Bioelectrochemistry 86 (2012) 78–86

Contents lists available at SciVerse ScienceDirect

Bioelectrochemistry

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

# Fabrication of a modified electrode based on $Fe_3O_4$NPs/MWCNT nanocomposite: Application to simultaneous determination of guanine and adenine in DNA

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ARTICLE INFO

*Article history:*
Received 18 November 2011
Received in revised form 5 February 2012
Accepted 11 February 2012
Available online 21 February 2012

*Keywords:*
$Fe_3O_4$ nanoparticles
Multi-walled carbon nanotube
Guanine
Adenine
DNA
Voltammetry

ABSTRACT

Multi-walled carbon nanotubes decorated with $Fe_3O_4$ nanoparticles ($Fe_3O_4$NPs/MWCNT) were prepared and used to construct a novel biosensor for the simultaneous detection of adenine and guanine. The direct electro-oxidation of adenine and guanine on the modified electrode were investigated by linear sweep voltammetry. The results indicate a remarkable increase in the oxidation peak currents together with negative shift in the oxidation peak potentials for both adenine and guanine, in comparison to the bare glassy carbon electrode (GCE). The surface morphology and nature of the composite film deposited on GCE were characterized by transmission electron microscopy, atomic force microscopy, cyclic voltammetry and electrochemical impedance spectroscopy. The $Fe_3O_4$NPs/MWCNT based electrochemical biosensor exhibits linear ranges of 0.01–10 μM and 0.05–8 μM with detection limits of 1 nM and 5 nM for adenine and guanine, respectively. The proposed method was successfully applied for a highly sensitive simultaneous determination of trace amounts of adenine and guanine in DNA of fish sperm samples with satisfactory results. The experimental detection limit was found to be equal to 3 ng $mL^{-1}$ DNA. The value of (G + C)/(A + T) in DNA was calculated to be 0.81. The fabricated electrode showed excellent reproducibility, repeatability and stability.



## 1. Introduction

Deoxyribonucleic acid (DNA) is an important substance for the storage of genetic information and protein biosynthesis. Adenine and guanine are two components of DNA, and most of the current electroanalytical protocols for DNA detection are based on these electroactive purine bases [1]. The abnormal changes in the concentration of these bases in organisms indicated efficiency and/or mutation of the immune system, and may be the sign of various diseases. Hence, determining individual concentrations of these components, or their ratios, in DNA has given rise to great interests in bioscience and clinical diagnosis [2–4].

The electroactivity of nucleic acids (NAs) was first discovered on mercury electrode which led to further understanding of the DNA double helix [5]. The electroactive groups in DNA oxidation were adenine and guanine residues which exhibit slow direct electron transfers [6,7] and irreversible adsorption on the electrode surface [8,9], resulting in low sensitivity for DNA detection. Over the past years, considerable efforts have been paid to the development of chemically modified electrodes for direct electrochemical investigation of NAs and their related nucleosides. For example, Fan et al. reported a facile approach to prepare $TiO_2$ nanocomposite uniformly embedded on graphene substrate, which provides an efficient microenvironment for the electrochemical reaction of these purine bases [10]. Abbaspour et al. used microwave irradiation for fast preparation of a sol–gel-derived CNT ceramic electrode for sensitive determination of guanine and adenine in DNA [11]. Tu et al. demonstrated a new strategy for preparation of carboxylated CNTs modified electrode with electrocatalytic activity for the determination of guanine and adenine in DNA solutions [12]. Ionic liquid modified electrode was also applied to the direct electrocatalytic oxidation of guanine and adenine [13]. Recent literature by P.A.M Farias et al. using stripping voltammetric determination of purines in the presence of copper ions has elaborated the analytical protocols based on acid DNA hydrolysis [14–17]. S. Hason et al. showed the determination of purines and their metabolites using roughened carbon electrodes. In this case the improved sensitivity of determination has been discussed in terms of microenvironments conferred by surface microstructures [18–20]. The previously attempted methods for the determination of guanine and adenine suffered from irreversible adsorption of the purine bases on the electrode surface which led to surface fouling.

Carbon nanotubes (CNTs) provide excellent performance in enhancing electrochemical reactivity, promoting electron-transfer reactions and alleviating surface fouling [21,22]. Nanoparticulate hybrid materials made from inorganic solids and CNTs are highly promising for applications in nanoscale devices and nanoelectronics

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1567-5394/$ – see front matter 
doi:10.1016/j.bioelechem.2012.02.004

[23,24]. Furthermore, uniformly dispersed nanoparticles on CNT surfaces yield highly effective nanocatalysts for chemically modified electrodes [25,26]. $Fe_3O_4$ nanoparticles have attracted an increasing interest in construction of sensors and biosensors because of their good biocompatibility, strong super paramagnetic property, low toxicity, easy preparation and high adsorption ability. Moreover, $Fe_3O_4$ nanoparticles exhibit high surface area and low mass transfer resistance. Yin et al. presented the development and application of glassy carbon electrodes modified with $Fe_3O_4$ magnetic nanoparticles for determination of trace amounts of bisphenol A and investigation of the electrochemical oxidation of guanosine [27,28].

In the present work, we synthesized a composite made of $Fe_3O_4$ nanoparticles immobilized on MWCNTs by a simple method. The excellent dispersibility of the resulting MWCNT with immobilized magnetite nanoparticles in dimethylformamide (DMF) leads to the formation of a stable colloidal dispersion, which is capable of forming a uniform and stable thin film on the surface of GCE. The modified electrode eliminates the disadvantage of the irreversible adsorption of purine bases on the electrode surface. The results presented here demonstrate that $Fe_3O_4$NPs/MWCNT modified electrode could bring new capabilities for the electrochemical devices by combining the advantages of carbon nanotubes and magnetite nanoparticles, and can be readily used for simultaneous determination of the relevant bases as well as ultra-high sensitive detection of DNA.

## 2. Experimental

### 2.1. Chemicals and reagents

MWCNTs with 10–20 nm o.d., 5–10 nm i.d., 0.5–200 nm tube length more than 95% purity were obtained from Nanostructured & Amorphous Materials (Houston, TX, USA). Adenine, guanine and fish sperm DNA were purchased from Sigma. All other chemicals were of analytical reagent grade from Merck. All aqueous solutions were prepared with doubly distilled de-ionized water**.** Stock solutions of adenine and guanine were freshly prepared as required in 0.1 M NaOH solution. The working solutions were prepared by diluting the stock solution with phosphate or acetate buffer solutions. In these experiments, 0.1 M acetate was used for preparation of pHs 4.0 and 5.0, and 0.1 M phosphate buffer solution (PBS) for pHs 3.0, 6.0 and 7.0.

### 2.2. Apparatus

Voltammetric experiments were performed using a Metrohm potentiostat/galvanostat model 797VA. A conventional three electrode system was used with a glassy carbon working electrode (unmodified or modified), a saturated Ag/AgCl reference electrode and a Pt wire counter electrode. A digital pH/mV/Ion meter (CyberScan model 2500) was used for preparation of the buffer solutions. The transmission electron microscope (TEM) images were obtained using a PHILIPS CM 200 by placing one drop of the sample dispersed in acetone (using an ultrasonic bath) on a carbon coated copper grid. Atomic force microscopy (AFM) experiments were carried out in ambient condition using Veeco CP Research instrument using Si cantilever. The composition(s) of $Fe_3O_4$NPs/MWCNT/GCE electrode was investigated by energy dispersive X-ray spectroscopy (EDS) (RONTEC, QUANTAX). Electrochemical impedance spectroscopy (EIS) measurements were performed with a Potentiostat/Galvanostat/Frequency response analyzer (FRA) EG&G model 273A between 100 mHz and 10 kHz using a 5 mV rms sinusoidal modulation in 0.1 M KCl solution containing 1 mM of both $K_4Fe(CN)_6$ and $K_3Fe(CN)_6$ (1:1 mixture), and at the $E_{1/2}$ of $[Fe(CN)6]^{3-/4-}$ (0.13 V vs. Ag/AgCl). Voltammetric experiments were carried out in the buffered solutions deoxygenated by purging with nitrogen (99.999%). Nitrogen gas was also maintained over the surface of the test solutions during the experiment.

### 2.3. Preparation of DNA samples

The DNA samples were prepared according to the literature [4,29,30]. A gentle treatment of DNA with 1 M HCl leads to selective removal of its purine bases by cleavage of purine glycoside bounds [31]. The fish sperm DNA for quantification of adenine and guanine was hydrolyzed as following: 3 mg of the fish sperm DNA was digested using 1 mL of 1 M HCl in a sealed 10 mL glass tube. After heating in boiling water bath (100 °C) for 80 min, 1 mL of 1 M NaOH was added. After cooling to room temperature, the solution was diluted to 10.0 mL using 0.1 M PBS (pH 7).

### 2.4. Preparation of $Fe_3O_4$NPs/MWCNT and modification of GCE

The MWCNTs were added to a mixture solution of sulfuric acid and nitric acid (3:1) in a reaction flask and refluxed for 24 h at 120 °C. The mixture was cooled, diluted with water and then filtrated. The product (MWCNT-COOH) was washed with water and dried at 60 °C for 3 h in a vacuum oven. Open-end MWCNTs with hydrophilic surfaces were thus obtained. Iron oxide–MWCNT composite was prepared according to a previously reported method [32]. Briefly, the functionalized MWCNTs were added into a solution of concentrated nitric acid containing iron(III)nitrate and then refluxed for 4.5 h in an oil bath at 120 °C. The mixture was cooled to room temperature and then ammonia solution (2.5 wt.%) was slowly added with vigorously stirring till the pH value reached 10. Then the solution was filtered on a 0.65 μm filter membrane and washed with distilled water repeatedly. The resulting product was dried overnight at 100 °C in an oven. The sample was then annealed at 250 °C for 1 h and 650 °C for 2 h in a steam of nitrogen.

Before modification, the GCE was polished with 0.1 μm alumina slurry on a polishing cloth, rinsed thoroughly with water, sonicated in water for 5 min and finally was dried in air. A 2.0 mg portion of the $Fe_3O_4$NPs/MWCNT was dispersed in 2.0 mL DMF and homogenized ultrasonically for 10 min. The suspension is quite stable for 8–9 months. Then, 4 μL of the suspension was placed on the GCE surface by micropipette and left to dry at room temperature to obtain $Fe_3O_4$NPs/MWCNT/GCE. The MWCNT/GCE without $Fe_3O_4$ NPs was also prepared according to the same procedure, using a suspension of MWCNT in DMF. Before voltammetric measurements, the modified electrode was cycled five times between 0 and 1.1 V (scan rate 100 mV $s^{-1}$) in a 0.1 M PBS of pH 7.0. When it was necessary, renewal of the electrode surface was easily accomplished by soaking the modified electrode in PBS and cycling the potential as mentioned above.

## 3. Results and discussion

### 3.1. Surface characterization of $Fe_3O_4$NPs/MWCNT/GCE

The microscopic structure of $Fe_3O_4$NPsimmobilisedon MWCNTs was explored using transmission electron microscopy (TEM) images. Fig. $S_1$ shows the well-distributed nanoparticles were deposited on the surface of MWCNTs. From the TEM images, the size of the iron oxide nanoparticles immobilized on MWCNTs was estimated to be 5–10 nm. In order to assess the thickness and topographical properties of the modified electrodes, atomic force microscopic (AFM) images of $Fe_3O_4$NPs/MWCNT, MWCNT films coated on the surface of GCE and bare GCE were obtained in contact mode. It can be seen in Fig. 1a, b and c that the surface morphology of the film is considerably changed in the presence of $Fe_3O_4$NPs and MWCNT with respect to bare GCE. From these measurements, a thickness of 400 nm was estimated, when 4 μL of the $Fe_3O_4$NPs/MWCNT suspension (2.0 mg $Fe_3O_4$NPs/MWCNT in 2.0 mL DMF) was deposited on the GCE surface. Moreover, the AFM images show that the surface of the electrode modified with $Fe_3O_4$NPs/MWCNT is entirely rough respect to MWCNT, which causes

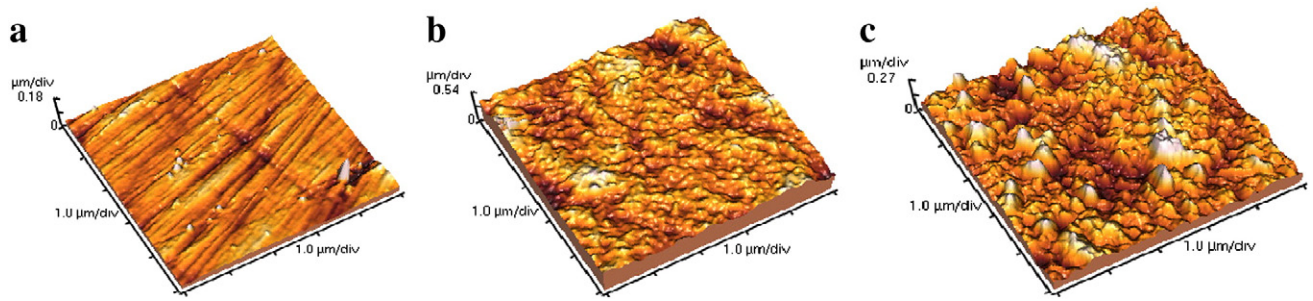


**Fig. 1.** AFM images of (a) bare GCE and the films obtained by depositing 4 μL suspensions of MWCNT (b) and $Fe_3O_4$NPs/MWCNT (c) on the surface of the GCE.

a remarkable increase in the microscopic active areas at the electrode surface and affect diffusion of the electroactive species and eventually response sensitivities of the voltammetric measurements [25,33]. We also carry out energy-dispersive X-ray spectrum (EDS) experiment to investigate the chemical composition of the hybrid nanostructure. The EDS results in Fig. 2 and Table 1 clearly show that Fe and O are the two main elements on the surface of MWCNT films.

### 3.2. Electrochemical behavior of $Fe_3O_4$NPs/MWCNT/GCE

Fig. 3 shows cyclic voltammetric (CV) curves obtained in nitrogen-purged 0.1 M PBS of pH 7. A pair of cathodic and anodic peaks, was observed at −0.104 and −0.365 V for $Fe_3O_4$NPs/MWCNT/GCE compared to bare GCE and MWCNT/GCE. These are characteristics of the redox behavior of $Fe_3O_4$ NPs proving that MWCNTs were successfully decorated with $Fe_3O_4$ NPs. Electrochemical impedance spectroscopy (EIS) was employed to investigate the impedance of different electrodes. Fig. $S_2$ shows the Nyquist plots of $K_3Fe(CN)_6/K_4Fe(CN)_6$ at the bare GCE, MWCNT/GCE and $Fe_3O_4$NPs/MWCNT/GCE. The EIS of the bare GCE displayed a semicircle with a very large diameter at higher frequencies, but $Fe_3O_4$NPs/MWCNT/GCE shows an almost straight line in the Nyquist plot, which is characteristic of a diffusion-limited electron-transfer process. The EIS observations reflect that $Fe_3O_4$NPs/MWCNTs at the GCE surface lower the electron transfer resistance and increase the electron transfer rate. The results also indicate that the $Fe_3O_4$NPs/MWCNTs are firmly immobilized on the electrode surface.

### 3.3. Electrochemical behavior of guanine and adenine on the surface of various electrodes

Fig. 4 illustrates linear sweep voltammetric (LSV) response of 10 μM guanine and adenine mixture on the surface of the bare GCE, MWCNT/GCE and $Fe_3O_4$NPs/MWCNT/GCE. The voltammogram of guanine on the bare GCE exhibits just a small hump and that of adenine is not observed. However, very distinct anodic peaks with high peak currents for adenine and guanine are observed at MWCNT/GCE and $Fe_3O_4$NPs/MWCNT/GCE. Compared to the bare GCE, substantial reduction in the overpotentials and remarkable enhancement in the peak currents were observed in the LSVs for both compounds on MWCNT/GCE and $Fe_3O_4$NPs/MWCNT/GCE (Table S1), indicating the catalytic role of MWCNTs in the electro-oxidation of guanine and adenine bases. The modified electrodes show a peak-to-peak separation of 300 mV in the LSV responses of guanine and adenine. Such a large peak separation can eliminate the mutual interfering interactions between guanine and adenine in simultaneous measurement of the two compounds and effectively increase the determination selectivity. These improvements can be interpreted based on a combination of the electrocatalytic effect and change of the diffusional regime of the porous thin film of MWCNTs, as previously reported on electrodes modified with thin films of nanotubes and fullerenes [34–39]. The increased peak currents and almost unchanged peak potentials of guanine and adenine on the surface of$Fe_3O_4$NPs/MWCNT/GCE with respect to MWCNT/GCE, indicate that $Fe_3O_4$NPs can effectively adsorb guanine and adenine on the electrode surface. In fact, $Fe_3O_4$NPs immobilized on the MWCNT/GCE surface with their large surface area increase the adsorptive sites, resulting in a significant increase in the oxidation current.

### 3.4. Optimization of the film thickness (choice of the amount of modifier)

The film thickness of the porous thin film modified electrodes affects both kinetics of the electrode processes and mass transfer mechanism via diffusion through the porous film [34–37] and thus, has a predominant role in the voltammetric response of these electrodes

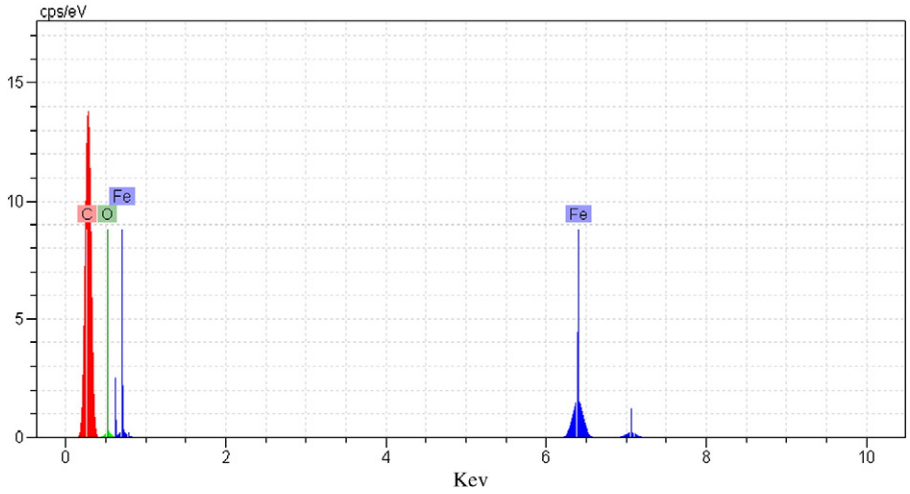


**Fig. 2.** The EDS pattern of $Fe_3O_4$NPs/MWCNT/GCE.

**Table 1**
EDS results for elemental composition of $Fe_3O_4$NPs/MWCNT/GCE.

| Element | Series | Unn. C [wt.%] | Norm. C [wt.%] | Atom. C [at.%] |
|---|---|---|---|---|
| Carbon | K series | 61.84 | 67.45 | 87.99 |
| Oxygen | K series | 3.77 | 4.11 | 4.02 |
| Iron | K series | 26.08 | 28.45 | 7.98 |

Total: 91.7%.

toward different analytes. To vary the thickness of the modifier, different volumes of $Fe_3O_4$NPs/MWCNT suspension in DMF (1 mg $mL^{-1}$, 1–5 μL) have been deposited on the electrode surface and their LSV response to guanine and adenine measured in the range of 0.01–10 μM. Based on the slope of calibration graph for guanine and adenine in the (0.01–10 μM) concentrations range (Figs. 5a and b), a volume of 4 μL of the modifier suspension provides reasonable sensitivity in the voltammetric responses (Fig. 5c), and was selected as the optimum volume for modification of the electrode surface.

### 3.5. Optimization of pH

In order to achieve the optimum pH and evaluate the ratio of electrons and protons involved in the anodic oxidation of guanine and adenine on the surface of the modified electrode, the electrochemical behaviors of these species were investigated in various pHs of the buffered solutions in the range of 3.0 to 8.0. As can be seen in Fig. S3a, the anodic peak potentials shift negatively by increasing the solution pH, suggesting the participation of $H^+$ in the oxidation processes. The slope values of 54.91 mV and 56.28 mV per pH unit (Fig. S3c) for guanine and adenine, respectively, indicate that equal numbers of electrons and protons are involved in the electro-oxidation of both compounds on the surface of the $Fe_3O_4$NPs/MWCNT/GCE. From the results of pH investigation shown in Fig. S3b maximum anodic peak currents for both guanine and adenine occur at pH 7.0, which is well suited for determinations in physiological conditions.

### 3.6. Effect of potential scan rate

Cyclic voltammetry responses of $Fe_3O_4$NPs/MWCNT/GCE at different potential scan rates, $\nu$/mV $s^{-1}$, for 10 μM guanine and adenine in 0.1 M PBS of pH 7.0 are shown in Fig. S4a. The plots of logarithm of peak currents, log ($I_P$/μA), versus logarithm of scan rates, log $\nu$ (mV $s^{-1}$), for guanine and adenine were linear (Fig. S4b). This behavior can be related to the adsorption of these species at the modified electrode surface and their diffusion through the porous $Fe_3O_4$NPs/MWCNT film [40]. The relationship between the oxidation peak potentials, $E_{p,a}$/V, and logarithm of the scan rates, log $\nu$ (mV $s^{-1}$), is also linear as shown in Fig. S4c with the corresponding Eqs. (1) and (2); indicates the irreversible nature of the electrochemical processes for both guanine and adenine.

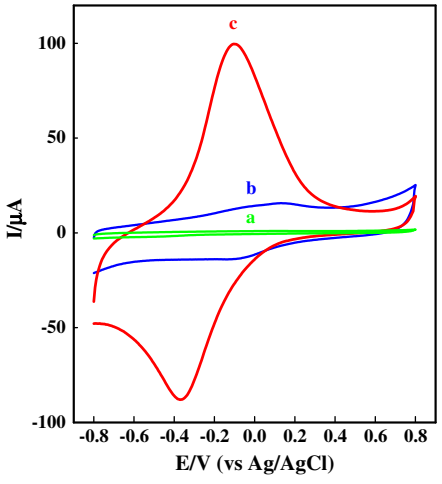


**Fig. 3.** CVs of (a) bare GCE (b) MWCNT/GCE and (c) $Fe_3O_4$NPs/MWCNT/GCE in 0.1 M PBS (pH 7).

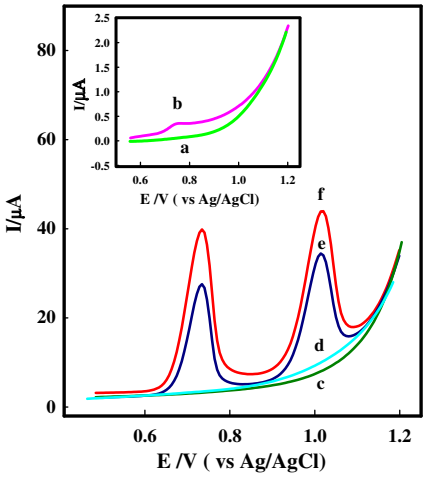


**Fig. 4.** LSVs of (a, b) bare GCE (the inset) (c, e) MWCNT/GCE (d, f) $Fe_3O_4$NPs/MWCNT/GCE in the presence (b, e, f) and absence (a, c, d) of10 μM of guanine and adenine in 0.1 M PBS (pH 7).

$$E_{p,Guanine}/mV = 49.72\log\left(\nu/mVs^{-1}\right) + 636.94\left(R^2 = 0.993\right) \quad (1)$$

$$E_{p,Adenine}/mV = 55.90\log\left(\nu/mVs^{-1}\right) + 909.53\left(R^2 = 0.992\right) \quad (2)$$

Integrating the results with those obtained in pH investigation, one can conclude that the electrochemical oxidation of guanine and adenine on $Fe_3O_4$NPs/MWCNT/GCE are two-electron and two-proton processes.

### 3.7. Effect of preconcentration (accumulation) parameters

Several reports have shown that guanine and adenine can be adsorbed on the surface of modified electrodes at open circuit condition [11,29,41,42]. However, in many accumulation steps, potential and time may significantly affect sensitivity of the determination by changing the amount of the adsorbed species at the electrode surface. Therefore, we investigated the effect of the accumulation potential in the range of −0.5 to 0.4 V on the LSV peak currents of 10 μM guanine and adenine in PBS of pH 7.0. The results showed that the oxidation peak current unchanged with respect to open circuit potential and the accumulations of guanine and adenine can be carried out under the open circuit condition.

The effect of accumulation time, $t_{acc}$/s was investigated for 1 μM and 10 μM of guanine and adenine mixture in various accumulation times under the foregoing optimal operating conditions. As shown in Fig. 6, the peak currents are linearly dependent on the accumulation time, before saturation of the electrode surface. For 10 μM of both guanine and adenine, before reaching the saturation condition (beyond ~210 s) the peak current increase by increasing the accumulation time. However, for 1 μM concentrations, the responses were linear up to 400 s (Fig. 6c). Apparently, lower concentrations of the analytes require longer accumulation time, meaning that the choice of accumulation time is dictated by the desired sensitivity. To avoid saturation of the electrode surface and to account for a reasonable sensitivity, an accumulation time of 240 s was used for the following experiments.

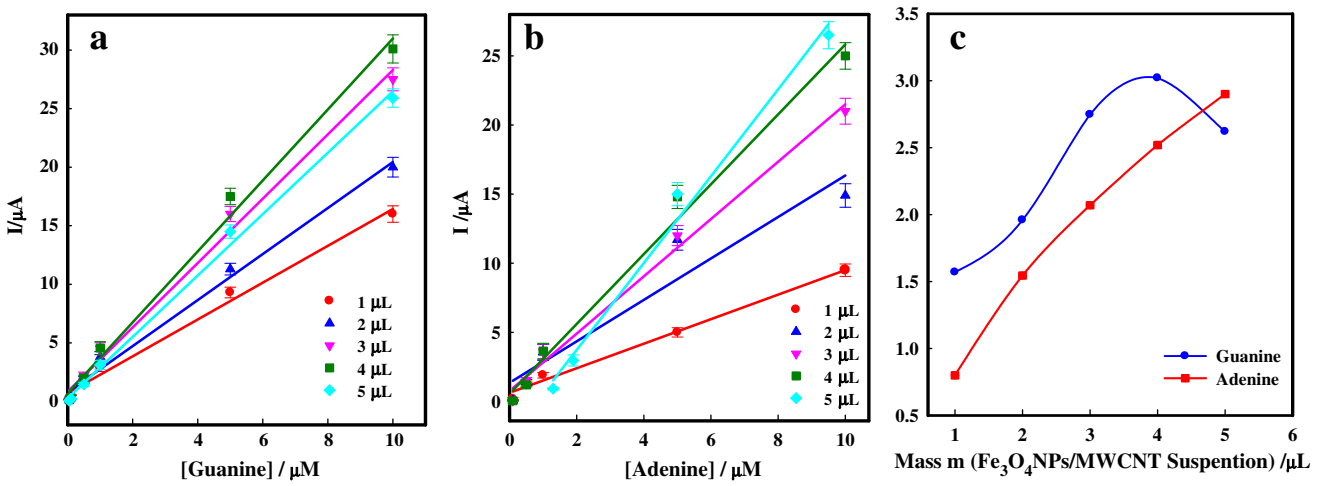


**Fig. 5.** Effect of content of $Fe_3O_4$NPs/MWCNT suspension (1 mg $mL^{-1}$), m/μl, deposited on GCE surface on the cyclic voltammograms for different concentrations of (a) guanine and (b) adenine (0.05–10 μM). (c) The plot of sensitivities LSVs for guanine and adenine versus content of $Fe_3O_4$NPs/MWCNT suspension.

### 3.8. Simultaneous determination of adenine and guanine

Possible mutual interferences due to adenine-guanine interactions were investigated by changing the concentration of one species at a constant concentration of the other species. Fig.7a shows the LSVs of various concentrations of guanine in the presence of a fixed concentration of adenine (1 μM) in 0.1 M PBS (pH 7.0) at $Fe_3O_4$-NPs/MWCNT/GCE. As can be seen, the peak currents, $I_p$/μA, of adenine are almost constant, but that of guanine increase with increasing its concentration in the mixture. The calibration curve for guanine shows a linear range of 0.01–10 μM with regression equation of $I_p$/μA = 3.3130 $C_{Guanine}$/μM + 0.0704 ($R^2$ = 0.995) and detection limit of 1 nM (Fig. 7b). Similarly, Fig. 8a shows LSVs of solutions containing a constant concentration of guanine (1 μM) and various concentrations of adenine. In this case, the peak currents are proportional to the concentration of adenine in the range of 0.05–8 μM with regression equation of $I_p$/μA = 3.17 $C_{Adenine}$/μM + 0.122 ($R^2$ = 0.994) and detection limit of 5 nM (Fig. 8b). The results indicate that due to relatively large peak potential separation of ~300 mV, the electrochemical signals of adenine and guanine at the modified electrode are independent of each other. For further evaluation of the feasibility of the $Fe_3O_4$NPs/MWCNT/GCE for simultaneous determinations, the fabricated electrode was applied to the determination of guanine and adenine by simultaneously changing their concentrations (Fig. 9a). As can be seen in Fig. 9b, the results indicated that the analytical characteristics of the electrode including response equations and detection limits do not change significantly in solutions containing varying concentrations of both guanine and adenine. The limits of quantifications (LOQs) are obtained at 0.01 μM and 0.05 μM for guanine and adenine, respectively. Fig. S5 demonstrates the LSVs of simultaneous determination of guanine and adenine in the concentrations of 0.01–1 μM. Fig. S6 shows the LSVs of various determination of guanine (0.01–1 μM) in the presence of 1 μM adenine. The corresponding responses are indicated in Tables S2, S3 and S4. Table 2 compares the response characteristics of $Fe_3O_4$NPs/MWCNT/GCE with those of other modified electrodes reported in the literature for the simultaneous determination of adenine and guanine. As can be seen, wide linear dynamic ranges (3 and more than 2 orders of magnitude for guanine and adenine, respectively) with a very low detection limits observed for the proposed electrode are remarkably better than those previously reported for these compounds.

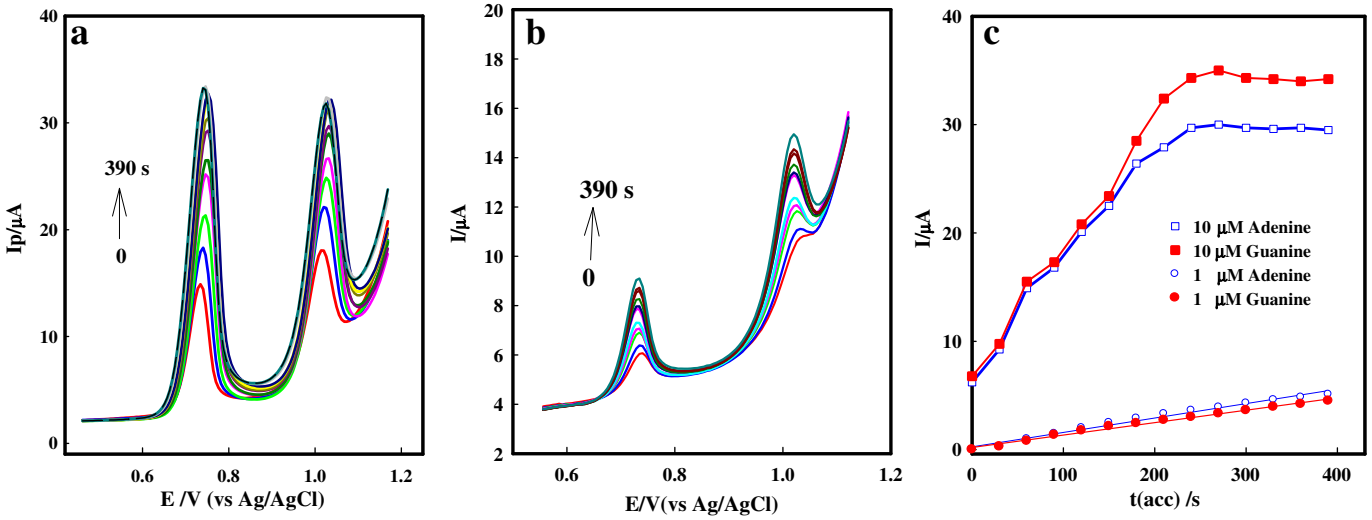


**Fig. 6.** Effect of accumulation time, t(acc)/s, on LSVs of (a) 10 μM and (b) 1 μM of guanine and adenine on the surface of the $Fe_3O_4$NPs/MWCNT/GCE in 0.1 M PBS of pH 7. (c) Plot of currents versus accumulation time for guanine and adenine at different concentrations.

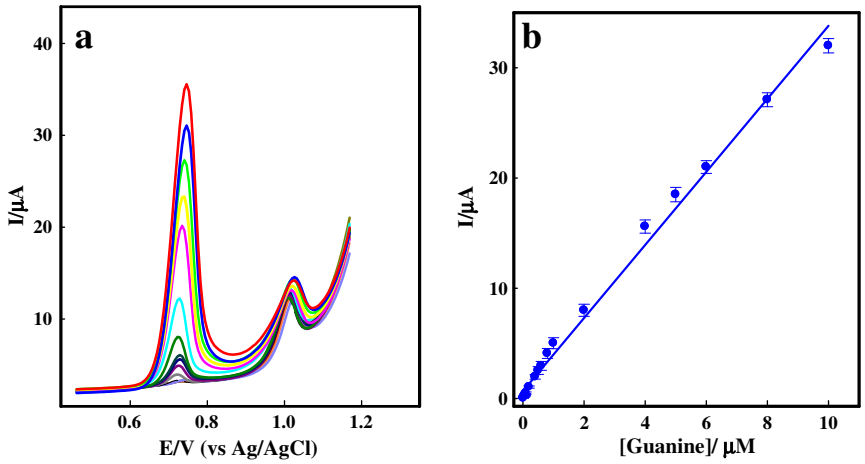


**Fig. 7.** (a) LSVs of various concentrations of guanine (0.01–10 μM) in the presence of 1 μM adenine, and (b) calibration plot for guanine.

### 3.9. Repeatability, reproducibility and stability of the modified electrode

The repeatability of the modified electrode was investigated by repetitive recording its response at guanine and adenine concentrations of 1 and 10 μM. The relative standard deviation of the peak currents for five replicate LSV determinations of 1 and 10 μM of guanine were 1.75% and 1.65%, respectively, and the corresponding values for adenine were 2.76% and 2.74%, respectively. The reproducibility of the electrode preparation procedure was estimated by preparing five modified electrodes based on the same fabrication procedure and applying for the simultaneous determination of guanine and adenine at 1and 10 μM concentrations. The corresponding RSD values were 2.47% and 3.46% for guanine, and 3.18% and 4.42% for adenine, respectively. The results indicate that the modified electrode has high reproducibility and excellent repeatability in both the preparation procedure and the voltammetric determinations. Also, on using the $Fe_3O_4$Nps–CNT/GC daily while storing the modified electrode under ambient conditions, the electrode retained 95.3% of its initial peak current response to 10 μM each of guanine and adenine after a period of one month, indicating long-term stability of the modifier film on the GCE surface. According to the previous reports [2,43], there may be severe drawbacks in electrochemical determination of purines, because these bases could irreversibly adsorb on the electrode surface and lower the precision and accuracy of the determinations. In the case of present electrode, however, the effect of any adsorbed species fouling problems is completely eliminated by applying only five potential cycles in the supporting electrolyte (PBS of pH 7.0) before each measurement. Another advantage of the $Fe_3O_4$NPs/MWCNT is its excellent dispersibility in the solvent, which leads to suspensions stable for 8–9 months and formation of highly reproducible films on the GCE, resulting in highly reproducible measurements. However, in the absence of $Fe_3O_4$ NPs, MWCNTs tend to aggregate, resulting in decreased stability of its suspensions.

### 3.10. Interference study

In the present work, the interference effects of 1 mM ascorbic acid (AA), 0.1 mM uric acid (UA), 10 μM dopamine (DP) and 1 mM glucose (Glu) were tested on the voltammetric response of 10 μM Guanine and adenine (Fig. 10. No changes in response currents of guanine and adenine were observed in the presence of AA, UA, DP, Glu solutions or the mixtures of all. In the mixture of all these compounds by using the modified electrode, five well-defined waves with a very good resolution are resulted. Among these interferences, glucose has no response but AA, UA, and DP showed oxidation process in the selected potential range. Therefore, in this study it was proved that this method can be successfully applied for the simultaneous determination of guanine and adenine in the presence of the other interference compounds in the clinical preparations.

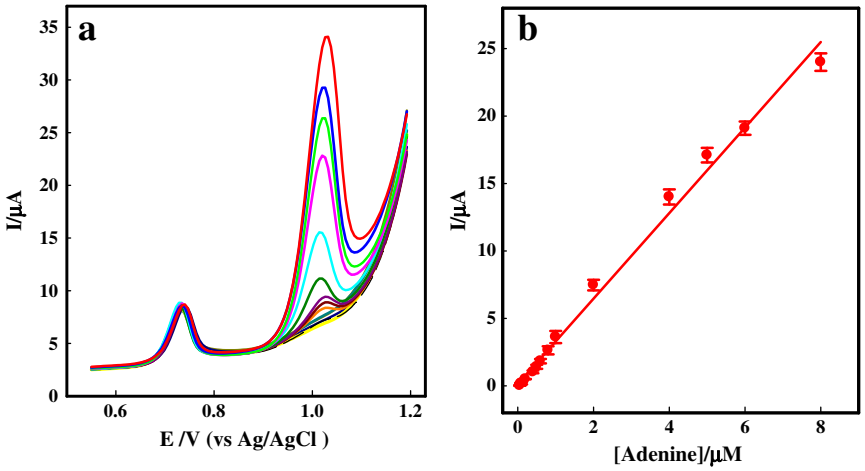


**Fig. 8.** (a) LSVs of various concentrations of adenine (0.05–8 μM) in the presence of 1 μM guanine, and (b) calibration plot for adenine.

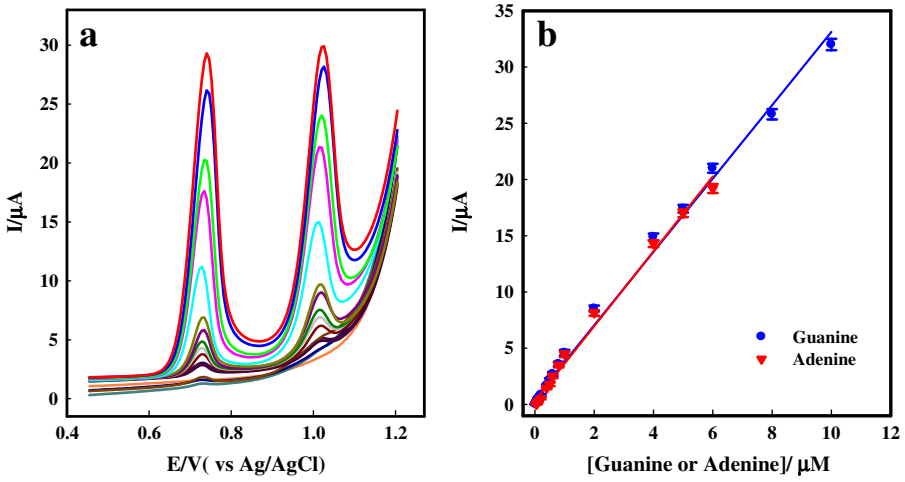


**Fig. 9.** (a) LSVs for the simultaneous determination of guanine and adenine in 0.1 M PBS at $Fe_3O_4$NPs/MWCNT/GCE with guanine and adenine concentration (0.01 to 10 μM); (b) calibration graphs for guanine (0.01–10 μM) and adenine (0.05–6 μM). Scan rate was 100 mV $s^{-1}$ and supporting electrolyte was 0.1 M in PBS of pH 7.

### 3.11. Analytical applications

The applicability of the modified electrode in biological samples was assessed by measuring adenine and guanine in DNA of fish sperm. Fig. 11a shows the LSVs of various concentrations of DNA in PBS (pH 7.0). As can be seen, the acid-denatured DNA gives two well-defined peaks due to the oxidation of guanine and adenine residues. Fig. 11 b shows calibration plots of adenine and guanine peak currents versus DNA concentration. There are linear relationships between DNA concentrations in the ranges of 0.04–5 and 0.06–5 μg $mL^{-1}$ vs. guanine and adenine peak currents, respectively. The experimental detection limit was found to be equal to 3 ng $mL^{-1}$ DNA. The determination of guanine and adenine concentrations was performed by standard addition method as following. Twenty microliters of denatured DNA was added in a cell containing 10 mL PBS of pH 7.0, and the peak currents of guanine and adenine residues were measured. Then a certain quantity of guanine or adenine solution was added and the corresponding peaks were measured again. From the differences between the peak currents, the concentrations of guanine and adenine in DNA were obtained using the corresponding calibration graphs. The contents of adenine and guanine in acid-denatured DNA were calculated to be 23.4% and 28.6% (mol%), respectively. Using the proposed method, the value (G + C)/(A + T)of 0.81 was obtained in HCl-denatured fish sperm DNA sample, which is in an acceptable agreement with the standard value of 0.77 [44].

**Table 2**
Comparison of different modified electrodes for simultaneous determination of adenine and guanine.

| Electrode | Guanine | | Adenine | | Method | Reference |
|---|---|---|---|---|---|---|
| | LR (μM) | DL (nM) | LR (μM) | DL (nM) | | |
| CILE[a] | 0.3–50 | 78.7 | 1.5–70 | 250 | CV | [13] |
| PTH/NPAu/MWNTs[b] | 0.05–5 | 10 | 0.05–5 | 8 | DPV | [23] |
| MW-CNCE[c] | 0.1–20 | 80 | 0.1–10 | 80 | DPV | [11] |
| MWCNT/GCE[d] | 0.05–10 | 20 | 0.05–10 | 80 | LSV | [12] |
| $TiO_2$-G/GCE[e] | 0.5–200 | 150 | 0.5–200 | 100 | DPV | [10] |
| $Fe_3O_4$NPs/MWCNT/GCE | 0.01–10 | 1 | 0.05–6 | 5 | LSV | This work |

[a] CILE: carbon ionic liquid on the carbon paste electrode.
[b] PTH/NPAu/MWNTs: polythionine electrodeposited on GCE modified with gold nanoparticles/CNT.
[c] MW-CNCE: sol–gel-derived CNT ceramic electrode prepared by microwave irradiation.
[d] MWCNT/GCE: GCE modified with carboxylated MWCNTs.
[e] $TiO_2$-G/GCE: $TiO_2$ nanoparticles-graphene nanocomposite modified GCE.

## 4. Conclusions

In this work, we used the excellent characteristics of $Fe_3O_4$NPs–MWCNT nano-composites to construct a highly sensitive sensor based on the GCE modified electrode for simultaneous determination of guanine and adenine. $Fe_3O_4$ NPs can effectively adsorb guanine and adenine on the electrode surface resulting in an increase in the oxidation currents respect to the MWCNT and also improve the solubility of the MWCNTs in solvent. The procedure enables preparation of stable and reproducible modifier films on the electrode surface, which leads to a considerable enhancement in repeatability and reproducibility in the voltammetric measurements. Remarkable improvements in the electron transfer kinetics of guanine and adenine were observed on the surface of the modified electrode, resulting in lowering the anodic overpotentials and considerably increasing sharpness of the waves and anodic peak currents. Very wide linear dynamic ranges, very low detection limits, high sensitivity, very good repeatability and reproducibility, and high stability, together with simple procedures for surface modification and determination can be presented as the advantages of the prepared sensor. The

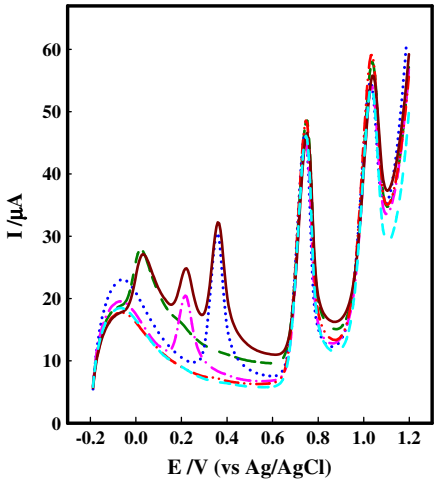


**Fig. 10.** LSVs for the simultaneous determination of 10 μM guanine and adenine in 0.1 M PBS (pH 7) at $Fe_3O_4$NPs/MWCNT/GCE in the presence of 1 mM AA (dashed line), 0.1 mM UA (dotted line), 10 μM DP(dashed-dotted line), 1 mM Glu (dashed-dotted-dotted line) and the mixture AA, UA, DP and Glu interferences (solid line). Scan rate: 100 mV $s^{-1}$.

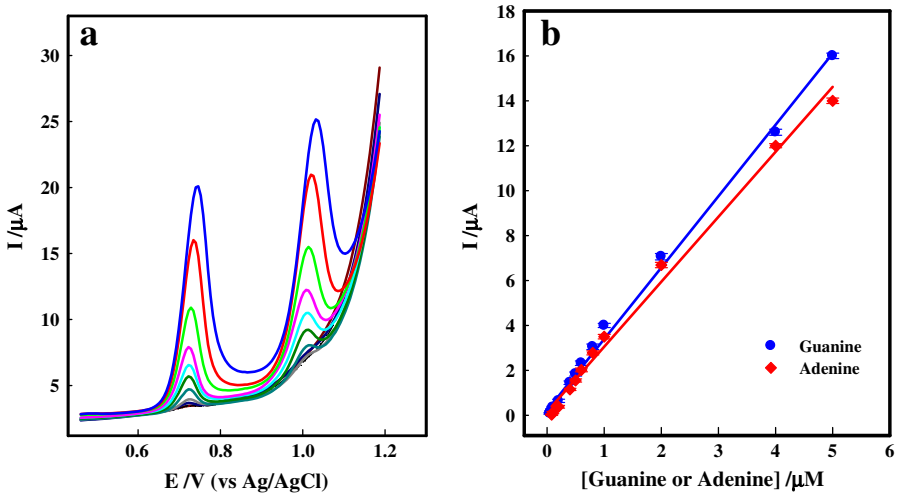


**Fig. 11.** (a) LSVs for various concentrations of DNA (0.04, 0.05, 0.06, 0.08, 0.1, 0.2, 0.4, 0.5, 0.6, 0.8, 1, 2, 4, 5 μg $mL^{-1}$) on $Fe_3O_4$NPs/MWCNT/GCE in 0.1 M PBS (pH 7), and (b) calibration graphs for adenine and guanine in DNA samples.

modified electrode can be used for the determination of trace amounts of guanine and adenine in fish sperm DNA. So, this simple and reliable strategy based on $Fe_3O_4$NPs/MWCNT/GCE is proposed for the development of an ultrasensitive voltammetric biosensor for simultaneous determination of guanine and adenine in biological systems.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at doi:10.1016/j.bioelechem.2012.02.004.

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