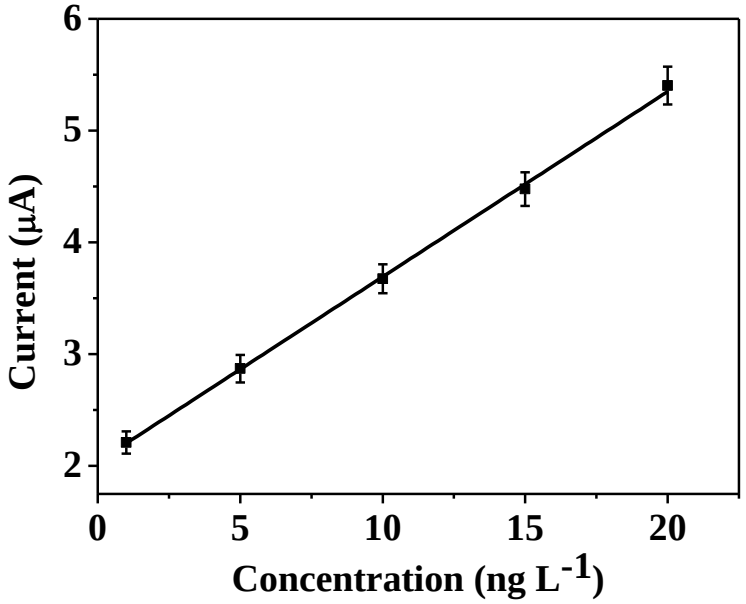
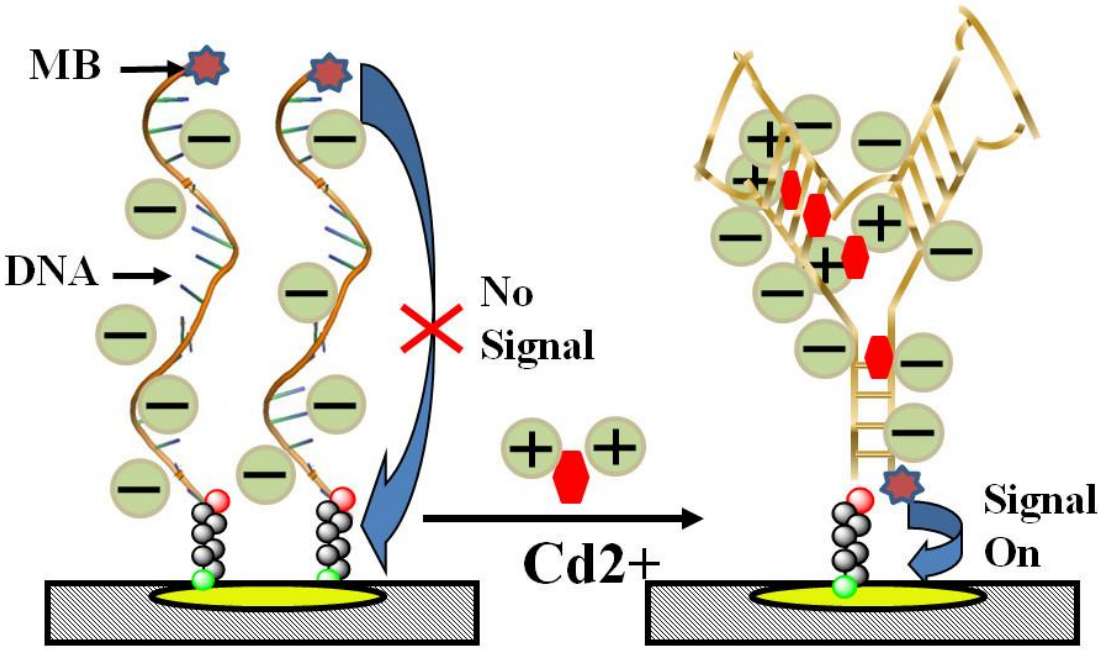


Figure 4. The current peaks of the ssDNA/Au biosensor for detecting of different concentrations of cadmium from 1 ng L^{-1} to 20 ng L^{-1}.

Based on the above advantages, it could be obtained high sensitive and selective detection for cadmium. Figure 4 showed the current peaks of the ssDNA/Au biosensor for the detection of different concentrations of cadmium. Following the increase of cadmium concentration (from 1 ng L^{-1} to 20 ng L^{-1}), the response signal of ssDNA/Au biosensor successively increased. The response signal of the ssDNA/Au also had a linear relationship with the cadmium concentration, ranging from 1 ng L^{-1} to 20 ng L^{-1}, and its detection limit (S/N=3) of cadmium reached 0.3 ng L^{-1}. These advantages of the

ssDNA/Au biosensor ensured it to be a potential "alarm" tool for effective detection of cadmium on spot.



Scheme 1. Schematic diagram of the ssDNA/Au biosensor interacting with cadmium ions.

The signal of MB on the ssDNA/Au increased obviously for detection of cadmium (Figure 4), which might be attributed to the cadmium bound with ssDNA and changed the 3-dimension structure of ssDNA. There is a possible interaction mechanism of the ssDNA/Au biosensor with cadmium ions, as shown in Scheme 1: Because of the strong interaction (electrostatic attraction, negative and positive electric attraction) between cadmium ions and ssDNA, those MB molecules might be closed to the surface of GC electrode, which resulted in the response signal increase.

UV-Vis spectroscopy method was used for identifying the possible interaction mechanism. The UV-Vis absorption peaks could be related with the types of bonds in a known molecule, which are also used for identifying the functional groups of a compound. The wavelength scan (from 230 to 290 nm) of PBS revealed no absorption peak (Figure 5). As the ssDNA was injected into the PBS solution, there was an obvious peak in 260 nm owing to the introduction of ssDNA into PBS. Following, cadmium ions with high concentration was injected into the above solution, resulting in hypochromic effects. Cadmium ions do not absorb at this scan range under study. There is a possible reason for a decrease in absorption after addition of cadmium ions: those cadmium ions bound ssDNA together might change the structure of ssDNA and decrease the UV-Vis absorption of ssDNA. The UV–vis spectrometer result was consistent with the electrochemical experimental results.

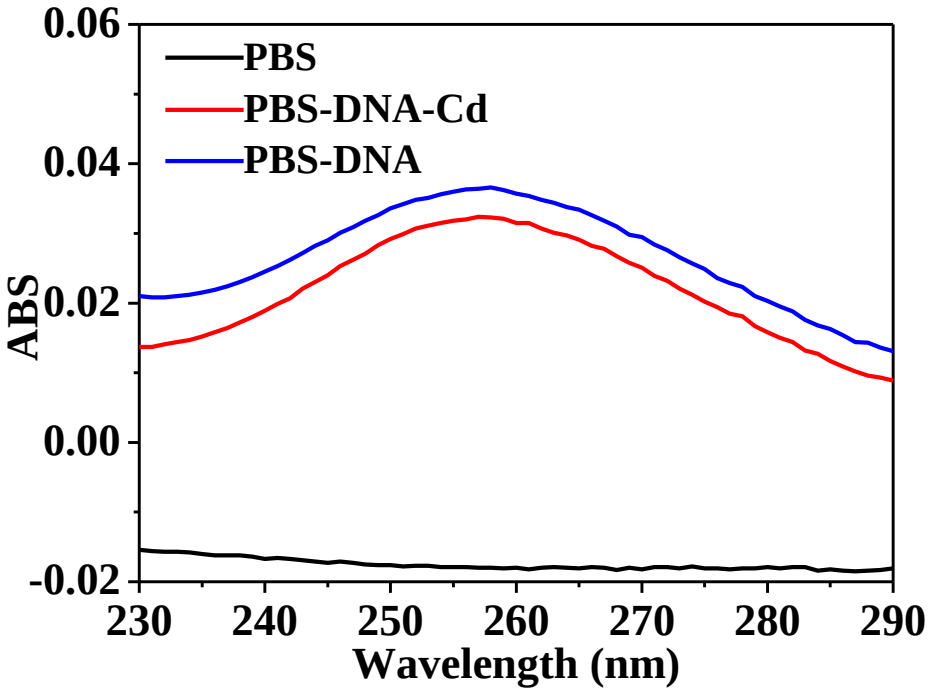


Figure 5. The ultraviolet-visible spectrum of PBS solution (50 mmol L^{-1}), DNA solution (10 nmol L^{-1}) and DNA solution with cadmium (100 ng L^{-1}).

3.3. Screening of Co-existing Interferent by ssDNA/Au Biosensor.

Zinc ions, copper ions, sodium ions, plumbum ions and magnesium ions were always co-exists with cadmium ions. The ssDNA/Au biosensor was also used for screening zinc ions, sodium ions, plumbum ions and magnesium ions at 10 μg L^{-1}. The response signals of ssDNA/Au for zinc ions, sodium ions, copper ions and magnesium ions were unapparently (almost acheving straight line, data not shown). So the ssDNA/Au biosensor indicated excellently potential for the monitoring of cadmium pollutants from co-existing interferent.

The ssDNA/Au biosensor was used for detecting of cadmium ions in sewerage. The test sewerage sample without enrichment was directly injected into the test container, and detected by the ssDNA/Au biosensor. The contents of cadmium ions determined by electrochemical method were 1.4 μg L^{-1} and 5.9 μg L^{-1} in the sewerage sample, respectively. The ssDNA/Au biosensor was recognized as a useful “pre-alarm” instrument for determination of cadmium contamination on spot.

4. CONCLUSIONS

In this work, a novel ssDNA modified gold electrode was prepared for determining of cadmium pollutants. The ssDNA/Au biosensor exhibited good performance due to its compact-packed self-assembly monolay and low redox potential of MB. The ssDNA/Au biosensor could successfully differentiate cadmium from interferent with high specificity. The interaction mechanism between

ssDNA and cadmium ions was deduced from the electrochemical method and ultraviolet-visible spectrophotometer. The ssDNA/Au biosensor was identified as a potential "pre-alarm" instrument for evaluation of food cadmium contamination on spot.

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