An electrochemical DNA biosensor for trace amounts of mercury ion quantification

Ferdaous Maâtouk, Mouna Maâtouk, Karima Bekir, Houcine Barhoumi, Abderrazak Maaref and Hedi Ben Mansour

ABSTRACT

In this work we report the development of an electrochemical DNA biosensor with high sensitivity for mercury ion detection. A new matrix based on gold nanoparticles (AuNPs)-glutathione (GSH)/cysteine was investigated. The interaction between DNA oligonucleotides and Hg^{2+} ions followed by the formation of Thymine–Hg^{2+}–Thymine (T–Hg^{2+}–T) structures was quantified using different electrochemical methods. It has been shown that the electrochemical impedance spectroscopy (EIS) measurements and the differential pulse voltammetry (DPV) confirmed the specific interaction between the oligonucleotide receptor layer and the Hg^{2+} ions. Besides, the developed sensor exhibited high sensitivity towards mercury among some examined metal ions such as Pb^{2+}, Cu^{2+} and Cd^{2+}. As a result, a high electrochemical response and low detection limit of 50 pM were estimated in the case of Hg^{2+} ions. The developed DNA biosensor was applied successfully to the determination of Hg^{2+}ions in wastewater samples.

Key words | DNA biosensor, DPV, EIS, gold nanoparticles, Hg^{2+} detection

Ferdaous Maâtouk
Houcine Barhoumi
Abderrazak Maaref
Laboratory of Interfaces and Advanced Materials, Faculty of Sciences,
University of Monastir,
Monastir, Tunisia

Mouna Maâtouk
Research Unit 03/UR/07 'Autoimmunity and Allergy', Faculty of Pharmacy,
University of Monastir,
Monastir, Tunisia

Karima Bekir
Laboratory of Analysis, Treatment and Valorisation of Pollutants of Environment and of Products, Faculty of Pharmacy,
University of Monastir,
Monastir, Tunisia

Hedi Ben Mansour (corresponding author)
Research Unit of Analysis and Process Applied to the Environmental – APAE Higher Institute of Applied Sciences and Technology Mahdia,
University of Monastir,
Monastir, Tunisia
E-mail: *hdbenmansour@gmail.com*

INTRODUCTION

The development of electrochemical sensors for the recognition of heavy metal ions is very important due to their fundamental role in biological, environmental and chemical processes (Prodi *et al.* 2000). Therefore, many sensor types have been used for environmental analysis, industrial quality control and clinical diagnostics (Andreescu & Sadik 2004). Among various types of sensors, electrochemical receptor layers, such as enzymes, nucleic acids, and antibodies, came to a special prominence. Recently, the interactions of heavy metal ions, in particular mercury, with nucleic acids have been studied, due to the possible toxicity and cancerogenicity of these ions (Anastassopoulou 2003). Furthermore, the mercury ion is a highly toxic environmental pollutant and has posed a serious threat to human health (Bolger & Schwetz 2002). Mercury can also affect many different areas of the brain and their associated functions, resulting in symptoms such as tremors, vision problems, deafness and loss of muscle coordination, sensation and memory (Stern 2005). In addition to the brain, inorganic mercury can damage the heart, kidney, stomach, and intestines (Zheng *et al.* 2003; Mutter *et al.* 2005; Wojcik *et al.* 2006). Due to their serious harm, the binding of metal ions to nucleic acids is used for the construction of biosensors for the detection of heavy metal ions (Liu *et al.* 2009). Recently, it has been reported that there is specific and strong coordination between $Hg^{2+}$and the two DNA thymine bases (T) to form a mediated base pair (T–Hg^{2+}–T) (Ono & Togashi 2004). Classical methods for mercury detection, such as atomic absorption spectroscopy (Li *et al.* 2006),

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colorimetric (Lin *et al.* 2010) and fluorescence (Chiang *et al.* 2008) are widely used. However, electrochemical methods have received particular attention due to their high sensitivity and selectivity, such as differential pulse stripping analysis (Yantasee *et al.* 2003; Wang *et al.* 2007), electrochemical impedance spectroscopy (EIS) (Lin *et al.* 2011) and square wave voltammetry (Jiang *et al.* 2015). In this study, we describe an electrochemical biosensor with high sensitivity and selectivity for Hg^{2+} detection based on DNA oligonucleotides. This probe report based on a DNA-AuNPs-glutathione/cysteine/Au modified electrode to capture mercury (II) ions. First, the interaction between thymine and mercury ions was evaluated by the EIS. Then, the electrochemical reduction of the surface provides a readout signal for the quantitative detection of Hg^{2+}. We also demonstrate that the sensitivity of this Hg^{2+} sensor could be significantly improved with the DNA oligonucleotides immobilized by using gold nanoparticles (AuNPs).

EXPERIMENTAL

Reagents

Cysteine (95%), glutaraldehyde (Glu, 25%), glutathione (GSH reduced form), hexacyanoferrate (II/III), gold colloid solution (20 nm), N-hydroxysuccinimide (NHS), phosphate buffered saline (PBS), trishydroxymethylaminomethane and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) were purchased from Sigma Aldrich (St. Louis, USA). $CuCl_2.2H_2O$, $3CdSO_4.8H_2O$, $Pb(NO_3)$ and $HgCl_2$ were purchased from Fluka-Chemika (Buchs, Switzerland). The DNA oligonucleotide was purchased from Bioneer Oligo Synthesis Report (Bioneer, BPS Bioscience, Biotools – Tunisia). The sequence is 5′-NH_2-$(CH_2)_6$-ATTTGTTCATGCCT-3′. All electrolytic solutions were prepared using ultra-pure water.

Apparatus

The electrochemical measurements were performed using an Autolab (PGSTAT 302 N, Eco Chemie). The measurements were made using a conventional three electrode electrochemical system consisting of a gold electrode, a platinum wire auxiliary electrode and Ag/AgCl/KCl reference electrode. The geometrical area of the working electrode was 0.031 cm^2. Impedance measurements were performed in the frequency range from 0.1 to 100,000 Hz. All electrochemical measurements were performed in a Faraday cage at room temperature (25 °C) to avoid any stray light or electrical perturbation from external sources.

Working electrode pretreatment and SAM formation

To obtain a clean, activated and reproducible electrochemical surface, the gold electrode was mechanically polished with alumina slurry followed by rinsing with distilled water and sonication in acetone for 2 min. After mechanical cleaning, the gold electrode was treated using a chemical process by immersion in a piranha solution (H_2SO_4/H_2O_2, 1:3 v/v) for 1 min and rinsed with ultrapure water. Afterwards, the clean gold electrode was immersed into 5 mM cysteine in 0.1 M PBS solution at pH 7.4 for 2 h. Then, the modified electrode was rinsed with ultrapure water to remove non-covalent attached cysteine molecules.

Immobilization of the probe DNA on the Au/cysteine modified electrode

The Au-cysteine modified electrode was activated in an atmosphere saturated with glutaraldehyde vapor for 1 h. The DNA probe was immobilized on the Au/cysteine modified electrode via two immobilization strategies:

1. In the first case, the DNA senor was fabricated via an adsorption process. The Au/Cysteine electrode was immersed in DNA probe solution for 1 h. Then, the modified electrode surface was washed with PBS solution for desorbing the non-attached DNA probes. Figure 1(a) shows the procedure of the preparation of the DNA/cysteine/Au modified electrode.
2. In the second case, the Au/Cysteine electrode was immersed for 1 h into a mixture of glutathione-AuNPs (1:1) solution previously stored for 16 h in a refrigerator. Then, the modified electrode was activated for 1 h in 1:1 (v/v) EDC/NHS mixture (10 mM EDC and 10 mM NHS, pH 5). Finally, the modified electrode was immersed in the DNA probe solution for 1 h and then washed with PBS solution to remove the unbound DNA. Figure 1(b) shows the preparation steps of the DNA biosensor based on the Au nanoparticles.

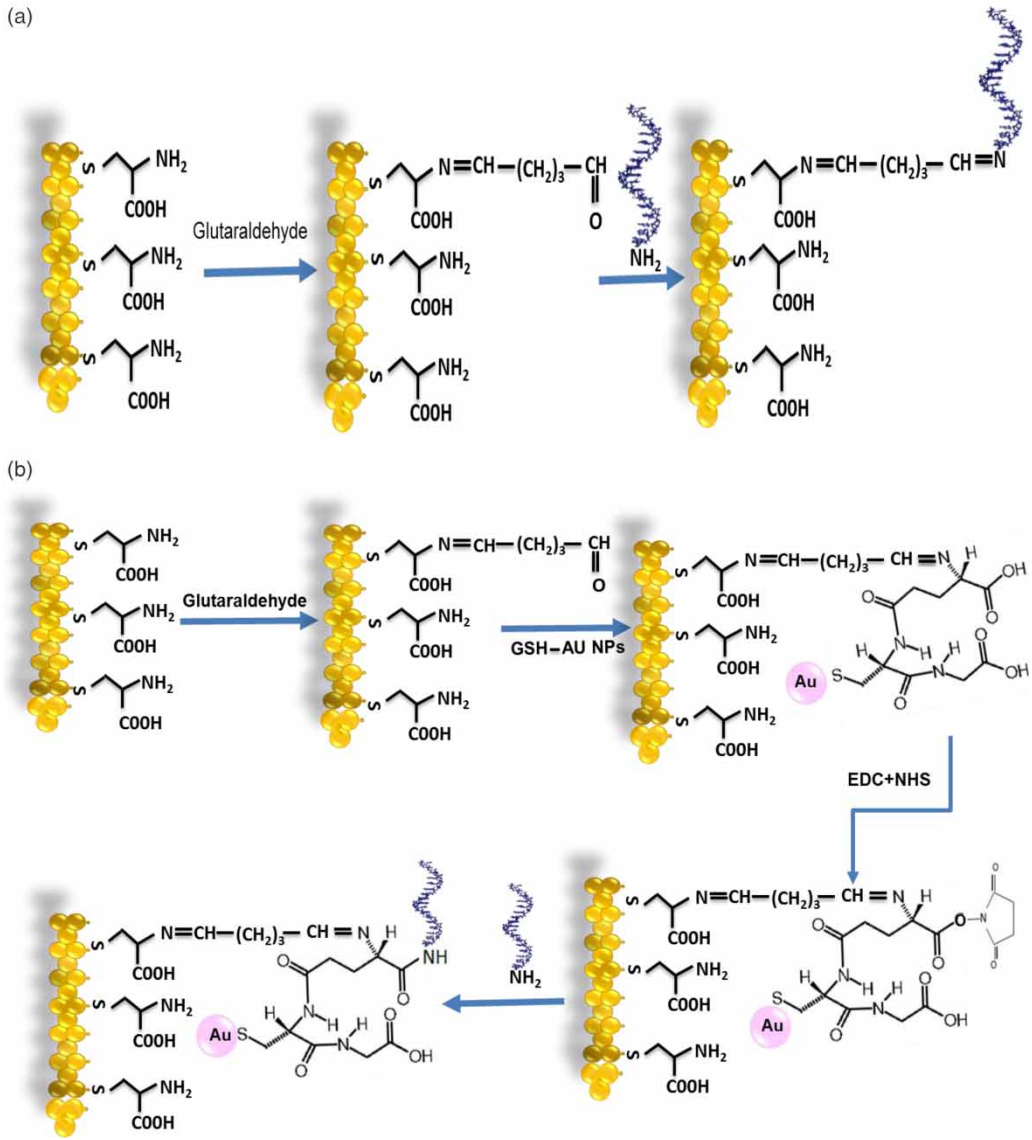

Figure 1 | The construction steps and design mechanism of the electrochemical sensor based on (a) Au/cysteine/DNA and (b) Au/cysteine/glutathione-Au NPs/DNA electrodes.

RESULTS AND DISCUSSION

Electrochemical characterization of the modified electrode

The immobilization of DNA on Au/cysteine using two approaches was characterized by cyclic voltammetry and impedance spectroscopy measurements in 0.1 M PBS solution containing 5 mM of the $[Fe(CN)_6]^{3-/4-}$ as redox agent at the scan rate of 50 mVs^{-1}. Figure 2(a) shows the CVs behavior of the bare and the modified gold electrode. The self-assembly of the cysteine monolayer on the electrode surface induces an increase of the peak current, explaining the diffusion controlled process for the $[Fe(CN)_6]^{3-/4-}$ redox agent. The significant change in current after DNA immobilization explains that the DNA probe has been successfully immobilized on the modified electrode surface. Thus, the decrease in current can be assigned to the electrostatic repulsion interaction between $[Fe(CN)_6]^{3-/4-}$and the negative charge phosphate backbone of the DNA probe.

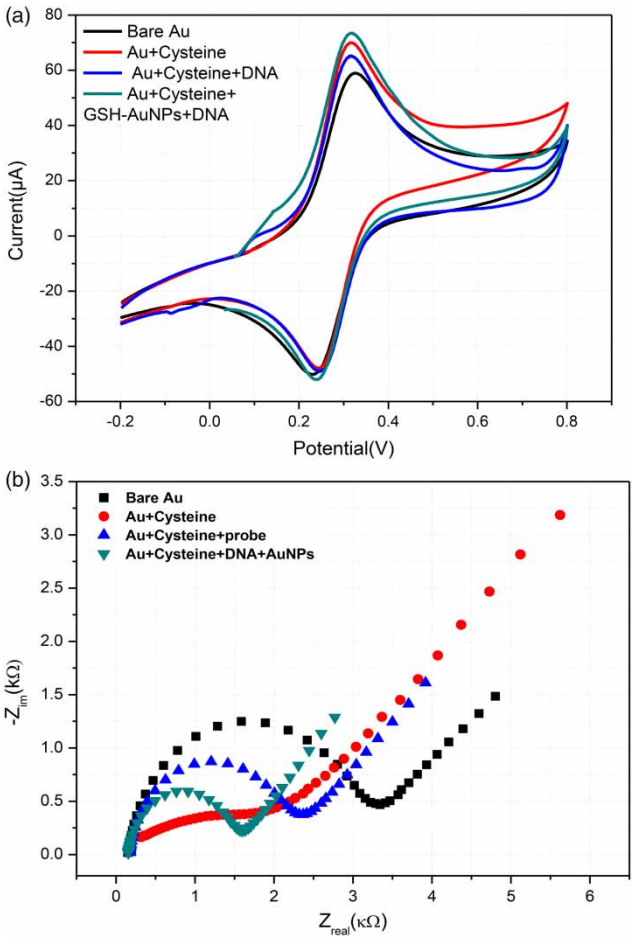

Figure 2 | (a) Cyclic voltammograms of Au bare electrode, Au/cysteine, Au/cysteine/DNA, Au/cysteine/GSH-Au NPs/DNA modified electrodes (scan rate: 50 mV/s) and (b) Nyquist plots for bare gold electrode, Au/cysteine, Au/cysteine/DNA and Au/cysteine/GSH-Au NPs/DNA modified electrode in 5 mM of ferri/ferrocyanide redox couple.

Furthermore, the incorporation of the gold nanoparticles in the sensing matrix enhanced the electrochemical performances of the DNA sensor by increasing the effective electrode surface area. This result improves the rate of electron transfer reaction, which was evidenced by an increase in the voltammetric responses of the $[Fe(CN)_6]^{3-/4-}$ agent in comparison with the probe/cysteine modified electrode (Du *et al.* 2009). In addition, the EIS method was used to explain and gain more information about the modified solid-liquid interface. Figure 2(b) shows the Nyquist plots, which represent the control of the charge transfer during the modification of the electrode surface. The lower semicircle diameter was obtained in the case of the DNA/GSH-Au NPS/cysteine modified electrode in comparison with the DNA/cysteine/Au modified electrode. This result demonstrates that the charge transfer resistance (R_{ct}) decreases in the presence of the gold nanoparticles, which enhances the specific surface and incidentally improves the electron transfer mechanism.

Electrochemical sensing of Hg^{2+} ions

The electrochemical detection of Hg^{2+} ions was performed by incubating the DNA modified electrode in different concentrations of Hg^{2+} ions dissolved in Tris-HCl (50 mmol.L^{-1}) for 1 h. The pH of the mercury solutions was adjusted to 6 in order to inhibit the formation of HgO species. After the immersion step, the modified electrode was carefully washed with Tris-HCl and the electrochemical measurements were conducted. In the presence of Hg^{2+} ions, the specific coordination between Hg^{2+} and thymine bases can change parallel DNA from linear to hairpin structures. This structure enhances the steric and coulombic interaction between adjacent DNA sequences (Liu *et al.* 2008). The electrochemical response of the modified electrode towards Hg^{2+} ions was evaluated using the differential pulse voltammetry (DPV) method. Thus, the reduction of surface confined Hg^{2+} ions was investigated. Figure 3 shows the voltammograms corresponding to the DNA/cysteine modified electrode upon incubating with various concentrations of Hg^{2+} from 1 nM to 0.1 μM. As can be seen in Figure 3,

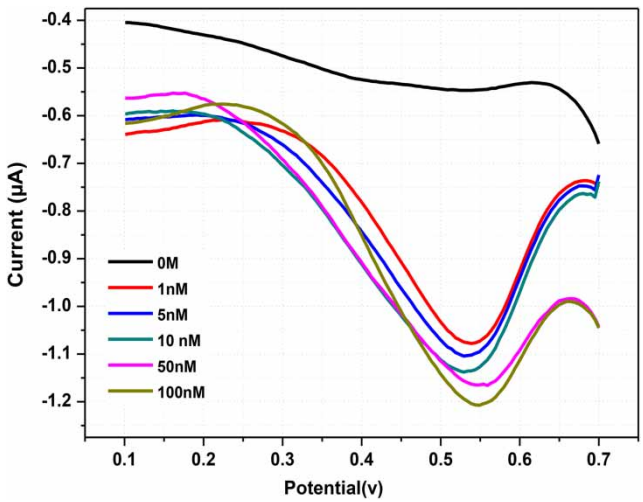

Figure 3 | DPV spectra of cysteine/DNA modified electrode for different concentrations of Hg^{2+}.

a reduction peak at 0.55 V (vs Ag/AgCl) was obtained, which can be attributed to the reduction potential of the surface confined Hg^{2+} (Zhu *et al.* 2009). Consequently, we observe that the peak reduction currents are intensified with an increase of the Hg^{2+} concentration.

Electrochemical sensing for Mercury (II) ions using gold nanoparticles

The electroanalytical performances of the T–Hg^{2+}–T based on biosensor were evaluated for different Hg^{2+}ion concentrations using the DNA/GSH-Au NPS/cysteine modified electrode. Figure 4(a) represents the DPV voltammograms

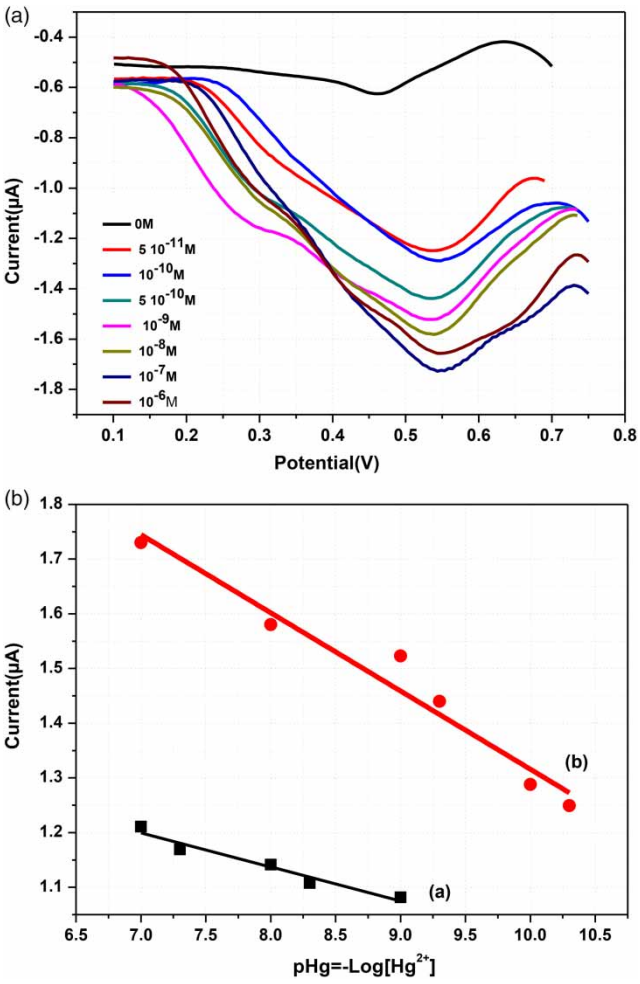

Figure 4 | (a) Differential pulse voltammograms of cysteine/GSH-Au NPs/DNA modified electrode for different concentration of Hg^{2+}. (b) Calibration curves for (a) cysteine/DNA and (b) cysteine/GSH-Au NPs/DNA modified electrode.

obtained before and after incubation of the DNA biosensor in Hg^{2+} solution in which the concentration varies from 50 pM to 1 μM. By plotting the concentration of Hg^{2+} versus the peak current, the analytical calibration curves are shown in Figure 4(b). The DNA/GSH-Au NPS/cysteine modified electrode response exhibit a good linear relationship with the Hg^{2+} concentration in the range of 50 pM to 0.1 μM, with a sensitivity of 0.143 μM/pHg and a detection limit around 50 pM. On the other hand, the DNA/cysteine modified electrode demonstrates a linear relationship in the range of 1 nM to 0.1 μM and a sensitivity of 0.053 μA/pHg. This comparison indicates that the modified electrode using the gold nanoparticles presents a higher sensitivity and a lower detection limit towards Hg^{2+} ions.

The value of 50 pM is below the maximum contaminant level for inorganic mercury in drinking water set by US EPA (0.002 mgL^{-1}, ca. 10 nmol L^{-1}) (US Environmental Protection Agency 2009).

In order to confirm the interaction between DNA thymine and mercury ion in the presence of gold nanoparticles, electrochemical impedance measurements were investigated. As shown in Figure 5(a), the charge transfer resistance (R_{ct}), which corresponds to the diameter of the semicircle in Nyquist plot, increases gradually when the mercury ions' concentration increases. Higher concentrations of Hg^{2+} ions result in higher charge transfer resistance (R_{ct}) of the negative charge redox indicator, $[Fe(CN)_6]^{3-/4-}$. This result can be attributed to the thymine–Hg^{2+}–thymine complexes formation, which inhibits the redox activity of the electroactive couple and limits access to the electrode surface. In addition, the impedance values are fitted to a standard Randle's equivalent circuit as shown in Figure 5(b). The components in the equivalent circuit included the solution resistance R_s, the charge transfer resistance R_{ct}, the constant phase element related to double layer capacitance (CPE) and the Warburg impedance (W). As summarized in Table 1, the charge transfer resistance values (R_{ct}) of DNA-modified electrodes before and after incubation in Hg^{2+} solution was obtained from the numeric simulation of the impedance plots. As a result, the addition of 1 μM of Hg^{2+} to the sensor leads to an apparent increase in the charge transfer resistance from 2 to 11.6 KΩ. Moreover, the Warburg impedance and the double layer capacitance decreased slightly with the

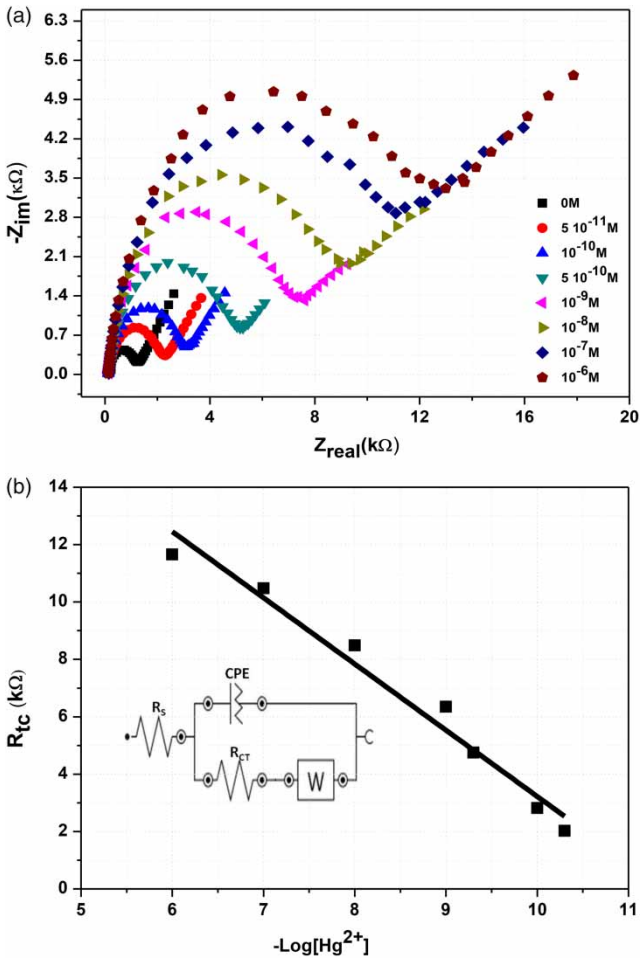

Figure 5 | (a) Nyquist spectra of the cysteine/GSH-AuNPs/DNA modified electrode for different Hg^{2+} concentrations. (b) Calibration curves for cysteine/GSH-AuNPs/DNA modified electrode and the corresponding equivalent circuit.

Table 1 | Impedance fitted parameters of DNA/AuNPs-glutathione/cysteine modified electrode after immersion in different concentrations of Hg^{2+}, obtained from the analysis of impedance data with the Randles circuit in Figure 5(b)

$[Hg^{2+}]$(M)	Rs (Ω)	CPE (μF)	N	R_{tc} (kΩ)	W(μF)	Θ(%)
0	136.3	1.876	0.875	1.033	625	0
5×10^{-11}	140.87	1.826	0.861	2.023	620	0.489
10^{-10}	26.64	1.634	0.891	2.816	563	0.633
5×10^{-10}	40.61	1.28	0.891	4.754	410	0.782
10^{-9}	35.75	1.174	0.891	6.832	314	0.848
10^{-8}	38.06	1.098	0.889	8.483	206	0.878
10^{-7}	36.62	1.038	0.887	10.475	136	0.901
10^{-6}	35.62	0.952	0.89	11.657	116	0.911

increase of mercury concentration. By plotting the charge transfer resistance versus the logarithm value of Hg^{2+} concentration, a calibration curve was obtained (Figure 5(b)). The R_{ct} values exhibit a linear relationship with the Hg^{2+} concentration in the range of 50 pM to 1 μM, with a sensitivity of 2.609 kΩ/pHg. From the R_{ct} values, the apparent electrode coverage (θ) of the mercury sensor can be approximately calculated according to Equation (1) (Troughton *et al.* 1982):

$$\theta = 1 - \frac{R_{ct}}{R_{ct'}} \quad (1)$$

where R_{ct} and $R_{ct'}$ are the charge transfer resistance of the bare and the AuNPs -GSH/cysteine modified electrodes, respectively. It can also be seen that the coverage surface increases considerably with the Hg^{2+} concentration. This result confirms the wide linear biosensor response range.

Selectivity of the electrochemical DNA biosensors for Hg^{2+} detection

The sensitivity of the developed DNA biosensors towards other heavy metal ions such as Cu^{2+}, Pb^{2+} and Cd^{2+} was investigated. As shown in Figure 6(a), the higher charge transfer resistance variation was obtained for the mercury ions, whereas a slight variation of R_{ct} was observed for the other tested metal ions. This result indicates that the electrochemical DNA biosensor has high sensitivity and more selectivity towards Hg^{2+} ions. It can be attributed to the specific coordination between thymine bases and Hg^{2+} ions. Moreover, Figure 6(b) illustrates the electrochemical response for Hg^{2+} ions with and without a mixture of three different interfering metal ions, such as Cu^{2+}, $Cd^{2+}$and Pb^{2+}, at a concentration of 1 μM. As shown in Figure 6(b), the current response for the Hg^{2+} ions, peak decreased when the modified electrode was immersed in the mixed ions solution. This decrease can be attributed to the ion interference effect. In a previous work it was demonstrated that the Pb^{2+} ion exhibits a significant affinity towards guanine by the formation of a G-quadruplex structure (Jarczewska *et al.* 2015). For this reason, the interference of Pb^{2+} ions cannot be excluded, as they interact specifically with guanine.

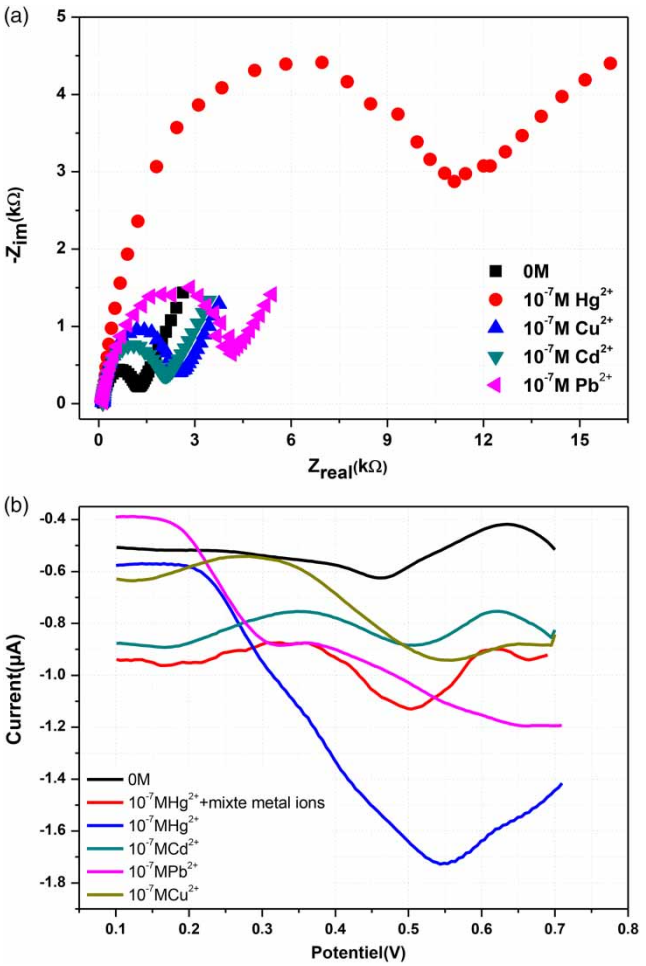

Figure 6 | (a) Nyquist plot of the cysteine/GSH-AuNPs/DNA senor for 0.1 µM Hg^{2+} against other metal cations (Cu^{2+}, Pb^{2+} and Cd^{2+} (0.1 µM)). (b) DPV corresponding to the detection of 0 M, 0.1 µM of Hg^{2+} in metal ion mixture (Cu^{2+},Pb^{2+} and Cd^{2+} (1 µM)) and 0.1 µM of Hg^{2+}.

Determination of Hg^{2+} in field samples

The analytical performance of the developed electrochemical DNA modified electrode was applied for the determination of the mercury ion in wastewater samples. The tested water samples were obtained from the Tunisian public industrial company ONAS, which is interested in the management of the sanitation sector. The wastewater samples were analyzed with voltammetric and impedance metric DNA sensors, and were compared to the results obtained using the spectrophotometric method. The results presented in Table 2 indicate the applicability of the voltammetric and impedancemetric methods to quantify mercury ions in wastewater samples. This result proves the usefulness of DNA/AuNPs-glutathione/cysteine modified electrodes for the detection of mercury in aqueous samples. Furthermore, in the present system the sensor probe can be regenerated in 100 mM of ascorbic acid solution for 1 hour to reduce Hg^{2+} into Hg^{+}, and might exhibit a weak coordination with thymine as described by Liu *et al.* (2009). The mercury sensor showed good reproducibility and efficacious regeneration (relative standard deviation below 5.0%).

Table 2 | Application of the DNA-Sensor to Hg^{2+} ions' determination in wastewater samples. The Hg^{2+} amount was given in $mg.L^{-1}$

Wastewater	Impedancemetric method	Voltammetric method	Spectrophotometric method
Sample 1	0.00459	0.00468	0.00462
Sample 2	0.00876	0.00880	0.00870
Sample 3	0.0120	0.0119	0.0122

CONCLUSIONS

In this work an electrochemical DNA biosensor has been successfully fabricated based on thymine-Hg^{2+}-thymine complexation for trace mercury ion quantification. Incidentally, the developed biosensor, based on an AuNPs-glutathione/cysteine-DNA matrix, was simple, sensitive and rapid. The Hg^{2+} ions were detected and identified at trace level quantities with a detection limit of 50 pM. A linear relationship of the biosensor response to the analyte concentration was obtained in the dynamic range from 50 pM to 0.1 µM. The obtained results revealed that this sensor exhibited a high sensitivity for mercury among other tested metal ions. The applicability of the DNA sensor for the determination of low concentration levels of Hg(II) ions in wastewater samples was successfully tested with a high reproducibility.

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