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Determination of Cadmium Ions Based on Electrochemical DNA Biosensors in Rat Tissues

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A highly sensitive electrochemical biosensor was fabricated for Cd^{2+} determination based on a dsDNA modified carbon paste electrode (CPE) and brilliant green (BG) indicator. The binding of Cd^{2+} on the modified dsDNA could result in the destabilization of the double helix structure of DNA, which was detected by the oxidation of a DNA hybridization indicator BG on the electrode surface. The reduction current of BG on the modified electrode increased significantly with the presence of Cd^{2+}. Based on such electrochemical response, this electrode was further used determine the concentration of Cd^{2+} with a linear range from 0.05×10^{-9} to 1.2×10^{-9} mol/L and a limit of detection of 0.1×10^{-12} mol/L. The portable, low-cost modified electrode showed good sensitivity, selectivity, and stability. The method developed in this study was also applied to the direct determination of Cd^{2+} in rat tissue samples with satisfactory results. This work reveals that the dsDNA-modified CPE is a promising tool for the food detection and animal diagnosis.

Keywords: DNA, Biosensor, Cadmium, Brilliant green, Rat

1. INTRODUCTION

Cadmium is an extremely harmful environmental toxicant that comes from industrial pollution. This metal easily accumulates in many organisms, especially molluscs and crustaceans. Low concentrations of cadmium have also been found in vegetables, cereals and starchy rhizomes. Humans

and animals can take up cadmium through respiration and digestion. Cadmium induces oxidative stress and accumulates in the brain parenchyma and neurons of humans and animals, causing neurological alterations and leading to behavioural disorders, poor olfaction and memory impairments. [1,2] Cadmium is well known to target multiple organ systems, particularly the kidneys and liver. [3] Recently, some studies have shown that cadmium has toxic effects on bone that may occur in parallel to nephrotoxicity.[4] Cadmium is harmful not only to human beings but also to animals. It is noteworthy that cadmium has been classified as a potential carcinogen and a secondary carcinogenic threat for humans by World Health Organization (WHO), [5] categorized as a carcinogen by the International Agency for Research on Cancer (IARC), [6] ranked as the seventh most harmful substance to human health by the United States Toxic Substances and Disease Registry (ATSDR), [7] listed as one of the key monitored indicators for the integrated waste water discharge control (below 0.1 mg/L) in China. [8]

Currently, diverse instruments, including atomic emission spectroscopy (AES), atomic absorption spectroscopy (AAS), NMR spectroscopy, X-ray fluorescence spectroscopy, and inductively coupled plasma mass spectrometry (ICP-MS), are often used in cadmium detection.[9] However, these detections are expensive and require complicated operational procedures. To overcome these limitations, biosensors based on biomacromolecule have been developed with the advantages of simplicity and low detection limits. [10,11] These biological recognition units in biosensors can be enzymes, antibodies and short single-stranded oligonucleotides (DNA or RNA) [12], which have good specific interactions to heavy metals. For example, DNA not only exhibits higher stability and lower molecular weight than other materials, but it also is low cost and easy to modify. Thanks to these superiorities, DNA has emerged as a promising biomaterial for the fabrication of biosensors. [13] It was found in Wong's work that cadmium could damage dsDNA by binding to the N(7) guanine base in ssDNA, leading to a destabilization and unwinding structure [14].

Although a small change in DNA structures can induce a response signal in the electrochemical method, the electrochemical signal is not strong enough for sensing application. In this case, methylene blue (MB) or ethyl green (EG) as a DNA hybridization indicator [15-17], has been used to amplify the electrochemical signals. The peak current of the hybridization indicator increased because its binding affinity for a destabilization and unwinding DNA was enhanced with the presence of heavy metal ions. Inspired by these studies, we have found that brilliant green (BG) could be used as a new DNA hybridization and electrochemistry indicator for the design of an electrochemical DNA biosensor. BG can preferentially bind to ssDNA through the interaction with the guanine base because cadmium destabilizes dsDNA to generate ssDNA. Significant increase of BG reduction peak current was measured on the dsDNA modified CPE without and with the presence of Cd^{2+}. Moreover, the reduction peak current of BG increases linearly with the increasing concentration of Cd^{2+}.

2. EXPERIMENTAL SECTION

2.1 Reagents

$CdCl_2$, $AgNO_3$, $CuCl_2$, $CaCl_2$, $Zn(NO_3)_2$, NaCl, $HgCl_2$, NaAc, HAc, HCL and HNO_3 were purchased from Sinopharm (Hong Kong). Tris was purchased from Solarbio (Beijing, China); BG was

purchased from Bangon Biotech (Shanghai, China). Two complementary ssDNAs with 10 bases for the synthesis of dsDNA were purchased from General Biotech (Shanghai, China). The sequences of the two complementary ssDNAs are listed below:

ssDNA1, poly-G: 5'-GGGGGGGGGG-3';

ssDNA2, poly-C: 5'-CCCCCCCCCC-3'.

A stock solution of 4×10^{-6} mol/L dsDNA was prepared in Tris-HCl solution (0.01 mol/L Tris-HCl, pH 8.0). A centrifuge tube containing ssDNA lyophilized powder was centrifuged at 12,000 rpm for 1 min at high speed to detach the ssDNA from the tube wall. DNA at the bottom of the tube was dissolved into a 1×10^{-4} mol/L solution with Tris-HCl buffer (pH 8.0). The buffer solution was mixed thoroughly on a vortex mixer. Then, the complementary ssDNA was added into the buffer solution and incubated in a 100 °C water bath for a period of approximately 10 min to prepare dsDNA. Finally, the as-prepared solution was cooled naturally to room temperature and stored at -20 °C. Different metal ion solutions were prepared by dissolving $CdCl_2$, $Ag(NO_3)$, $CuCl_2$, $CaCl_2$, $Zn(NO_3)_2$ and $HgCl_2$ in Tris-HCl buffer solution (0.05 mol/L Tris, 0.2 mol/L NaCl, pH 7.4), respectively. The BG solution was prepared with Tris-HCl buffer (0.05 mol/L Tris, 0.2 mol/L NaCl, pH 7.0).

2.2 Apparatus

Electrochemical measurements for cadmium determination were carried out with a PalmSens (Netherlands) electrochemical workstation connected to a three-electrode cell. To perform the experiments, the dsDNA modified CPE was used as the working electrode, a platinum wire ass the counter electrode and the saturated calomel electrode (SCE) as the reference electrode. All of the electrodes were purchased from GSRL (Wuhan, China). A METTLER-TOLEDO FE20 meter was used to measure pH of the buffer solution.

2.3 Laboratory procedure

2.3.1 Preparation and activation of CPE

The CPE was prepared according to the reported literature procedures without any modification. Briefly, graphite and liquid paraffin oil were mixed in a certain ratio and then filled into a PVC pipe to form the carbon paste electrode (CPE). For activation of the CPE surface, several cyclic voltammograms (CVs) of CPE were collected by scanning from 0-1.2 V in the Tris-HCl buffer solution. SCE reference electrode was placed in acetate buffer solution (0.5 mol/L NaAc, pH 4.8) containing 0.02 mol/L NaCl to activate for 5 min.

2.3.2 DNA immobilization

Using the electrochemical enrichment method, dsDNA was immobilized on the activated CPE surface by applying 0.5 V to the working electrode in the stirring Tris-HCl buffer (pH 8.0) solution containing 4×10^{-6} mol/L dsDNA for 5 min. Then, the modified CPE was rinsed thoroughly with ultrapure water.

2.3.3 Interaction with cadmium

For the interaction of Cd^{2+} and dsDNA, the dsDNA-modified electrode was immersed in the stirring Cd^{2+} solution for 5 min without applying any potential to the electrode. After that, the CPE was rinsed thoroughly with ultrapure water.

2.3.4 Accumulation of BG

After the interaction with Cd^{2+}, the dsDNA modified CPE was further placed into a 1.0×10^{-3} mol/L BG hybridization indicator solution. To facilitate the accumulation of BG on the electrode surface, the electrode was kept in the solution for 5 min with gentle stirring. Similarly, no potential was applied in this step. Then, the electrode was rinsed with ultra-pure water, and water remained on the electrode surface was carefully removed by the absorption of filter paper.

2.3.5 Electrochemical measurement –Different pulse voltammetry

The concentration of Cd^{2+} was determined by differential pulse voltammetry (DPV) according to the change in the reduction current peak of BG in the Tris-HCl buffer solution (pH 7.4) after the interaction between dsDNA and Cd^{2+}. The interference investigation was conducted upon the interaction of interfering metal ion and dsDNA under the same condition. The selectivity for each metal ion was evaluated based on the electrochemical signals recorded with the DNA biosensor. The setup data for DPV detection was listed in Table 1 as follows.

Table 1. The setup data of DPV

Cathodic potential (V vs. SCE)	Amplitude	Modulation time (s)	Interval (s)	Step potential (V vs. SCE)
0.7 ~ 0.2	0.04995	0.05	0.5	0.01005

2.3.6 Detection of cadmium contents in real samples

Sprague-Dawley (SD) rats were fed cadmium solutions of 0 mg/L, 25 mg/L and 75 mg/L for three months. After euthanasia, the tails of SD rats were treated by the microwave digestion method. The resulted sample solutions were adjusted to pH 7.4 and used to prepare the test solutions with Tris-HCl buffer solution. The cadmium contents of the test solutions were determined with the DNA biosensor.

2.3.7 Recovery test

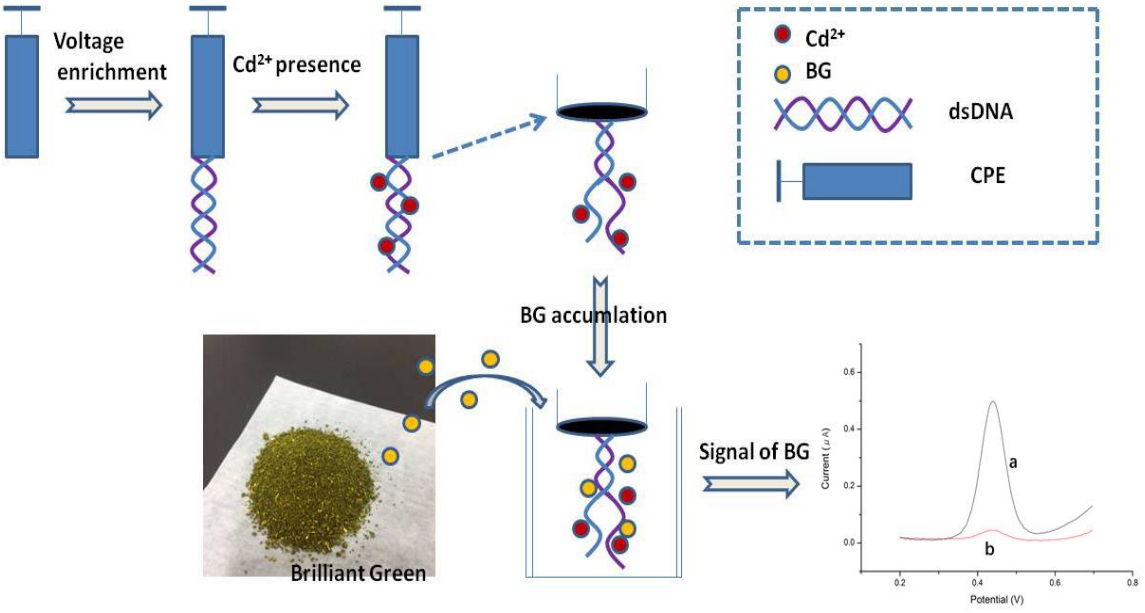
A blank chicken sample solution was prepared by the microwave digestion method. The resulted blank solution was adjusted to pH 7.4 and used to prepare the test solution with Tris-HCl buffer solution.

Following the addition of 0.5×10^{-9} mol/L, 1.0×10^{-9} mol/L and 1.5×10^{-9} mol/L cadmium solutions, ICP-MS and DNA biosensors were used to detect the content of cadmium and calculate the recovery percentage from the blank material.

3. RESULTS AND AISCUSSION

3.1 Electrochemical determination of cadmium

Scheme 1 depicted the procedure and principle of the electrochemical DNA biosensor for cadmium determination based on the dsDNA modified CPE and BG indicator. As shown in DPV curves, the reduction peak current of BG signal (curve a) on the dsDNA modified CPE was much higher with the presence of Cd^{2+} than the one (curve b) without the presence of Cd^{2+}. Such reduction peak current increase up to 10.4 times revealed that more BG indicators were binding to the dsDNA modified CPE surface due to the destabilization and unwinding dsDNA structure caused by Cd^{2+}.[18] Then, ssDNA and dsDNA modified CPEs were prepared to investigated the binding affinity of BG upon the DPV measurements. As shown in Fig. 1, the reduction peak current of BG on ssDNA modified CPE (curve a) was about 4.2 times higher than the reduction peak current on dsDNA modified CPE (curve b). This phenomenon suggested that BG has stronger binding affinity to ssDNA than dsDNA. Therefore, these results demonstrated that BG, as a new hybridization and electrochemistry indicator of the dsDNA modified CPE, could be used to design an electrochemical biosensor for the selective detection of Cd^{2+}.



Scheme 1. The procedure and principle of cadmium detection by electrochemical DNA biosensor on account of DNA destabilization induced by Cd^{2+}.

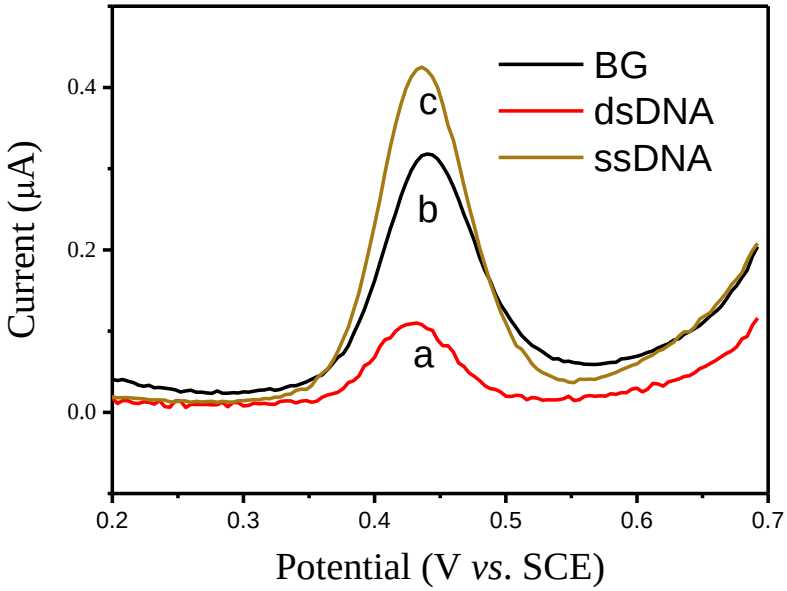


Figure 1. DPV of the a) dsDNA-modified CPE, b) bare CPE and c) ssDNA-modified CPE in Tris-HCl buffer solution (pH 7.4) containing 1.0×10^{-3} mol/L BG.

The interaction of dsDNA and Cd^{2+} was further studied by changing the amount of dsDNA modified on the CPE. It was clearly showed in Fig. 2 that the reduction peak current of BG increased by changing the concentration of dsDNA from 2×10^{-6} to 6×10^{-6} mol/L used for the CPE modification. This is because that Cd^{2+} at a given concentration result in an increasing amount of damaged dsDNA among these CPEs. Thus, the reduction peak current of BG was related to the amount of damaged dsDNA on the modified CPE as well as the presence of Cd^{2+}. Such electrochemical response of BG could be used to detect Cd^{2+} with the proposed electrochemical DNA biosensor.

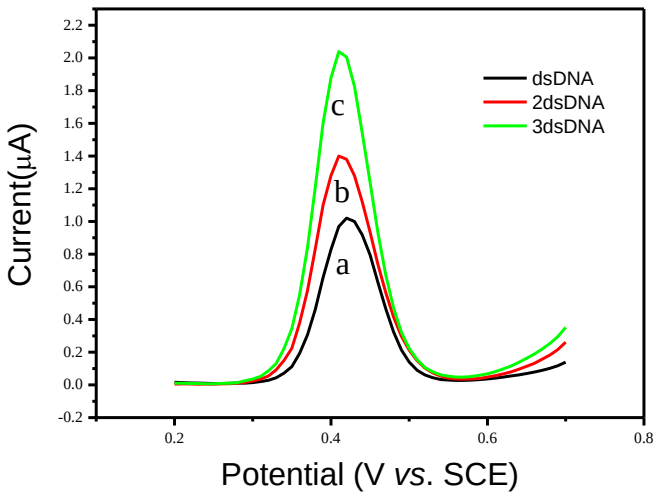


Figure 2. DPV on the a) the CPE modified in 2×10^{-6} mol/L dsDNA solution, b) the CPE modified in 4×10^{-6} mol/L dsDNA solution and c) the CPE modified in 6×10^{-6} mol/L dsDNA solution after interacting with the same concentration of Cd^{2+} in Tris-HCl buffer (pH 7.4) containing 1.0×10^{-3} mol/L BG.

In order to verify the feasibility of the electrochemical biosensor for Cd^{2+} detection, we have prepared the dsDNA modified CPE in 4×10^{-6} mol/L dsDNA solutions. The detection of Cd^{2+} at different concentrations was performed using the dsDNA modified electrode. Fig. 3 showed the detection results that the reduction peak current of BG increased linearly with the Cd^{2+} concentration in the range of 0.5 ~ 1.2 nM. The detection limit was calculated to be 0.1 pM by 3σ/S (where σ represents the standard deviation of the blank signal and S is the slope of the calibration curve).

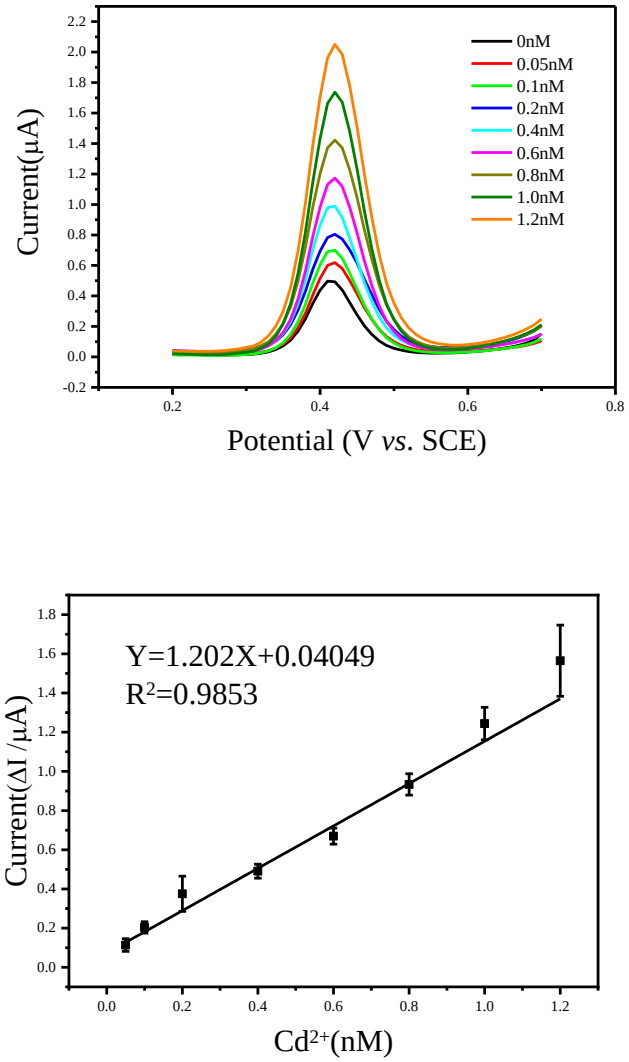


Figure 3. The DPV peaks of 1.0×10^{-3} mol/L accumulated BG on the 4×10^{-6} mol/L probe dsDNA-modified CPE after interacting with 0 ~ 1.2×10^{-9} mol/L Cd^{2+} in Tris-HCl buffer (pH 7.4).

In contrast with other biosensors listed in Table 1, [14, 19-29] the limit of detection (LOD) of the proposed electrochemical sensor in this work is lower than one of most other sensor. The interfacial material is the key unit of sensor as it involves in the signal generation and transformation. [30] Even though other biomaterials, such as antibody and enzyme,[19, 25] have been used to design diverse

electrochemical biosensor, the helix structure of DNA is stable and provide more binding sites for the detection target.[31] Besides, the biological activity of antibody and enzyme is easily influenced by the external factors, including temperature, acidity and toxicity in the detection solution.[32] The sensitivity for Cd^{2+} detection might be improved by taking the advantages of some nano-materials, including gold nanoparticles, carbon nanotube, grapheme, metal–organic framework (MOFs).[26, 27, 33-35] But the synthesis of these nano-materials is time-consumed and complicated, which may not be suitable for massive detections. Recently, similar electrochemical DNA biosensor was also reported by Ebrahimi [21] and used for Cd^{2+} detection with LOD of 0.3 pM. However, the sensitivity for Cd^{2+} detection could be further improved by our proposed electrochemical biosensor with BG as a new indicator.

Table 1. Comparison of other cadmium detection biosensors

Type of interface	Interface material	Limit of detection (LOD)	Reference
POU/PGM	Exonuclease III	5×10^{-12} mol/L	[19]
Surface-enhanced Raman spectroscopy	AuNPs-TMT	2.9×10^{-9} mol/L	[20]
Hybridization indicator	Ethylgreen-DNA	0.3×10^{-12} mol/L	[21]
UPD	Au-ssDNA	1.0×10^{-11} mol/L	[14]
Macro-and micro-interface	Calex[4] arene	1.0×10^{-6} mol/L	[22]
	Screen printed electrodes	5×10^{-7} mol/L	[23]
	Bacillus badius	1.0 μg/L	[24]
	Arthrospira platensis Antibody 2A81G	1.0×10^{-20} mol/L	[25]
Nano-interface	Multiwall carbon nanotubes with cyclic dipeptide	2.749×10^{-8} mol/L	[26]
	Gold nanoparticle amalgam	2.6 ppb	[27]
	Pristine single-walled carbon nanotube	0.7 ppb	[28]
	Carbon nanotubes	0.7 μg/L	[29]

3.2 Selectivity of electrochemical DNA sensors

The selectivity of the DNA biosensor was studied by using other interfering metal ions, including Hg^{2+}, Pb^{2+}, Zn^{2+}, Ca^{2+}, Ag^{+}, and Cu^{2+}. Compare to the bank solution, the change of the reduction peak current of BG on the dsDNA modified CPE was negligible in each interfering metal ion solution even at much higher concentrations (1×10^{-5} mol/L) (Fig. 4). The addition of 1×10^{-9} mol/L Cd^{2+} into the solution could significantly increase the reduction peak current of BG on the modified CPE (Fig. 4). These results indicate that the DNA biosensor has excellent selectivity for Cd^{2+} detection. Lead and cadmium often enter the environment from many natural and artificial pollution sources at the same time, which causes compound pollution and animal disease and poisoning. In addition to simultaneous pollution, lead and mercury can produce specific binding reactions with oligonucleotides. Pb^{2+} can induce the formation of G-quadruplexes [36], and Hg^{2+} can mismatch thymine (T-T) in dsDNA to form a stable T-Hg^{2+}-T mismatch structure [37]. The T-T can block DNA double-stranded charge transfer, and the function of DNA double-stranded charge transfer can be restored after the formation of the T-Hg^{2+}-T complex [38]. Therefore, the sequence of DNA which has specific binding to Cd^{2+} was selected to design the electrochemical biosensor. Nonetheless, other DNA sequences for Pb^{2+} and Hg^{2+} can be easily prepared and used for sensing applications.

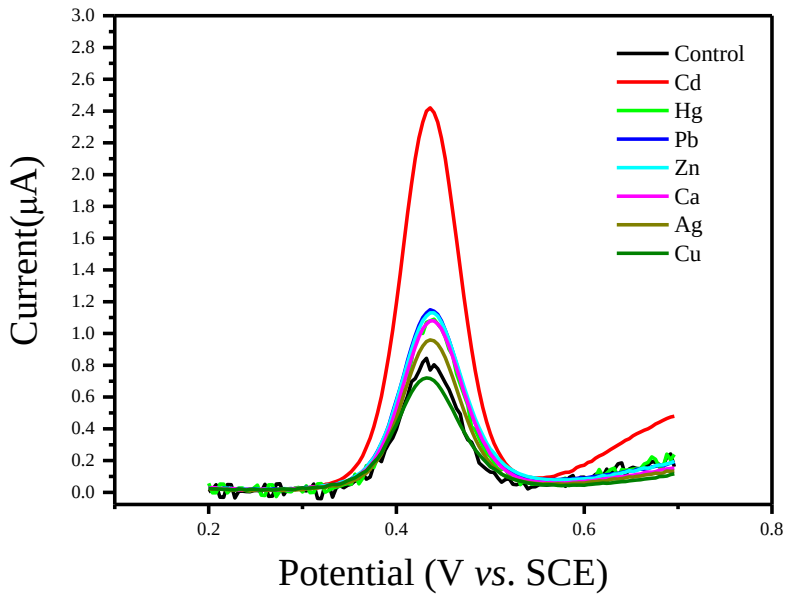


Figure 4. DPV curves on the dsDNA modified CPE with the presence of 1×10^{-5} mol/L Hg^{2+}, Pb^{2+}, Zn^{2+}, Ca^{2+}, Ag^{+}, Cu^{2+} and 1×10^{-9} mol/L Cd^{2+} in Tris-HCl buffer (pH 7.4).

3.3. Analysis of real sample

Chicken and rat samples were used to verify the practical applications of the electrochemical biosensor. The detection results of Cd^{2+} in chicken tissues were listed in Table 2. The concentration of Cd^{2+} in the sample solution was detected to be 0.121 μg/L, which was in a good agreement with the value detected by ICP-MS. Moreover, by adding the standard Cd^{2+} into the sample solutions, good

percent recovery values of the electrochemical biosensor were also obtained and comparable to the values of ICP-MS. The detection of Cd^{2+} in rat tail were also executed by the same electrochemical biosensor as shown in Table 3. After feeding with Cd^{2+} drinking water for 3 months, the concentration of Cd^{2+} detected by the electrochemical biosensor was obviously higher than that of the chicken sample solution. The accumulated Cd^{2+} in the rat tail increased with the increasing concentration of Cd^{2+} drinking water. Similar concentration values of Cd^{2+} in the rat tail samples were detected by both the electrochemical biosensor and ICP-MS. All these results indicated that the electrochemical biosensor exhibited good accuracy in the real sample detections.

Table 2. Recovery of Cd^{2+} in chicken tissues

Add (nM)	DNA biosensor (μg/L)	Recovery (%)	RSD(n=3) (%)	IPC-MS[b] (μg/L)
0[a]	0.121	101.1	0.09	0.120
0.5	0.164	93.2	0.28	0.155
1.0	0.225	97.1	0.20	0.191
1.5	0.278	96.2	0.50	0.257

[a.] The chicken tissues obtained from Poultry Institute, Chinese Academy of Agricultural Sciences (Yangzhou, China).

[b.] The IPC-MS data obtained from Test Center, Yangzhou University.

Table 3. Analysis of Cd^{2+} in real rat tail samples

Rat Sample[a] (mg/L)	DNA biosensor (μg/L)	RSD (n=3) (%)	ICP-MS[b] (μg/L)
0	0.215	6.63	0.207
50	8.739	2.85	8.847
75	12.419	2.54	12.726

[a.] The sample was obtained from rat tail tissue. The rats were freely provided drinking water containing cadmium acetate (0 mg/L, 50 mg/L and 75 mg/L) for 3 months.

[b.] The IPC-MS data from Test Center, Yangzhou University.

4. CONCLUSIONS

In summary, an electrochemical biosensor was fabricated based on the dsDNA modified CPE as the working electrode and BG as a new indicator. Due to the destabilization and unwinding of dsDNA modified on CPE by cadmium, the reduction peak current of the BG was linearly increased by increasing the Cd^{2+} concentration. The DNA biosensor has stronger specific binding to Cd^{2+}, higher sensitivity and

simpler operation for Cd^{2+} detection than reported methods. The portability of the biosensor is also an advantage. Cd^{2+} was determined in Tris-HCl buffer (pH 7.4) within a 0.05×10^{-9} to 1.2×10^{-9} mol/L detection range with a 0.1×10^{-12} mol/L detection limit. For practical use, the electrochemical DNA biosensor was successfully applied to detect the Cd^{2+} concentration in the chicken tissue and rat tail samples. Satisfactory results were achieved by comparing with ICP-MS. The combination of the dsDNA modified and BG new indicator results in sensitive electrochemical signals, making them a promising system for analytical and biological applications.

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