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Sensitive electrochemical DNA-based biosensors for the determination of Ag$^+$ and Hg^{2+} ions and their application in analysis of amalgam filling

Maryam Ebrahimi[1] · Jahan Bakhsh Raoof[1] · Reza Ojani[1]

Abstract

In the present paper, we used single-stranded poly-T (100% thymine bases) and poly-C (100% cytosine bases) nucleic acids as DNA probes for selective and sensitive individual electrochemical determination of Hg^{2+} and Ag$^+$, respectively, on the multi-walled carbon nanotube paste electrodes (MWCNTPEs) using $[Fe(CN)_6]^{3-/4-}$ as electroactive labels. In the presence of Hg^{2+} and Ag$^+$, the probe–Hg^{2+}/Ag$^+$ interactions through T–Hg^{2+}–T and C–Ag$^+$–C complexes formation could cause the formation of a unimolecular hybridized probe. This structure of probe led to its partial depletion from electrode surface and facilitation of electron transfer between $[Fe(CN)_6]^{3-/4-}$ redox couple and electrode surface, resulting in the enhanced differential pulse voltammetry (DPV) oxidation current of $[Fe(CN)_6]^{3-/4-}$ at the probe-modified electrode surface. We applied the difference in the oxidation peak currents of $[Fe(CN)_6]^{3-/4-}$ before and after Hg^{2+}/Ag$^+$–DNA probe bonding (ΔI) for electrochemical determination of these heavy metal ions. Detection limits were 8.0×10^{-12} M and 1.0×10^{-11} M for Hg^{2+} and Ag$^+$ ions determination, respectively. The biosensors were utilized to determine the weight percent of toxic metals, i.e., silver and mercury in dental amalgam filling composition. The results of their practical applicability in analysis of the amalgam sample were satisfactory.

Keywords $[Fe(CN)_6]^{3-/4-}$ · T–Hg^{2+}–T coordination · C–Ag$^+$–C coordination · Differential potential voltammetry · Intramolecular DNA hybridization biosensor · Dental amalgam

Introduction

Nowadays, heavy metal pollution is one of the most serious environmental problems which raised major public health concerns [1, 2]. Heavy metals such as mercury and silver play an important role in industrial and biological fields [3–5]. However, their toxic nature is a major threat to the surrounding ecosystems and human beings' health [5–7]. An important and common application of these ions is in dental amalgam fillings (also known as silver fillings) composed of mercury (42–52%), silver (20–34%), tin (8–15%) copper (1–15%) and other metals (0–5%) by weight [3, 7–9]. Due to the large amount of mercury in amalgam

fillings and its adverse effects even at very low concentrations, the use of amalgam fillings in most developed countries has been banned or restricted [10–12]. However, these materials are still used extensively in dental clinics because of their durability compared to other fillings and hence mercury emission from dental amalgam remained one of the largest challenges [11–13]. Several solutions have been suggested to reduce the harmful effects of amalgam [12]. The most important methods are based on the use of amalgam separators which can diminish the amount of mercury in wastewater of dental clinics [12] and replacement of alternatives filling material instead of amalgam, such as glass ionomer, porcelain, gold and, especially composite resin, due to its white color and cost [12]. Because of the hazardous effects of mercuric and silver ions, development of an effective and valid approach for sensitive and selective determination of these toxic ions is of significance [2, 14]. Up to now, several analytical methods have been developed for the detection of Hg^{2+} and Ag$^+$ ions [2, 15]. Among them, the most common techniques used for both

✉ Jahan Bakhsh Raoof
j.raoof@umz.ac.ir

[1] Electroanalytical Chemistry Research Laboratory, Department of Analytical Chemistry, Faculty of Chemistry, University of Mazandaran, Babolsar, Iran

ions are atomic absorption spectroscopy [16, 17] and inductively coupled plasma mass spectrometry (ICPMS) [18, 19]. However, complicated methods, expensive equipment and the necessity of time-consuming sample pretreatment are the most important drawbacks of the mentioned techniques [20, 21]. Electrochemical methods can surpass these defects with advantages such as simplicity, low cost, high selectivity and sensitivity and hence have attracted extensive attention of researchers [2, 15, 22, 23].

In the recent years, the metal ion–DNA interactions have become the base of many efforts for designing of several nucleic acid-based electrochemical biosensors for determination of heavy metal ions [1, 2, 6, 7]. For example, several researchers have reported new biosensors based on cytosine (C)–Ag^+–C coordination [24, 25] and thymine (T)–Hg^{2+}–T [15, 23, 26, 27] for detection of Ag^+ and Hg^{2+}, respectively.

Various strategies have been proposed for the immobilization of nucleic acid probes on the electrode surface as an important step in designing electrochemical nucleic acid-based biosensors [28, 29] such as covalent bonding [6, 24], electrochemical adsorption [30, 31], physical adsorption [32], avidin (or streptavidin)–biotin interaction [33] and probe entrapment [34]. Although covalent bonding is the most common probe immobilization strategy, it requires complicated, costly and time-consuming modification of DNA and/or electrode surface [28, 35]. On the other hand, adsorption of probes on the electrode surface eliminates these tedious and time-consuming modifications and is the simplest method to immobilize the probe on the electrode [35, 36], especially carbonaceous electrodes, which due to the unique chemical and physical properties such as porosity and high internal surface area are considered as very suitable electrodes for this immobilization procedure of probe [35].

In our previous works [2, 37], we have also reported two individual electrochemical DNA biosensors for the determination of Hg^{2+} and Ag^+ ions, based on the differences in voltammetric signal of ethyl green dye before and after Hg^{2+}/Ag^+-induced intermolecular DNA hybridization at the surface of silver and gold nanoparticle-modified carbon paste electrodes, respectively. In the present work, first, we have investigated the ability of Hg^{2+} and Ag^+ ions to intramolecularly hybridize probes with 100% T or 100% C, respectively. Then, the changes in electrochemical signal of $[Fe(CN)_6]^{3-/4-}$ labels before and after DNA–Hg^{2+}/Ag^+ interaction were applied for the determination of these ions. Cao et al. [22] and Zhang et al. [24] have reported DNA-based biosensors for the detection of Hg^{2+} and Ag^+ ions using electrochemical signal of $[Fe(CN)_6]^{3-/4-}$ with electrochemical impedance spectroscopy (EIS) and square wave voltammetry (SWV) techniques, respectively.

However, compared with those biosensors, our present sensors have some advantages. For example, their sensors were costly and led to time-consuming processes of thiol-functionalization of probe and electrode. While we applied multi-walled carbon nanotube paste electrode (MWCNTPE) as working electrode not only to profit from useful properties of carbon paste electrode (CPE) such as simple, fast and very low cost preparation, low ohmic resistance, renewability, low background current, wide potential range and ability to introduce various modifiers during paste preparation [35, 38], but also to use electrochemical adsorption method for DNA immobilization on working electrode as a simple and economic immobilization procedure. Furthermore, the improved performances [larger linear ranges of designed biosensors in low concentration levels and lower limit of detections (LODs)] are important advantages of our proposed biosensors. The two linear ranges in both the present biosensors allowed the determination of target ions in real samples in both low and high level of concentrations. These improvements could be attributed to the use of sensitive DPV technique and the presence of CNTs in biosensor substrate matrix. Although several electrochemical sensors have been reported for the determination of mercuric ion in dental amalgam fillings [39–41], to the best of our knowledge, electrochemical sensor for the determination of silver ions in amalgam filling has not been reported. Furthermore, except our previously developed DNA-based biosensor for Hg^{2+} ions [37], no electrochemical DNA-based sensor for analysis of Hg^{2+} or Ag^+ in amalgam has been developed. In the present work, we have successfully applied the proposed biosensors for the analysis of both toxic metals in dental amalgam filling. Electrochemical impedance spectroscopy (EIS), cyclic voltammetry (CV) and DPV techniques were applied to prove sensitive Hg^{2+}/Ag^+ recognition and to design electrochemical biosensors with high sensitivity and selectivity for the detection of these ions through an intramolecular DNA hybridization process.

Experimental

Chemicals

The probe DNAs, a 20-mer oligonucleotide (5′-TTTT TTTT TTTTTTTTTTTT-3′) and a 20-mer oligonucleotide (5′-CCCC CCCC CCCCCCCCCCCC-3′), were prepared from MWG-BIOTECH (Germany). Silver (I) nitrate, $HAuCl_4$, copper (II) chloride and nickel chloride with analytical grade were supplied from Merck. Sodium citrate, lead (II) nitrate, magnesium chloride, mercuric chloride, ferric chloride, potassium ferricyanide and potassium ferrocyanide

with analytical grade were purchased from Fluka. Stock solutions of 1.0 mM metal ions were prepared in 50.0 mM Tris–HCl buffer-supporting electrolyte (pH = 7.40). MWCNT was purchased from Notrino Company. Dental amalgam fillings were supplied from local dental clinic in Babolsar, Iran, and its components, i.e., mercury and dental amalgam alloy, were purchased from Cookson (England) and Septodont (France) companies, respectively. A stock solution of 10^{-4} M of probe was prepared in TE buffer solution (10 mM Tris–HCl, 1.0 mM EDTA, pH = 8.00) and kept frozen. More diluted probe solutions were prepared using Tris–HCl buffer solution (50.0 mM, pH = 7.40) containing 20 mM NaCl.

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 MWCNTPE as working electrode, a platinum wire as the auxiliary electrode and a saturated calomel electrode (SCE) as the reference electrode. A Hitachi S-1460 field-emission scanning electron microscope using an AC voltage of 15 kV was employed to characterize the morphology of carbon nanotubes, and an Ion Analyzer 250 Corning pH meter was used for pH measurements.

Procedure

Working electrode preparation for electrochemical studies

CPE was prepared according to the method reported in our previous works [2, 37]. For preparation of MWCNTPE, graphite powder, paraffin oil and MWCNT were thoroughly mixed in a ratio of 60:33:7% (w/w) [42]. Subsequent steps for the preparation of this electrode were similar to the preparation of conventional CPE. Figure 1A shows field-emission scanning electron microscopy (FE-SEM) image of multi-walled carbon nanotubes used for the preparation of modified electrode.

For both biosensors, first, the surface of working electrode was electrochemically activated under optimized potential of 1.80 V vs. SCE [2, 37] in 0.50 M acetate buffer solution (pH 4.8) containing 20 mM NaCl without stirring for 5 min. Then, the immobilization of DNA probe on the activated electrode was performed by applying a potential of 0.50 V to the electrodes for 5 min in a solution of 10^{-6} M DNA with stirring, followed by rinsing the electrodes with sterilized and deionized water.

Intramolecular hybridization of probe

In both biosensors, hybridization occurred by incubation of corresponding probe-modified working electrodes in buffer solution of 0.05 M Tris–HCl (pH = 7.40) and 20 mM NaCl containing different concentrations of Hg^{2+} or Ag^+ during 600 s. Then the electrodes were rinsed with sterilized and deionized water. Selectivity experiments of present biosensors were also conducted by the same procedure.

Electrochemical measurements

To demonstrate the feasibility of electrochemical detection of Hg^{2+} and Ag^+ ions, EIS, DPV and CV techniques were employed. For this purpose, the probe-modified electrode corresponding to each Hg^{2+} or Ag^+ ion was incubated in a buffer solution of 0.05 M Tris–HCl (pH = 7.40) and 20 mM of NaCl containing different concentrations of Hg^{2+} or Ag^+ for 600 s and then CV curves, DPV curves and Nyquist plots of electrodes were recorded in a buffer solution of 0.01 M Tris–HCl, (pH 7.0) and 0.1 M KCl containing 1.0 mM $K_3[Fe(CN)_6]/K_4[(CN)_6]$. Cyclic voltammetry was

Fig. 1 **a** FE-SEM image of multi-walled carbon nanotubes and **b** applied alloy and mercury for dental amalgam filling preparation
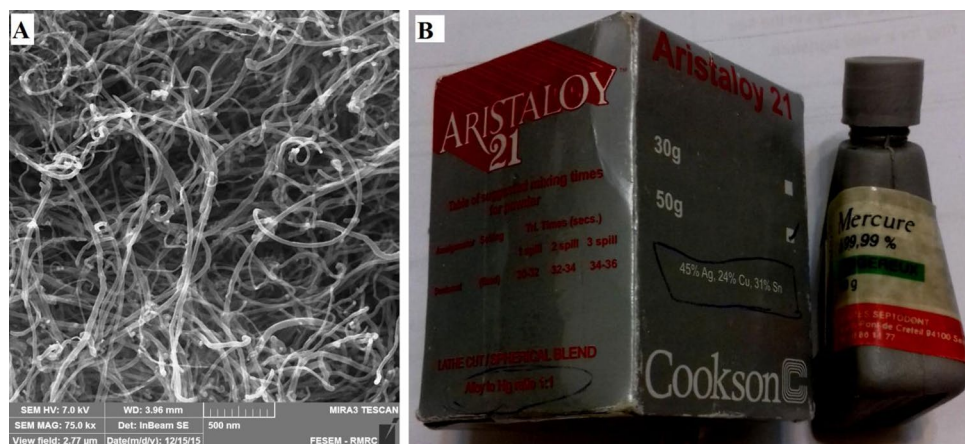
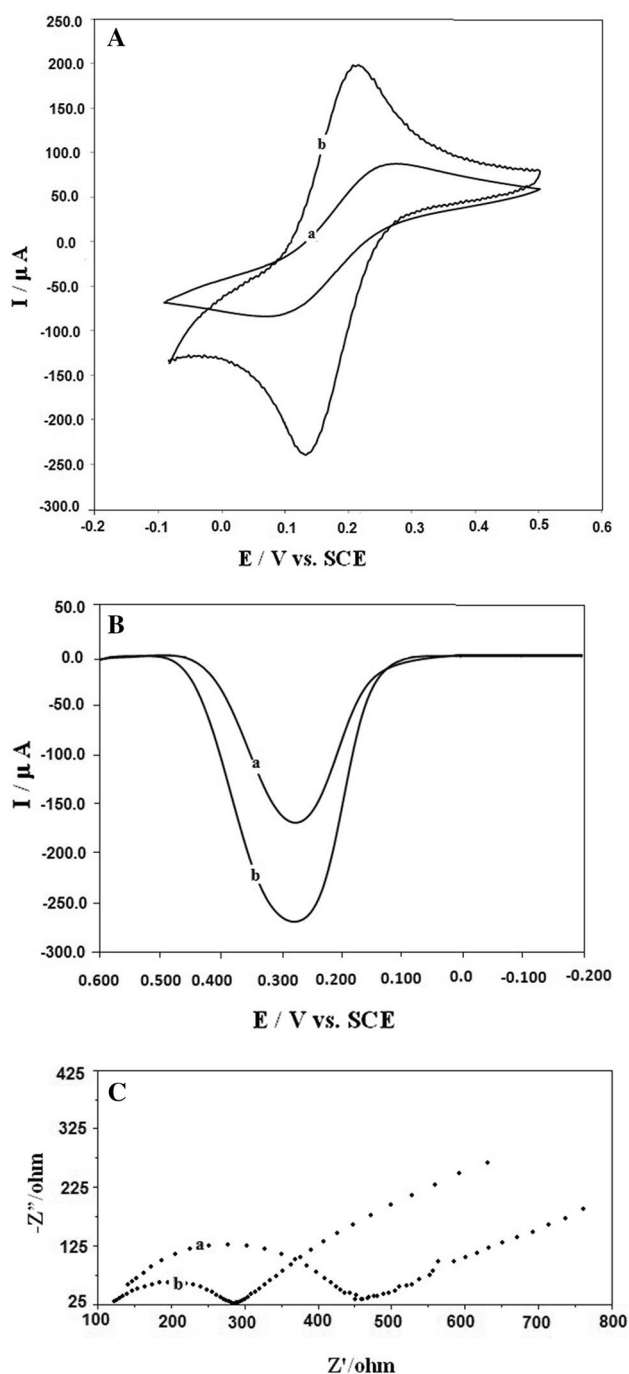**Fig. 2 A** Cyclic voltammograms, **B** differential pulse voltammograms and **C** Nyquist plots of 1.0 mM $[Fe(CN)_6]^{3-/4-}$ in 10 mM Tris–HCl buffer solution (pH 7.0) at (a) bare CPE and (b) MWCNTPE

conducted at a scan rate of 20 mV s^{-1} in the potential range of −0.2 to 0.6 V vs. SCE, while differential pulse voltammetry was recorded at modulation amplitude of 50 mV, step potential of 10 mV, modulation time of 0.05 s and interval time of 0.5 s in the potential range of 0.6 to −0.2 V vs. SCE.

Preparation of dental amalgam filling as real sample

To avoid the emission of mercury vapor, dental amalgam was put in the tight container containing the X-ray fixer [43, 44]. Dental amalgam sample was prepared according to the method reported in the literature [45]. 0.35 g of amalgam was accurately weighed and dissolved in 60% nitric acid. The solution was placed in steam bath until complete disintegration of the amalgam. The known volume of obtained solution was then diluted to a total volume of 10 ml by adding Tris–HCl buffer and the pH of the solution was adjusted to 7.4. The remaining undissolved white precipitate was meta-stannic acid which is related to tin metal. All other metals went into solution under this condition. The solution was filtered and used for voltammetric measurement.

Results and discussion

Electrochemical investigation of working electrode surfaces

As can be seen in Fig. 2, the results of the cyclic voltammograms, differential pulse voltammograms and Nyquist plots of $[Fe(CN)_6]^{3-/4-}$ at bare CPE and MWCNTPE, clearly indicated the improved surface properties of the MWCNTPE compared to CPE. As compared to CPE, an enhancement in voltammetric peak currents and a decrease in peak separation of $[Fe(CN)_6]^{3-/4-}$ at the surface of MWCNTPE confirmed the increased conductivity of the electrode surface due to the presence of MWCNT (Fig. 2A, B). Moreover, a significant decrease in electron transfer resistance, R_{ct}, of $[Fe(CN)_6]^{3-/4-}$ redox couple at the MWCNTPE (Fig. 2C) clearly shows the improved surface properties of the modified electrode.

Electrochemical determination of Hg²⁺ and Ag⁺

In both biosensors, due to the specific interaction with Hg^{2+} or Ag^+, single-strand DNA (ss-DNA), as the probe, undergoes an intramolecular hybridization through T–Hg^{2+}–T or C–Ag^+–C complex formation, leading to partial depletion of DNA probes from the crowded surface of probe-modified working electrodes because of the repulsion force between probes [46]. EIS, CV and DPV techniques were applied to illustrate this phenomenon. Figure 3A, A' shows the Nyquist plots of MWCNTPE and probe-modified MWCNTPE before and after incubation in different concentrations of Hg^{2+} or Ag^+ solutions, respectively. As can be seen in EIS studies of both biosensors (Fig. 3A, A'), upon DNA immobilization at the surface of working electrodes (curve

b), the semicircle associated with electron transfer resistance of $[Fe(CN)_6]^{3-/4-}$ significantly increased. However, after incubation of probe-modified electrode in 1.0 nM Hg^{2+} or Ag^+ solution (curve c), the charge transfer resistance decreased. This can be attributed to the decrease in negative charge of the phosphate group of probe on electrode surface due to the present of positive ions of mercury or silver. In addition, releasing of some probes from probe-modified electrode decreased the repulsion between DNA probe and $[Fe(CN)_6]^{3-/4-}$. Both these reasons led to a

decrease in the R_{ct} value of Nyquist plot proportional to the increase of Hg^{2+} or Ag^+ concentration. For further investigation, CV (Fig. 3B, B') and DPV (Fig. 3C, C') measurements in both biosensors were also performed. Consistent with the EIS results, both corresponding probe-modified electrodes (curve b in Fig. 3B, B', C, C') exhibited a remarkably lower peak currents of $[Fe(CN)_6]^{3-/4-}$ than that of the bare electrode (curve a). The repulsion force between phosphate group of DNA and $[Fe(CN)_6]^{3-/4-}$ indicator was responsible for the decrease in electrochemical response of
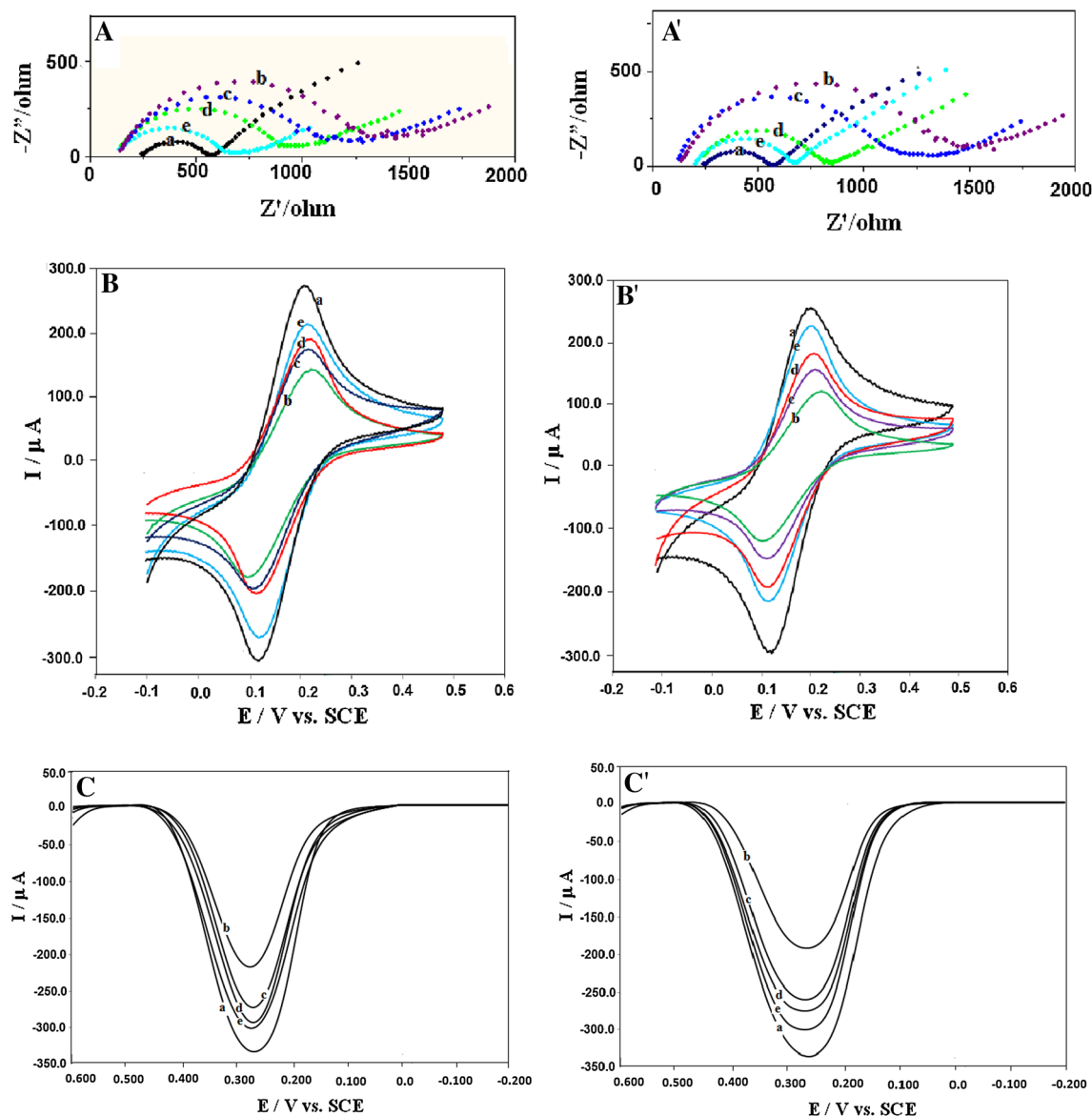

Fig. 3 **A** and **A'** Nyquist plots, **B** and **B'** cyclic voltammograms and (**C** and **C'**)) differential pulse voltammograms of 1.0 mM $[Fe(CN)_6]^{3-/4-}$ in 10 mM Tris–HCl buffer solution (pH 7.0) at different modified electrodes: MWCNTPE (a), probe modified MWCNTPE before (b) and after incubation in 0.05 M Tris–HCl buffer solution, pH 7.40 and 20 mM of NaCl containing (c) 1.0 nM,(d) 0.1 μM and (e) 10.0 μM of Hg^{2+}/Ag^+ during 600 s. (**A**, **B** and **C** at Ag^+ sensor; **A'**, **B'** and **C'** at Hg^{2+} sensor)

$[Fe(CN)_6]^{3-/4-}$ at the surface of probe-modified electrodes. However, after incubation for 600 s, the peak currents of both biosensors significantly increased proportional to the concentration of Hg^{2+} or Ag^+ in the solution (curves c–e in Fig. 3B, B', C, C').

The above results demonstrated that electrochemical response of probe-modified electrodes to mercuric and silver ions relied on the changes in electron transfer rates of $[Fe(CN)_6]^{3-/4-}$ due to the intramolecular DNA hybridization at the electrode surface. Hence, The oxidative DPV responses of $[Fe(CN)_6]^{3-/4-}$ at the working electrodes before and after Hg^{2+}- or Ag^+-induced probe intramolecular hybridization (ΔI) were employed for quantitative determination of these metal ions.

Effect of incubation time of probe-modified electrode in Hg^{2+} and Ag^+ solution

The influence of incubation time of each probe-modified electrode on the change in voltammetric response of $[Fe(CN)_6]^{3-/4-}$ (ΔI) was investigated by measuring the current responses before and after incubation in Tris buffer
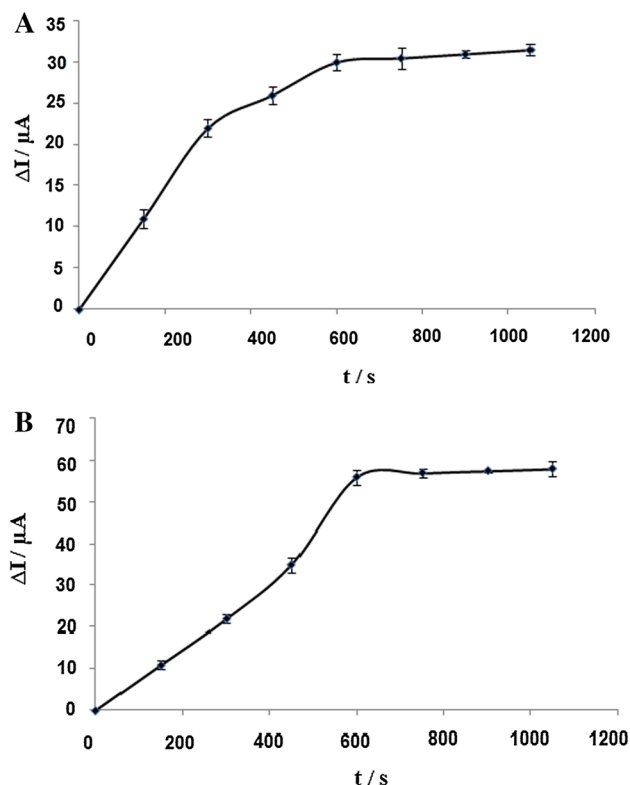

Fig. 4 The effect of Hg^{2+}/Ag^+-probe binding time on DPV response of 1.0 mM $[Fe(CN)_6]^{3-/4-}$ to 1.0 nM Hg^{2+}/Ag^+ at **a** Ag^+ sensor and **b** Hg^{2+} sensor. Error bars show the standard deviations of measurements taken from three independent experiments

solution containing 1.0 nM Hg^{2+} or Ag^+. As shown in Fig. 4, in both biosensors, ΔI gradually increased as the incubation time increased from 0 to about 600 s and maintained nearly constant during 600–1050s. Therefore, an incubation time of 600 s was suggested as optimum time for the incubation of probe-modified electrodes in Hg^{2+} or Ag^+ solution for all analyses of these metal ions.

DPV determination of Hg^{2+} and Ag^+

For determination of Hg^{2+} or Ag^+, the difference in DPV anodic peak currents (ΔI) of 1.0 mM $[Fe(CN)_6]^{3-/4-}$ before and after incubation of corresponding probe-modified electrodes in 0.05 M Tris–HCl buffer solution (pH = 7.40) and 20 mM of NaCl containing different concentrations of Hg^{2+} or Ag^+ was obtained. As can be seen in Fig. 5A, A', the anodic peak current, I, was gradually increased with the increase of the concentrations of Hg^{2+} or Ag^+. Figure 5B, B' shows the relationship between ΔI and the concentrations of Hg^{2+} or Ag^+ at the surfaces of corresponding biosensors. As shown in these figures, in both biosensors, ΔI is linearly dependent on Hg^{2+} or Ag^+ over concentration ranges from 2.0×10^{-11} M to 1.0×10^{-9} M and 1.0×10^{-5} to 1.0×10^{-3} M. The detection limits (based on a three signal-to-noise ratio) calculated were 8.0×10^{-12} M and 1.0×10^{-11} M for Hg^{2+} biosensor and Ag^+ biosensor, respectively.

Selectivity of oligonucleotides-based sensors

The selectivity of Hg^{2+} biosensor was tested through substituting Hg^{2+} in the incubation buffer with other heavy metal ions, including Cu^{2+}, Ag^+, Mg^{2+}, Ni^{2+} and Fe^{2+}. Also, the selectivity of Ag^+ biosensor was investigated through other heavy metal ions, including Cu^{2+}, Hg^{2+}, Pb^{2+}, Fe^{2+} and Ni^{2+} at the surface of the biosensor. As shown in Fig. 6, the DPV responses of $[Fe(CN)_6]^{3-/4-}$ solution at both DNA-modified working electrodes did not show remarkable change after incubation in these interfering metal ion solution even at a relatively high concentration (10^{-5} M), but mercuric or silver ions could change the signal of label on the surface of corresponding electrodes. The diminishing of negative charge of the probe-modified electrode in the presence of positive ions was responsible for the negligible increase in DPV response for $[Fe(CN)_6]^{3-/4-}$ after incubation in the interfering ion solution. These results indicated a satisfactory selectivity of both the developed biosensors for Hg^{2+} and Ag^+ determinations.

Analysis of real samples

The analysis of Hg^{2+} and Ag^+ ions in tap water was performed to demonstrate the practical usage of the
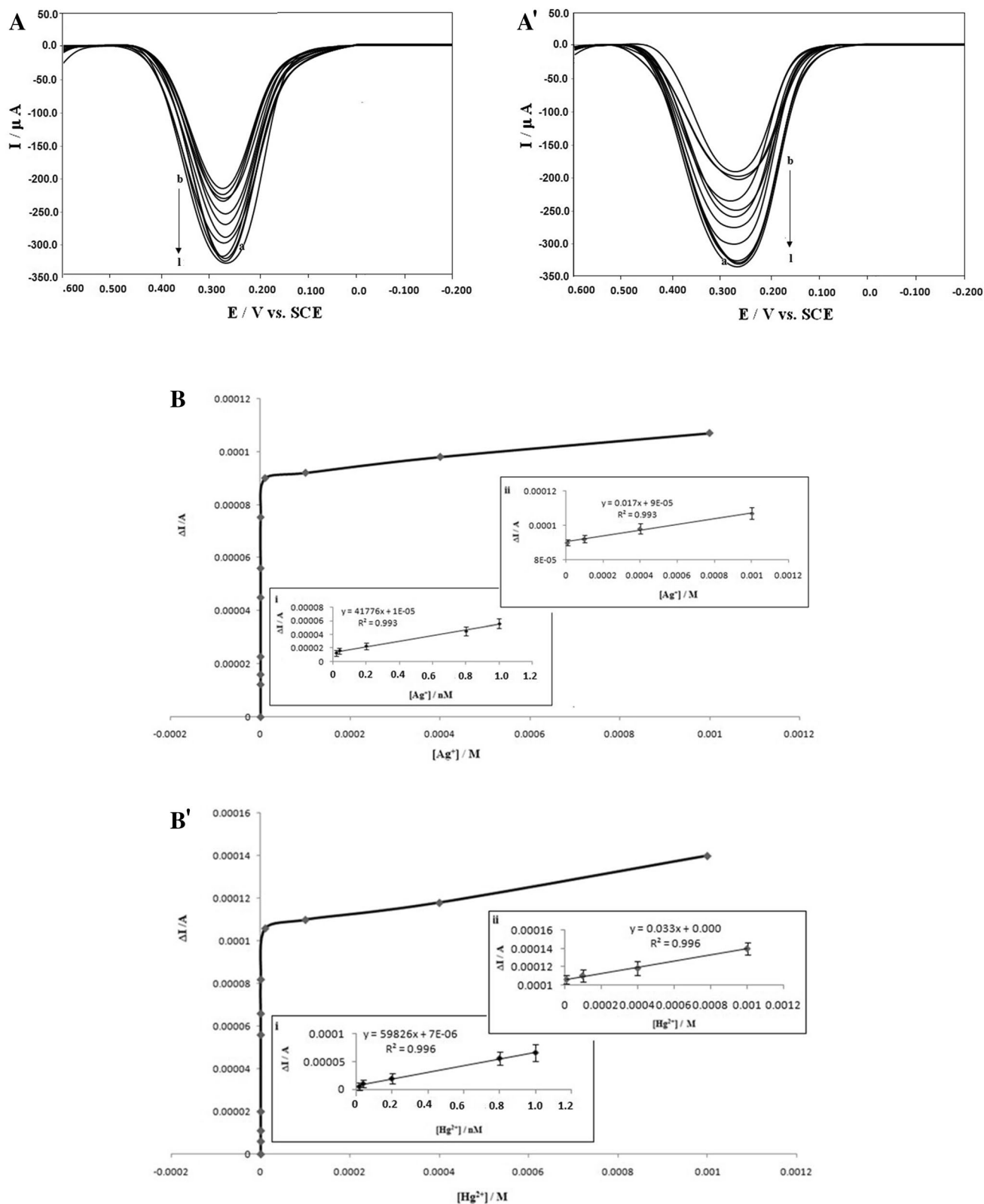
Fig. 5 Differential pulse voltammograms of 1.0 mM $[Fe(CN)_6]^{3-/4-}$ solution at MWCNTPE (a) and probe-modified MWCNTPE before (b) and after immersing in different concentrations of Hg^{2+}/Ag^+ from 20.0 pM to 1.0 mM (c–l) at (**A**) Ag^+ biosensor and (**A'**) Hg^{2+} biosensor. The plot of the relationship between ΔI and different concentrations of Hg^{2+}/Ag^+ from 0.0 to 1.0 mM at (**B**) Ag^+ biosensor and (**B'**) Hg^{2+} biosensor, inset: related calibration plot of Ag^+ (**B**) and (**B'**) Hg^{2+} concentration in the range of (1) 20.0 pM–1.0 nM and (2) 0.01–1.0 mM. Error bars show the standard deviations of measurements taken from three independent experiments
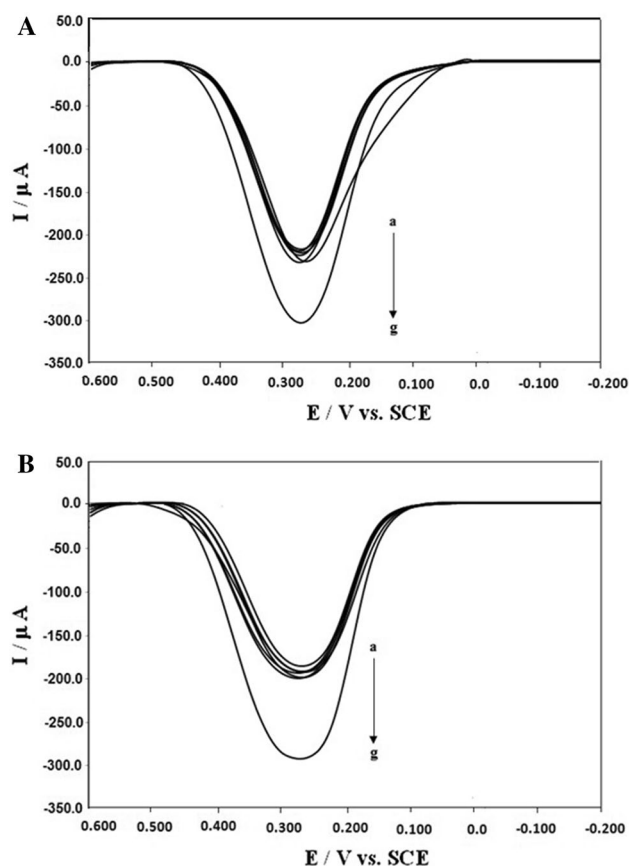
Fig. 6 Differential pulse voltammograms of 1.0 mM $[Fe(CN)_6]^{3-/4-}$ solution at DNA-modified electrode before (a) and after immersing in 0.01 mM different interfering metal ions, including Ni^{2+} (b), Cu^{2+} (c), Hg^{2+} (d), Fe^{2+} (e), Pb^{2+} (f) and target metal ion Ag^+ (g) at (**A**) Ag^+ biosensor and including Ni^{2+} (b), Cu^{2+} (c), Ag^+ (d), Fe^{2+} (e), Mg^{2+} (f) and target metal ion Hg^{2+} (g) at (**B**) Hg^{2+} biosensor

Table 1 The ability of proposed biosensors for electrochemical determination of Hg^{2+}/Ag^+ in spiked tap water samples

Hg^{2+}/Ag^+ added/M	Hg^{2+}/Ag^+ found/M[a]	Recovery (%)	Biosensor
0.5×10^{-9}	$0.48\ (\pm 0.01) \times 10^{-9}$	96.0	Ag^+ biosensor
0.5×10^{-9}	$0.53\ (\pm 0.03) \times 10^{-9}$	106.0	Hg^{2+} biosensor
1.0×10^{-9}	$0.92\ (\pm 0.03) \times 10^{-9}$	92.0	Ag^+ biosensor
1.0×10^{-9}	$0.95\ (\pm 0.02) \times 10^{-9}$	95.0	Hg^{2+} biosensor
10.0×10^{-6}	$10.4\ (\pm 0.3) \times 10^{-6}$	104.0	Ag^+ biosensor
10.0×10^{-6}	$9.8\ (\pm 0.4) \times 10^{-6}$	98.0	Hg^{2+} biosensor

From drinking water system of Babolsar, Iran

[a]Mean value $\pm$ SD (standard deviation), $n = 5$

biosensors. The tap water samples were spiked with 0.5, 1.0 nM and 10 μM of Hg^{2+} or Ag^+ ion. In both biosensors, the recovery values were satisfactory and results showed the utility of the proposed method for the detection of both low and high concentration levels of these ions in real water samples (Table 1). The obtained results are comparable with our previous works [2, 37].

The designed biosensors were also used in the detection of mercury and silver, and in the determination of their weight percentage (Wt%) in dental amalgam filling. As shown in Fig. 1B, the alloy of dental amalgam which was prepared from local dental clinic was composed of Ag (45%), Cu (24%) and Sn (31%), and alloy to mercury ratio was 1:1. Thus, the resulting dental amalgam filling should be composed of approximately 50% Hg and 22.5% Ag by weight. The amalgam solution for analysis was prepared as described in "Preparation of dental amalgam filling as real sample". The voltammetric analysis of prepared amalgam solution exhibited a mercury ion concentration of 8.6×10^{-4} M which was equivalent to 49.14% of mercury in amalgam. For silver, these values were evaluated to be 7.13×10^{-4} M and 22%, respectively.

The analytical performances of some electrochemical DNA-based biosensors for silver and mercuric ion determination are shown in Table 2. As can be seen, present biosensors indicated satisfactory analytical performance.

Conclusion

Rapid and simple electrochemical DNA biosensors for the determination of Hg^{2+} and Ag^+ were developed. The intramolecular hybridization of oligonucleotide through T– Hg^{2+}–T and C– Ag^+–C coordination led to the significant decrease in charge transfer resistance on the corresponding probe-modified electrodes and consequently increase in the DPV anodic peak currents of $[Fe(CN)_6]^{3-/4-}$. The electrochemical determination of Hg^{2+} and Ag^+ relied on the difference in the values of the DPV peak currents of $[Fe(CN)_6]^{3-/4-}$ before and after Hg^{2+}- or Ag^+-induced DNA intramolecular hybridization (ΔI) which was linearly related to Hg^{2+}/Ag^+ concentrations with low LODs. This decrease in charge transfer resistance of the probe-modified electrodes due to probe–Hg^{2+}/Ag^+ binding was also investigated using EIS and CV techniques. Analysis of these toxic metals in the dental amalgam was performed using these designed biosensors and the results proved the accuracy of developed systems.

Table 2 Comparison of the electrochemical biosensors for the detection of mercuric and silver ions

Electrode	Determined ion	Determination technique	LOD	Linear range(s)	References
Cu2O@NCs[a]-modified Au	Hg^{2+}	EIS	0.15 nM	1–100 nM	[15]
CS@3D–rGO@DNA[b]-modified Au	Hg^{2+}	EIS	0.16 nM	0.1–10 nM	[23]
SWCNT-modified Au	Ag^+	SWV	1.5 nM	2–100 nM	[24]
Au	Hg^{2+}	DPV	0.6 zM	5 zM–55 pM	[26]
Graphene oxide-modified Au	Hg^{2+}	DPV	0.12 nM	0.5–50 nM	[27]
MWCNT-modified Au	Ag^+	DPV	1.3 nM	10–60 nM	[47]
MWCNTPE	Hg^{2+}	DPV	8 pM	20 pM–1.0 nM 0.01–1 mM	Present paper
MWCNTPE	Ag^+	DPV	10 pM	20 pM–1.0 nM 0.01–1 mM	Present paper

[a]Cuprous oxide and nano-chitosan composites

[b]DNA-modified nanocomposite of three-dimensional reduced graphene oxide and chitosan particles

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