International Journal of Biological Macromolecules 108 (2018) 1237–1241

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

journal homepage: www.elsevier.com/locate/ijbiomac

Design of an electrochemical DNA-based biosensor for selective determination of cadmium ions using a DNA hybridization indicator

Maryam Ebrahimi, Jahan Bakhsh Raoof*, Reza Ojani

Eletroanalytical Chemistry Research Laboratory, Department of Analytical Chemistry, Faculty of Chemistry, University of Mazandaran, Babolsar, Iran

ARTICLE INFO

Article history:
Received 17 September 2017
Received in revised form 6 November 2017
Accepted 6 November 2017
Available online 7 November 2017

Keywords:
Cadmium ion
Ethyl green
Double strand DNA
Differential pulse voltammetry
DNA biosensor

ABSTRACT

A simple electrochemical DNA- based biosensor was designed for determination of Cd^{2+} using ethyl green (EG) as a DNA hybridization indicator on the surface of carbon paste electrode (CPE). The interaction of Cd^{2+} with double strand DNA (dsDNA) led to the destabilizing of dsDNA and the increase in the reduction peak currents of EG. The difference in the values of electrochemical responses of EG before and after DNA helix destabilization in the presence of Cd^{2+} (ΔI) was used for Cd^{2+} determination and was linearly related to the concentration of Cd^{2+} over the ranges of 1.0 pM–1.0 nM and 10.0 nM–1.0 μM. Detection limit was achieved as low as 0.3 pM using simple and low cost working electrode and without applying any materials for modification of electrode or DNA. The proposed biosensor was utilized successfully to real sample analysis of Cd^{2+}.

1. Introduction

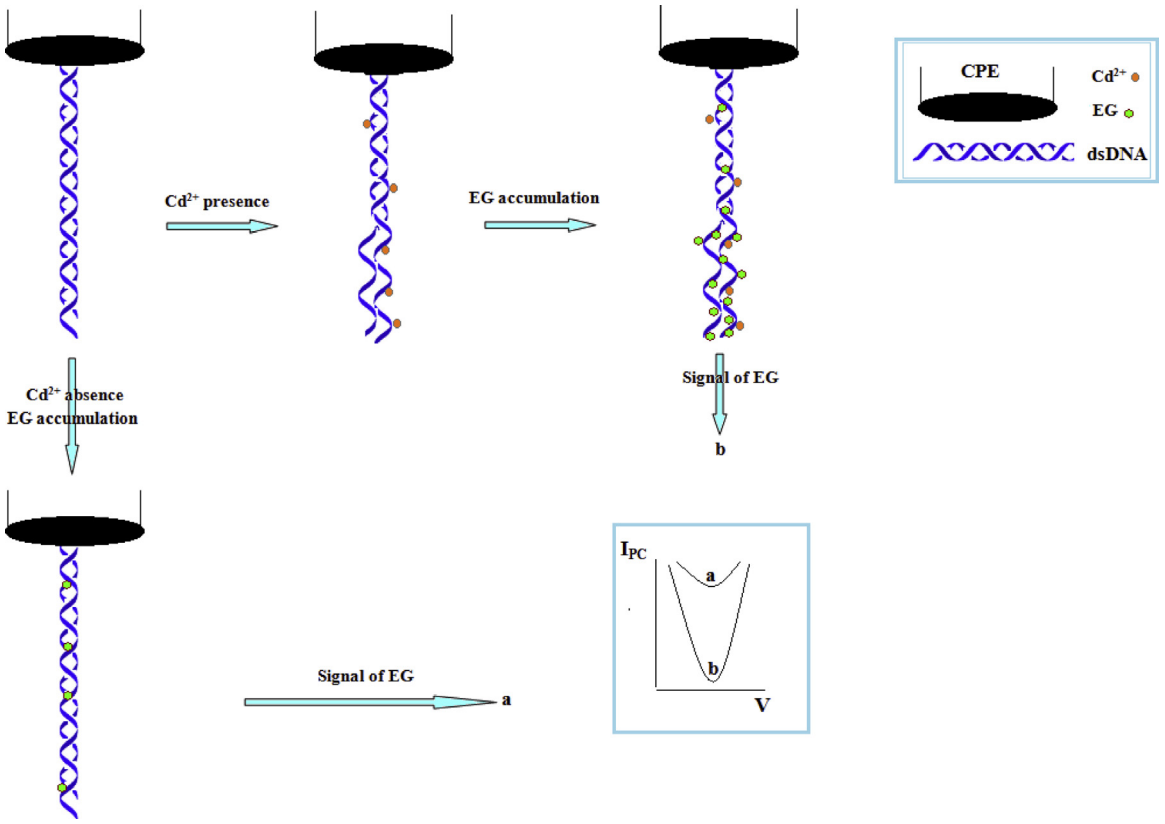
Cd^{2+} is one of the most toxic heavy metal ions and is known human and animal carcinogen [1–3]. Cd^{2+} inhibits DNA repair proteins [3–5] and can cause DNA lesions [6]. Exposure to cadmium can cause hyper tension [3] and cardiovascular [7] diseases and disturb the function of several tissues of animals and humans such as kidney, lung, liver and prostate [3]. According to United States Environmental Protection Agency (EPA) and the World Health Organization (WHO), the upper admissible limits of cadmium in drinking water are 5.0 μg/L (44.5 nM) and 3.0 μg/L (26.7 nM) [8]. Therefore, development of the sensitive and rapid approach for determination of Cd^{2+} is very urgent. Various sensitive techniques such as X-ray spectrometry [9], atomic absorption spectroscopy (AAS) [10,11], inductively coupled plasma-mass spectrometry (ICP-MS) [12], spectrophotometry [13] and inductively coupled plasma-optical emission spectroscopy (ICP-OES) [14], have been used for Cd^{2+} determination. These methods suffer from complexity of sample preparation and expensive instruments [13,15,16]. However, electrochemical methods using for Cd^{2+} detection not only are sensitive, but also have relatively simple instruments and procedure [15–17].

In recent years, the interaction of heavy metal ions with DNA as an important biological macromolecule is the base of the important class of electrochemical metal ions biosensors [18]. However, to the best of our knowledge, DNA-based biosensors for electrochemical determination of Cd^{2+} are little. Wong et al. [19] determined Cd^{2+} electrochemically on the single strand DNA (ssDNA) modified gold electrode based on Cd^{2+}-DNA interaction. Analytical performance of their method was appropriate. Recently, Qu et al. [16] developed a DNA-based biosensor for selective electrochemical detection of Cd^{2+} that was based on the Cd^{2+}-induced structural switching of ssDNA probe, which tagged with methylene blue as an electroactive label. In these sensors, not only a long time for covalent immobilization of DNA probe on the surface of gold electrode was required, but also the costly and time consuming prior modification of probe or electrode by thiol group for probe immobilization was necessary.

Cd^{2+} is capable of binding to dsDNA through N7 atoms of guanine bases of DNA, leading to the breaking of base pairs and dsDNA destabilization [4,20]. On the other hand, some dyes can detect hybridized DNA (double strand DNA (dsDNA)) in order to different affinities for ss- and ds-DNAs [21,22]. In previous work [23], we could show ethyl green (EG) dye as a DNA hybridization indicator prefers binding to ssDNA compared to dsDNA. Therefore, its electrochemical signal decreased after DNA hybridization. Thus, the destabilization and unwinding of DNA helixin the presence of Cd^{2+} [1] can cause increase the electrochemical signal of EG. Therefore, we can introduce a novel and simple strategy for rapid electrochemical determination of Cd^{2+} based on a DNA hybridization indicator without any modification of probe or working electrode.

* Corresponding author.
E-mail address: j.raoof@umz.ac.ir (J.B. Raoof).

https://doi.org/10.1016/j.ijbiomac.2017.11.023
0141-8130/



Scheme 1. The principle of the electrochemical approach for Cd^{2+} determination based on Cd^{2+}-induced DNA destabilization.

The differential pulse voltammetry (DPV) technique was used for electrochemical measurements.

2. Experimental

2.1. Materials and reagents

Two 10–mer oligonucleotides for preparation of dsDNA were supplied from MWG-BIOTECH (Germany). The sequences of applied oligonucleotides were as follows:

ssDNA1: poly C DNA: 5′-CCC CCC CCC C –3’ (100% cytosine bases)

ssDNA2: poly G DNA: 5′-GGG GGG GGG G –3’ (100% guanine bases)

EG and $HgCl_2$ were purchased from Fluka. $CdCl_2$, $AgNO_3$, $Zn(NO3)_2$, $CaCl_2$ and $CuCl_2$ were purchased from Merck company. Stock solutions of 10^{-4} M of probes were prepared in TE buffer solution (10 mM Tris-HCl, 1.0 mM EDTA, pH = 8.00) and kept frozen. Tris-HCl buffer solution (50.0 mM, pH = 7.40) containing 20 mM NaCl were used for preparation of more diluted probe solutions. In order to preparation of dsDNA, the solution containing ssDNA1 and its complementary DNA (ssDNA2) was placed in a water bath at 100 °C and heated for 10 min. Then the solution was allowed to reach the room temperature.

2.2. Apparatus

Electrochemical measurements were carried out using an AUTOLAB PGSTAT 30 electrochemical analysis system and GPES 4.9 software package and FRA software (Eco Chemie, The Netherlands) in a single compartment electrochemical cell with carbon paste electrode (CPE) as working electrode, a platinum wire as the auxiliary electrode and a saturated calomel electrode (SCE) as the reference electrode. An Ion Analyzer 250 Corning pH–meter was used for pH measurements.

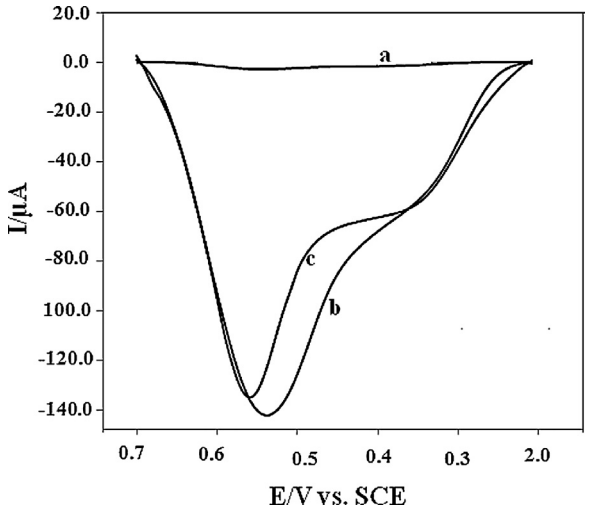


Fig. 1. DPV curves of 1.0 mM accumulated EG on the a) dsDNA-immobilized CPE, b) ssDNA1 probe-immobilized CPE and c) ssDNA2 probe-immobilized working electrode in 10.0 mM Tris-HCl buffer solution (pH 7.0).

2.3. Procedure

2.3.1. Working electrode preparation for electrochemical studies

CPE was prepared according to the procedure described in our previous works [21,22]. In order to preparation of electrode for electrochemical analyses, first, the surface of working electrode was electrochemically activated under optimized potential of 1.80 V vs. SCE [21,22] in 0.50 M acetate buffer solution (pH 4.8) containing

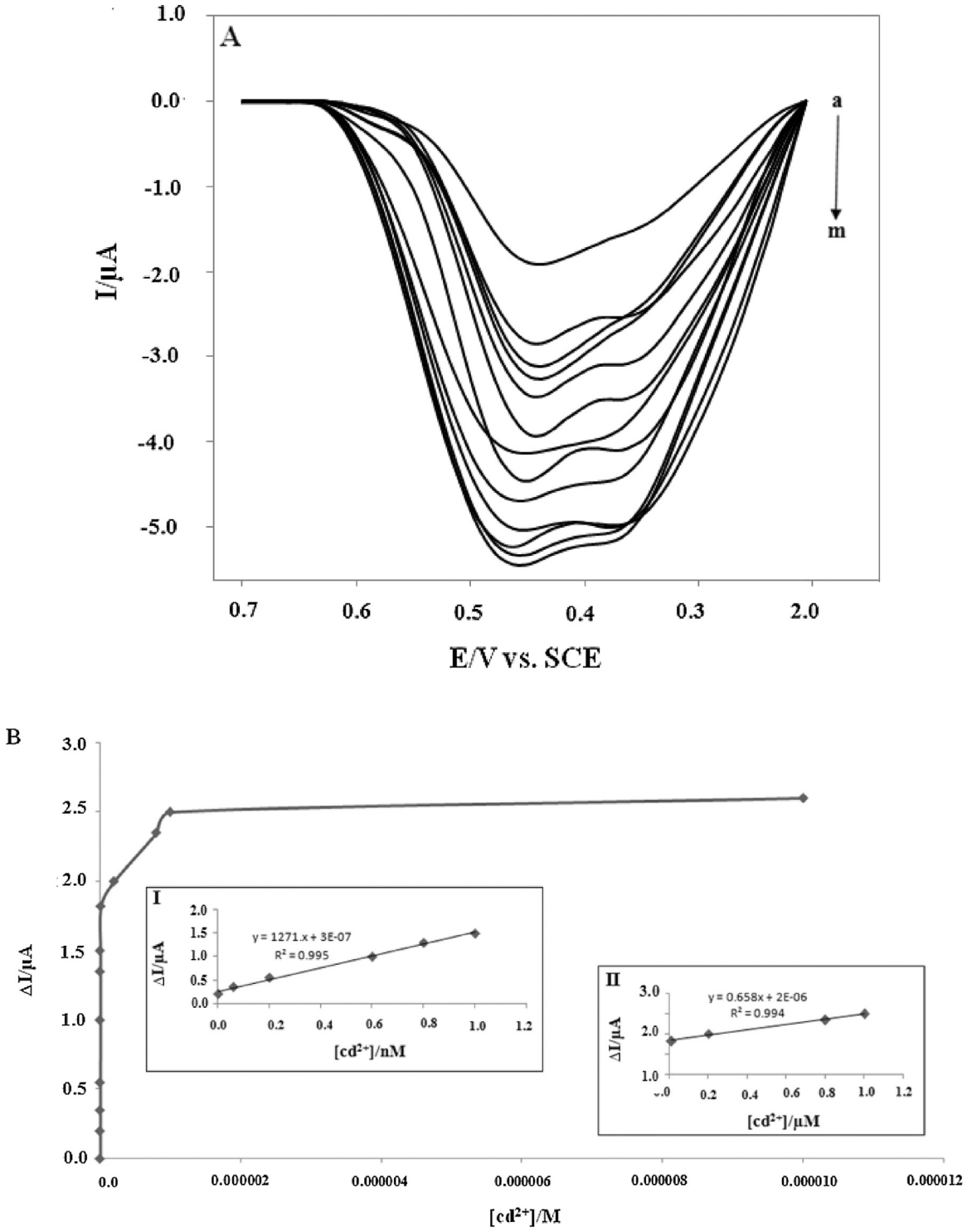


Fig. 2. (A) DPV signals of 1.0 mM accumulated EG on the bare CPE (a), dsDNA probe-immobilized CPE after destabilization in the presence of different concentrations of Cd^{2+} from 0 to 10.0 µM (b-m) in 10.0 mM Tris-HCl buffer solution (pH 7.0). (B) The differences plot of DPV responses vs. Cd^{2+} concentrations at, inset: related calibration plot of Cd^{2+} concentration over the ranges (I) 1.0 pM–1.0 nM and (II) 10.0 nM–1.0 µM.

20 mM NaCl without stirring for 5 min. Then, we applied a potential of 0.50 V to the electrodes for 5 min in order to the immobilization of dsDNA on the activated electrode in a solution of 10^{-6} M DNA with stirring. Finally, the electrode was rinsed with sterilized and deionized water.

2.3.2. Cd-dsDNA interaction

The Cd^{2+} and dsDNA interacted by incubating the dsDNA-modified CPE in the stirring Cd^{2+} solution (0.05 M Tris-HCl buffer solution, pH 7.40) for 5 min (the optimum time, data not shown) without applying any potential to the electrode. CPE was rinsed with sterilized and deionized water.

2.3.3. Accumulation of EG on the electrodes surface

In order to the accumulation of EG as an electroactive label on the electrode surface after immobilization of dsDNA on the CPE, the electrode was immersed into 10 mM Tris-HCl buffer solution (pH 7.0) containing 1.0 mM EG for 5 min with stirring. In this step, no

potential was also imposed to the dsDNA modified CPE. Finally, the electrode was rinsed with sterilized and deionized water.

2.3.4. Electrochemical measurement

The voltammetric determination of cadmium ions was carried out by DPV measurements of reduction peak current of EG in Tris–HCl buffer solution before and after DNA-Cd^{2+} interaction under cathodic potential scan from 0.7 to 0.2 V vs. SCE at the modulation amplitude of 0.04995, modulation time of 0.05 s, interval time of 0.5 s and step potential of 0.01005 V vs. SCE. Selectivity experiments of present biosensor were also conducted by recording signal of EG after interfering metal ions-DNA interaction under the same condition.

3. Results and discussion

3.1. Electrochemical determination of Cd^{2+}

Scheme 1displayed the strategy of preparation of the biosensor for electrochemical determination of Cd^{2+}. The cathodic peak current of EG accumulated on the DNA biosensor increased after DNA-Cd^{2+} interaction, in order to the destabilization of DNA duplex through breaking of base pairs. As can be seen in Fig. 1, EG prefers binding to ssDNAs (curves b and c) compared to dsDNA (curve a).

For determination of Cd^{2+}, the difference in DPV peak currents (ΔI) of 1.0 mM EG before and after incubation on the probe modified electrodes in 0.05 M Tris-HCl buffer solution (pH = 7.40) and 20 mM of NaCl containing different concentrations of Cd^{2+} was obtained. As can be seen in Fig. 2A, the peak current, I, was gradually increased with the increase of the concentrations of Cd^{2+}. Fig. 2B shows the relationship between ΔI versus concentrations of Cd^{2+} at the surfaces of CPE. As shown in Fig. 2B, ΔI is linearly related to Cd^{2+} concentrations over the range of 1.0 pM–1.0 nM and 10.0 nM–1.0 μM. The detection limit (based on a three signal-to-noise ratio) was calculated 0.3 pM.

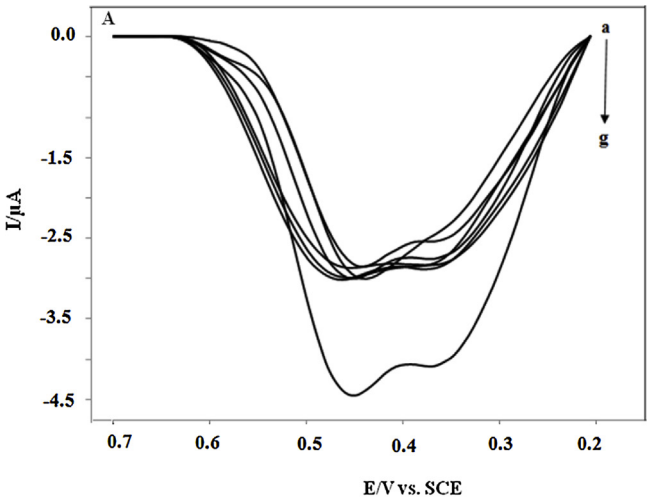
3.2. Selectivity study of biosensor

The selectivity of Cd^{2+} biosensor was tested through substituting Cd^{2+} in the incubation buffer with other heavy metal ions, including Cu^{2+}, Ca^{2+}, Hg^{2+}, Zn^{2+} and Ag^{+}. As shown in Fig. 3A, the DPV responses of EG solution at DNA-modified working electrode did not show remarkable change on the signal of label on the surface of the biosensor after incubation of these interfering metal ions even at a relatively high concentration (10 μM), but Cd^{2+} in low concentration (1 nM) could significantly change the signal of label. These results indicated a satisfactory selectivity of developed biosensor for Cd^{2+} determination (Fig. 3B).

3.3. Analysis of real sample

The analysis of Cd^{2+} ions in tap and Caspian Sea water samples was performed to demonstrate the practical usage of the biosensor. The recovery values were satisfactory and results showed the utility of the proposed method for the detection of Cd^{2+} ions in real water samples (Table 1).

The analytical performances of other electrochemical sensors for Cd^{2+} determination are given in Table 2. As seen, despite using very simple working electrode and without applying complex procedure or any modifier for modification of electrode or DNA probe, the sensor is capable for detection and determination of cadmium ion at very lower concentrations than maximum allowed amount of cadmium recommended by WHO and EPA.



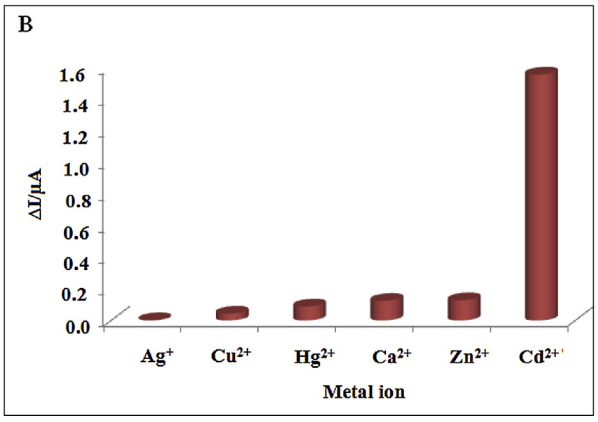


Fig. 3. DPV signals of 1.0 mM accumulated EG on the probe dsDNA-immobilized CPE before (a) and after interaction with 10.0 μM Ag^{+} (b), Cu^{2+} (c), Hg^{2+} (d), Ca^{2+} (e), Zn^{2+} (f) and 1.0 nM Cd^{2+} (g).

Table 1
Real analysis of Cd^{2+} in water samples.

Water sample	Cd^{2+} added/M	Cd^{2+} found/M[a]	Recovery (%)
Tap water[b]	0.0	Not detectable	–
	1.0×10^{-9}	$0.92\ (\pm 0.03) \times 10^{-9}$	92.0
	1.0×10^{-6}	$0.95\ (\pm 0.02) \times 10^{-6}$	95.0
Caspian Sea water[c]	0.0	$0.712\ (\pm 0.05) \times 10^{-6}$	–
	1.0×10^{-6}	$1.75\ (\pm 0.3) \times 10^{-6}$	102.21

[a] Mean value ± SD (standard deviation), n = 5.
[b] From drinking water system of Babolsar, Iran.
[c] From Caspian Sea, Babolsar, Iran.

4. Conclusion

We introduced a novel approach for electrochemical determination of Cd^{2+} based on DNA-Cd^{2+} interaction and EG without applying complicated method and costly materials. The destabilization of dsDNA in the presence of Cd^{2+} ions led to open the DNA helix and the increase in the electrochemical responses of EG as an electroactive label. Cd^{2+} ions were determined selectively and sensitively by the difference in the value of DPV cathodic peak currents of EG before and after dsDNA destabilization. Analytical performance of developed biosensor compared other electrochemical sensor for cadmium determination was satisfactory.

Table 2
Comparison of the electrochemical biosensors for detection of cadmium ions.

Electrode	Determination technique	LOD	Linear range(s)	Reference
ssDNA modified Au electrode	Cyclic voltammetry	2.62 pM	8.89 pM–0.18 nM	[16]
kaolin platinum electrode	Square wave voltammetry	5.4 nM	0.9nM– 8.3 μM	[17]
ssDNA modified Au electrode	Osteryoung square wave voltammetry	10.0 pM	10.0 pM–500.0 nM	[19]
PEDOT/PSS modified GCE[a]	Linear sweep anodic stripping voltammetry	0.13 μM	0.17–0.88 μM	[24]
Cd-ion imprinted polymer modified CPE	Differential pulse stripping voltammetry	2.74 nM	17.7nM– 0.17 μM	[25]
SWCN[b] film electrode	Square wave stripping voltammetry	6.22 nM	0.293–2.0 μM	[26]
CPE	Differential pulse voltammetry	0.3 pM	1.0pM–1.0 nM 10.0 nM–10.0 μM	Present paper

[a] Poly(3,4-ethylenedioxythiophene)/poly (4-styrenesulphonate) modified glassy carbon electrode.
[b] Single-walled carbon nanotube.

References

[1] S.C.B. Oliveira, O. Corduneanu, A.M. Oliveira-Brett, In situ evaluation of heavy metal–DNA interactions using an electrochemical DNA biosensor, Bioelectrochemistry 72 (2008) 53–58.
[2] M. Airoldi, G. Gennaro, M. Giomini, A.M. Giuliani, M. Giustini, Interaction of the alternating double stranded copolymer poly(dA-dT)poly(dA-dT) with $NiCl_2$ and $CdCl_2$: solution behavior, J. Biomol. Struct. Dyn. 25 (2007) 77–84.
[3] C. Giaginis, E. Gatzidou, S. Theocharis, DNA repair systems as targets of cadmium toxicity, Toxicol. Appl. Pharmacol. 213 (2006) 282–290.
[4] P. Wang, A.B. Guliaev, B. Hang, Metal inhibition of human alkylpurine-DNA-N-glycosylase activity in base excision repair, Toxicol. Lett. 166 (2006) 237–247.
[5] Y. Nzengue, R. Steiman, C. Garrel, E. Lefebvre, P. Guiraud, Oxidative stress and DNA damage induced by cadmium in the human keratinocyte HaCaT cell line: role of glutathione in the resistance to cadmium, Toxicology 243 (2008) 193–206.
[6] Y. Hou, P. Wu, N. Zhu, The protective effect of clay minerals against damage to adsorbed DNA induced by cadmium and mercury, Chemosphere 95 (2014) 206–212.
[7] N. Bashir, V. Manoharan, S. Miltonprabu, Cadmium induced cardiac oxidative stress in rats and its attenuationby GSP through the activation of Nrf2 signaling pathway, Chem. Biol. Interact. 242 (2015) 179–193.
[8] M.K. Zeinu, H. Hou, B. Liu, X. Yuan, L. Huang, X. Zhu, J. Hu, J. Yang, S. Liang, X. Wu, A novel hollow sphere bismuth oxide doped mesoporous carbon nanocomposite material derived from sustainable biomass for picomolar electrochemical detection of Lead and Cadmium, J. Mater. Chem. A 4 (2016) 13967–13979.
[9] E. Margui, I. Queralt, M. Hidalgo, Determination of cadmium at ultratrace levels in environmental water samples by means of total reflection X-ray florescence spectrometry after dispersive liquid liquid microextraction, J. Anal. At. Spectrom. 28 (2013) 266–273.
[10] E.Z. Jahromi, A. Bidari, Y. Assadi, M.R.M. Hosseini, M.R. Jamali, Dispersive iquid-liquid microextraction combined with graphite furnace atomic absorption spectrometry: ultra trace determination of cadmium in water samples, Anal. Chim. Acta 585 (2007) 305–311.
[11] T. Daşbaşı, Ş. Saçmacı, A. Ülgen, Ş. Kartal, A solid phase extraction procedure for the determination of Cd (II) and Pb (II) ions in food and water samples by flame atomic absorption spectrometry, Food Chem. 174 (2015) 591–596.
[12] S. Christopher, R. Thompson, Determination of trace level cadmium in SRM 3280 Multivitamin/Multielement Tablets via isotope dilution inductively coupled plasma-mass spectrometry, Talanta 116 (2013) 18–25.
[13] M.J. Ahmed, T. Zannatand, Z. Fatema, A simple spectrophotometric method for the determination of trace level of cadmium inreal environmental, biological, tobacco, fertilizer and samples using 2', 3, 4', 5,7-pentahydroxyflavone, ACSJ 4 (2014) 481–503.
[14] I. Kula, M.H. Soylak, M. Ugurlu, M. Isiloglu, Y. Arslan, Determination of mercury cadmium, lead, zinc, selenium and iron by ICP-OES in mashroom samples from around thermal power plant in Mugla, Turkey, Bull. Environ. Contam. Toxicol. 87 (2011) 276–281.
[15] G. Fan, D. Zhai, D. Zou, Highly sensitive electrochemical determination of cadmium (II) in environmental water based on the electrodeposited bismuth nanoparticles, Int. J. Electrochem. Sci. 11 (2016) 4362–4370.
[16] J. Qu, L. Wu, H. Liu, J. Li, H. Lv, X. Fu, Y. Song, A novel electrochemical biosensor based on DNA for rapid and selective detection of cadmium, Int. J. Electrochem. Sci. 10 (2015) 4020–4028.
[17] M.A. El Mhammedia, M. Achak, M. Hbid, M. Bakasse, T. Hbid, A. Chtaini, Electrochemical determination of cadmium(II) at platinum electrode modified with kaolin by square wave voltammetry, J. Hazard. Mater. 170 (2009) 590–594.
[18] L. Cui, J. Wu, H. Ju, Electrochemical sensing of heavy metal ions with inorganic, organic and biomaterials, Biosens. Bioelectron. 63 (2015) 276–286.
[19] E.L.S. Wong, E. Chow, J.J. Gooding, The electrochemical detection of cadmium using surface-immobilized DNA, Electrochem. Commun. 9 (2007) 845–849.
[20] D.O. Zharkov, T.A. Rosenquist, Inactivation of mammalian 8-oxoguanine-DNA glycosylase by cadmium(II): implications for cadmium genotoxicity, DNA Repair 1 (2002) 661–670.
[21] M. Ebrahimi, J.B. Raoof, R. Ojani, Novel electrochemical DNA hybridization biosensors for selective determination of silver ions, Talanta 144 (2015) 619–626.
[22] M. Ebrahimi, J.B. Raoof, R. Ojani, Z. Bagheryan, A novel electrochemical biosensor for selective determination of mercury ions based on DNA hybridization, Anal. Biochem. 488 (2015) 13–14.
[23] J.B. Raoof, R. Ojani, M. Ebrahimi, E. Hamidi-Asl, Developing a nano-biosensor for DNA hybridization using a new electroactive label, Chin. J. Chem 29 (2011) 2541–2551.
[24] S. Anandhakumar, J. Mathiyarasu, K.L.N. Phani, V. Yegnaraman, Simultaneous determination of cadmium and lead using PEDOT/PSS modified glassy carbon electrode, AJAC 2 (2011) 470–474.
[25] H. Ashkenani, M.A. Taher, Determination of cadmium(II) using carbon paste electrodemodified with a Cd-ion imprinted polymer, Microchim. Acta (2012) 53–60.
[26] M.P. Bui, C.A. Li, K.N. Han, X.H. Pham, G.H. Seong, Electrochemical determination of cadmium and lead on pristine single-walled carbon nanotube electrodes, Anal. Sci. 28 (2012) 699–704.