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Journal of Pharmaceutical and Biomedical Analysis
35 (2004) 905–912
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Electrochemical genosensor for Mitomycin C–DNA interaction based on guanine signal

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Received 4 December 2003; received in revised form 1 March 2004; accepted 2 March 2004

Available online 24 May 2004

Abstract

The interaction of mitomycin C (MC) with fish sperm or calf thymus DNA immobilized onto carbon screen-printed electrodes (CSPE) and carbon paste electrode (CPE) have been studied by using electrochemical techniques as square wave voltammetry (SWV) and differential pulse voltammetry (DPV). After the interaction was occurred between DNA and MC on electrode surface, it was observed that the guanine signal was higher with bare electrode than DNA-modified one. The changes in the experimental parameters such as the concentration of MC, and the accumulation time of MC were studied by using SWV and DPV. In addition, reproducibility, and detection limit parameters were determined using both electrodes. The partition coefficient of MC was also calculated before and after interaction of MC with dsDNA at CPE surface. These results showed that these two different DNA biosensors could be used for the sensitive, rapid and cost effective detection of MC–DNA interaction.

Keywords: DNA interactions; Electrochemical DNA biosensors; Mitomycin C; Guanine; DNA; Carbon screen-printed electrode; Carbon paste electrode

1. Introduction

There has been groving interest in studying DNA-targetted biosensors and DNA interactions in recent years. Such studies have application in the development of a technology for the detection and quantitation of some anticancer drugs found, as for several kinds of carcinogens and toxins. Some anticancer drugs and DNA interactions studies have been studied by using a variety of techniques [1–4] and there is a increasing interest in the electrochemical investigations of these interactions [5–19].

The antibiotic mitomycin C [20] [1aS-(1aα,8β, 8aα,8bα)]-6-amino-8-[[(aminocarbonyl)oxy]methyl)-1,1a,2,8,8a,8b-hexahydro-8a-methoxy-5 methylazirino [2′-4]pyrrolo[1,2-a]-indole-4,7-dione is an antitumor agent used in clinical chemoteraphy against a broad spectrum of solid tumors [21]. Mitomycin

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doi:10.1016/j.jpba.2004.03.001

isolated from *Streptomyces caespitosus*, is a natural antitumor antibiotic and used in anticancer chemoteraphy (especially for gastrointestinal cancer) [22,23]. Mitomycin C has sitotoxic character and this molecule also give a damage to normal human cells. Paz et al. reported [24] damage to DNA is generated by mono- and bifunctional alkylation of guanine residues by mitomycin C (MC), leading to MC-guanine monoadducts and MC-gunanine bisadducts; the latter constitute DNA interstrand and intrastrand cross-links.

Many scientists presented the electrochemical behavior of MC by using pulse polarography [25], pulse radiology [26,27], enzymatic methods [28–31] and voltammetry [32–35]. The interaction between the acid activated MC and DNA was performed by using hanging mercury drop electrode (HMDE) based on MC signal or guanine signal [6–8,36].

Marin et al. [7] showed the acid activated MC binds to DNA at pH 3.9 by using HMDE surface as detected by the decrease of guanine signal. Additionally they reported that the quinone group in the occured DNA–MC adduct was reversibly reduced at HMDE resulting with a 10 fold higher and 50 mV more negative cathodic peak. In another work of Marin et al. [36], direct determination of submicromolar concentrations of MC with an excess of 5-flurouracil or cisplatin in urine without any cleaning up step by using DNA biosensor.

In another study related to MC–DNA interaction, the cyclic voltammetry in connection with HMDE used for detecting the interaction between MC and DNA was studied based on the cathodic responses of MC both in single-sweep and repeated cycle modes [6].

Perez et al. [8] showed as the first time the electrochemical detection of DNA–MC adducts at HMDE by using different procedure such as the potential controlled interaction for MC with DNA at electrode surface.

There have not yet been any literature reports about the electrochemical detection of the interaction between MC and DNA by using solid electrodes such as, carbon screen printed electrode (CSPE) and carbon paste electrode (CPE) based on the changes of guanine signal. In this study, it was shown that the detection of MC–DNA interaction could be done faster, more sensitive and less laborious techniques by using these kinds of solid electrochemical genosensors.

2. Materials and methods

2.1. Apparatus and reagents

Electrochemical measurements for CSPE were performed by using square wave voltammetry (SWV) with an Autolab PGSTAT 10 electrochemical analysis system, with a GPES 4.5 software package (Ecochemie, Utrecth, Holland) in connection with a VA-Stand 663 (Metrohm, Milan, Italy).

The planar screen-printed electrochemical cell (1.5 cm × 3.0 cm) consist of three main parts which are a graphite working electrode, a graphite counter electrode and a silver pseudo reference electrode [37]. The procedure and reagents to make screen printed electrodes were reported elsewhere [38] The graphite working screen printed electrode surface is 3 mm in diameter. Each of them were used as disposable.

Electrochemical measurements performed with for CPE were investigated by using differential pulse voltammetry (DPV) with an AUTOLAB PGSTAT 30 electrochemical analysis system (Eco Chemie, The Netherlands). The three electrode system consisted of the carbon paste electrode (CPE) as the working electrode, a reference electrode (Ag/AgCl) and a platinum wire as the auxiliary electrode. The body of CPE was a glass tube (3 mm i.d.) tightly packed with the carbon paste. The electrical contact was provided by a copper wire inserted into the carbon paste. Carbon paste was prepared in the usual way by hand-mixing graphite powder (Fisher) and mineral oil (Acheson 38) in a 70:30 mass ratio. The surface was polished on a weighing paper to a smoothed finish before use. The convective transport was provided by a magnetic stirrer. The raw voltammograms were treated by using the Savitzky and Golay filter (level 2) included in the General Purpose Electrochemical Software (GPES) of Eco Chemie (The Netherlands) with moving average baseline correction defined as in the literatures [39,40] using a “peak width” of 0.01 V.

Double-stranded calf thymus DNA was obtained from Sigma (Milan, Italy) for CSPE studies. Double-stranded fish sperm DNA was obtained from Serva, (Germany). dsDNA stock solution (1000 μg/ml) was prepared with TE solution (10 mM Tris–HCl, 1 mM EDTA, pH 8.00) and kept frozen. More diluted solutions of DNA were prepared with 0.50 M acetate buffer solution (pH 4.80) (ABS). Other

chemicals were of analytical grade and they were purchased from Merck (Darmstadt, Germany).

MC stock solution (1000 μg/ml) was prepared in water and diluted MC solution was prepared in 20 mM Tris–HCl buffer solution (TBS) (pH 7.00).

3. Procedure

3.1. Procedure for voltammetric assay

All the experiments were performed at room temperature (25.0 ± 0.5) °C.

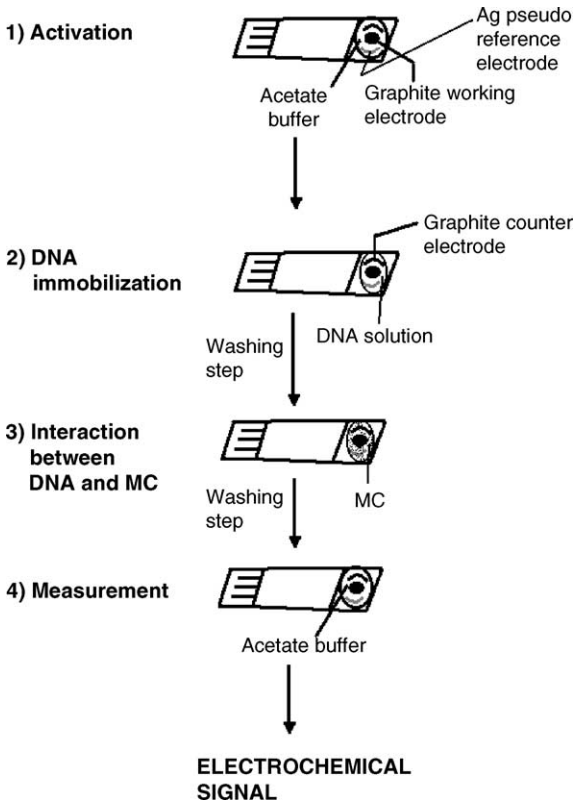
3.1.1. Interaction of MC with DNA at CSPE surface (shown in scheme)

This procedure was performed by following the similar steps in the study by Lucarelli et al. [16]. SCPE was activated by applying +1.80 V for 1 min in 100 μl drop of ABS. dsDNA was immobilised onto pretreated CSPE surface at a fixed potential of +0.50 V, for 5 min without stirring (Scheme 1). Each disposable strip was covered with a droplet containing 20 μg/ml of dsDNA in ABS. After this step, 80 μl new droplet of ABS for 5 s. Eighty microliter drop of MC solution which was prepared in 20 mM Tris–HCl buffer solution (pH 7.00) containing 1 μg/ml MC and 20 mM NaCl was put onto the CSPE surface for 2 min at open circuit system. After the interaction of MC was washed with 80 μl droplet of TBS for 5 s. SWV technique was used in order to evaluate oxidation signal of guanine on the CSPE surface. After washing step, electrode surface was covered with a droplet of TBS to obtain an oxidation signal of guanine. Square wave voltammetry parameters were used as 200 Hz frequency, 40 mV amplitude, 15 mV step potential and 0.2–1.35 V potential range versus Ag-Pseudo-reference electrode.

3.1.2. Interaction of MC with DNA at CPE surface

This procedure was performed by following the similar steps in the study by Karadeniz et al. [17]. CPE was activated +1.70 V for 1 min in ABS for electrode surface pretreatment. Fish sperm DNA was immobilised onto the pretreated CPE surface by applying potential at +0.5 V during 5 min in 0.5 M acetate buffer solution containing 10 μg/ml DNA and 20 mM NaCl with 200 rpm stirring. The electrode was then cleaned with blank ABS solution for 5 s for the removing of the unbound DNA at the electrode surface. Fish sperm DNA modified CPE was then immersed into 20 mM Tris–HCl buffer solution (pH 7.00) containing 5 μg/ml MC during 2 min with 200 rpm stirring. After the accumulation of MC, the electrode was rinsed with blank Tris-HCl buffer solution for 5 s. The oxidation signal of guanine was measured by using DPV in ABS containing 20 mM NaCl after the interaction with MC. The same protocol was applied for measurement of guanine signal before interaction with MC. Differential pulse voltammetry parameters were used as 50 mV amplitude, 15 mV step potential, 0.2–1.45 V potential range versus Ag/AgCl reference electrode.

The interaction procedure was repeated three times at CPE surface by using this procedure at above. The interaction between denatured DNA and MC was also studied by using the same method in order to ob-



Scheme 1. The procedure for CSPE consist of the following steps: (1) activation of electrode surface; (2) DNA immobilization on electrode surface; (3) interaction between MC and DNA; (4) measurement.

serve difference of the guanine oxidation signal after the interaction with MC. Before the interaction step, fish-sperm DNA was denatured at 95 °C by heating in the water bath during 6 min and then freezing this solution in the ice bath for 2 min. The denatured DNA was immobilized onto the electrode surface and then the difference in guanine peak high magnitude was showed after the measurements were done before/after MC interaction with denatured DNA.

Repetitive measurements were carried out by refreshing the surface and repeating the above assay formats by using both electrochemical transducers.

4. Results and discussion

Both electrochemical methods involves the monitoring the oxidation signal of guanine, which decreases in these experiments in the presense of MC.

4.1. The detection MC interaction with dsDNA at CSPE surface by SWV

The SWV peak currents of guanine were measured before and after interaction with MC at CSPE surface, shown in Fig. 1). The oxidation peak potential of the guanine was obtained at +0.9 V.

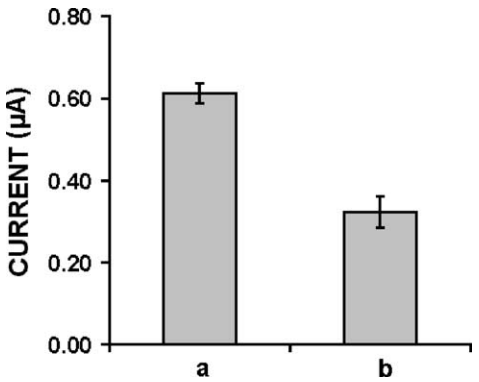


Fig. 1. Histomograms for the magnitude of guanine oxidation signals before interaction with MC (a); after interaction with MC (b) at surface of dsDNA-modified CSPE by using SWV. 20 μg/ml calf thymus-dsDNA immobilization on CSPE surface by applying at +0.5 V potential during 5 min; 1 μg/ml MC accumulation at open circuit system during 2 min; DPV measurement, scanning between +0.2 and +1.4 V in TBS with 20 mM NaCl.

The guanine peak obtained with the dsDNA modified electrode was higher than that one obtained after interaction with MC. After the interaction with MC, there was observed a decrease as 50% dramatically at guanine signal (Fig. 1a and b) A series of three repetitive SVW measurements of the interaction at 20 μg/ml concentration level of fish-sperm dsDNA with 1 μg/ml concentration level of MC at CSPE surface resulted in reproducible results such as a mean response 32.8 nA with a relative standard deviation of 8.7% was obtained. At this concentration level of MC and dsDNA, the detection limit estimated from S/N=3, corresponds to 33 ng/ml for MC at dsDNA modified CSPE in 2 min accumulation time.

The effect of experimental parameters including MC accumulation time and the effect of MC concentration on DNA were also studied in order to find optimum analytical performance. The concentration of MC has a pronounced effect its interaction with dsDNA at CSPE surface (Fig. 2). It was shown that guanine oxidation signal decreased gradually with increasing concentration of MC up to 1 μg/ml and then it levelled of.

Fig. 3 shows the effect of interaction time of MC with dsDNA. After interaction with MC there was a decrease on guanine signal till 2 min but it levelled of between 2 and 5 min.

After MC interaction with DNA at CSPEs surface, there was a decrease at guanine signal. This decrease marked a possible damage in the oxidizible groups of guanine that could be caused mutations basically on guanine bases. In parallel results to the study performed by Marin et al. [7], here we obtained a de-

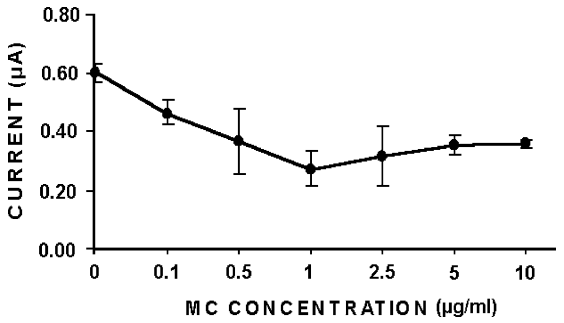


Fig. 2. The effect of MC at different concentrations at oxidation signal of guanine by using ds DNA modified CSPE. Other conditions are as in Fig. 1.

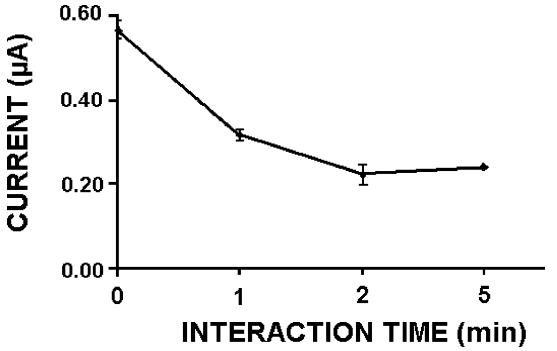


Fig. 3. The effect of interaction time of MC with DNA upon the SWV response based on oxidation signal of the guanine at dsDNA-modified CSPE surface. Other conditions are as in Fig. 1.

crease at guanine oxidation signal after MC interaction with dsDNA and/or ssDNA. After the interaction between MC and DNA, it was shown here that the maximum decrease at guanine peak was observed in 1 μg/ml concentration level of MC.

4.2. The detection of MC interaction with dsDNA and denatured DNA at CPE surface by DPV

Fig. 4 shows the DPV signals of guanine observed with dsDNA modified electrode (A) and denatured dsDNA modified CPEs (B). When MC was interacted with dsDNA guanine oxidation signal was decreased gradually (Fig. 4A(a) and A(b)). We also observed similar results with denatured DNA-modified CPE (Fig. 4B). This dramatic decrease may claim that this interaction between DNA and MC by through possible interaction with guanine.

In comparison to previous studies performed by HMDE [6–8,36], our results showed that the utility of these electrodes such as CSPE and CPE for the determination of interaction between DNA and MC is more simpler, much more cost-effective, and also they provide rapid detection of the interaction between DNA and MC by non-toxic agents.

A series of three repetitive DPV measurements based on guanine signal for the interaction at 10 μg/ml concentration level of fish sperm DNA with 5 μg/ml MC and 10 μg/ml concentration level of denatured DNA with 5 μg/ml MC at CPE surface resulted in reproducible results, respectively, such as a mean response of 164.3 nA and 220 nA with a relative standard deviation of 7.2% and 6.9% was obtained. At 10 μg/ml concentration level of fish sperm DNA with 5 μg/ml concentration level of MC, the detection limit estimated from S/N=3, corresponds to 30 ng/ml and at 10 μg/ml concentration level of denatured DNA with 5 μg/ml concentration level of MC, the detection limit estimated from S/N=3, corresponds to 19 ng/ml for MC at DNA modified CPE in 2 min accumulation time.

The guanine oxidation signal obtained with dsDNA modified CPE dramatically decreased after interaction

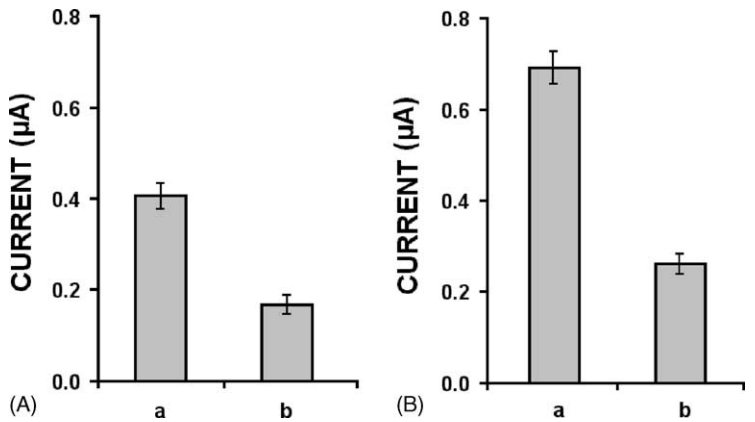


Fig. 4. Histomograms for the magnitude of guanine oxidation signals before DNA interaction with MC (a); after DNA interaction with MC (b) at CPE by using DPV, with (A) 10 μg/ml fish sperm DNA, (B) 10 μg/ml denatured fish sperm DNA. Fish sperm DNA or denatured DNA immobilization on CPE surface +0.5 V during 5 min with stirring; MC accumulation at open circuit system during 2 min in solution containing 5 μg/ml MC with stirring; measurement scanning between +0.2 V and + 1.35 V in ABS with 20 mM NaCl.

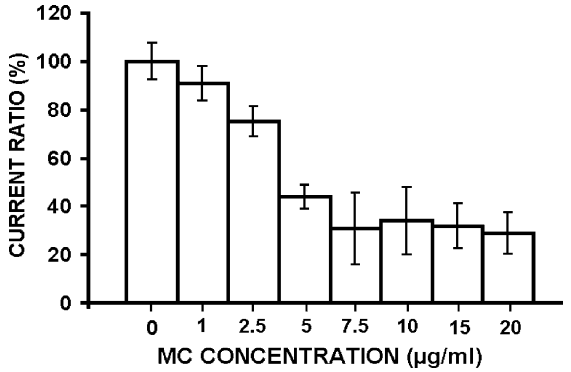


Fig. 5. The effect of various concentrations, such as 0, 1, 2.5, 5, 7.5, 10, 15 and 20 μg/ml of MC for interaction with DNA based on the calculated current ratio using the peak heights of guanine before and after DNA interaction with MC at CPE surface. Other conditions are as in Fig. 4.

of DNA with concentration of MC from 1 μg/ml to 5 μg/ml. It was almost leveled of between 5 and 20 μg/ml (Fig. 5).

Fig. 6 displays the accumulation time study for MC at dsDNA immobilized CPE based on the changes in the guanine signal before/after interaction with MC at electrode surface. Different accumulation time of MC in the range from 1 to 10 min was applied by using DNA-modified CPE. There was a gradual decrease at guanine signal till 1 min and then it was sharply decreased till 2 min. It levelled off after 2 min.

The partition coefficient is an important factor in order to discover an idea about interaction mechanism

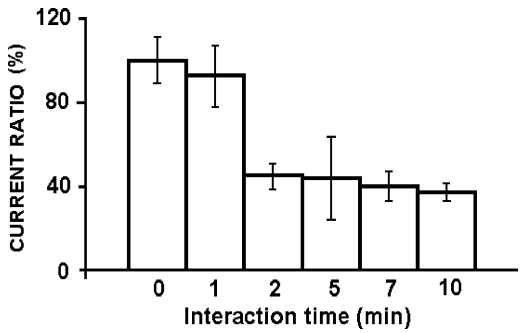


Fig. 6. The effect of different interaction times, such as 0, 1, 2, 5, 7 and 10 min for interaction of MC with DNA based on the calculated current ratio using the peak heights of guanine before and after DNA interaction with MC at CPE surface. Other conditions are as in Fig. 4.

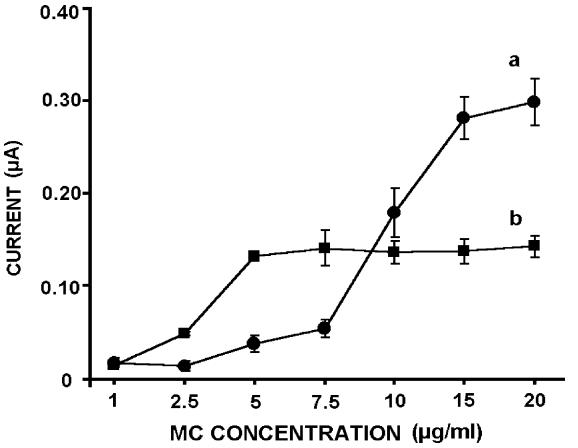


Fig. 7. Calibration data for the oxidation signals of MC obtained in various concentrations after MC interaction with 10 ppm dsDNA-modified CPE (a) and with bare CPE (b). Other conditions are as in Fig. 4.

of drug with DNA. Here, it was investigated by using DNA-modified CPE to determine the behavior of bound and free MC molecules towards DNA.

According to the Millan and Mikkelsen's reference method [41], the calibration data obtained at the dsDNA modified (Fig. 7a) and at bare CPE (Fig. 7b) were used to estimate the partition coefficient of MC on the CPE surface as in the following equation:

$$\text{Partition coefficient} = \text{MC}_{\text{bound}}/\text{MC}_{\text{free}} = |(i_{\text{bound}} - i_{\text{free}})/i_{\text{free}}|$$

i_{free} is the oxidation peak current of MC obtained at bare CPE i_{bound} the oxidation peak current of MC obtained at dsDNA-modified CPE after its interaction with DNA. The oxidation peak current of MC was obtained at +0.78 V by using bare CPE (not shown). The partition coefficient of MC at dsDNA modified CPE was found 0.71 as calculating i_{free} (Fig. 7b) and i_{bound} (Fig. 7a) for MC before and after DNA interaction with MC of 5 μg/ml concentration level.

4.3. The comparison of the performance between CSPE and CPE

Our results have shown that both electrodes might be used for the detection of MC interaction with DNA directly. CPE is more suitable and more reproducible than CSPE; on the other hand, CSPE is disposable and

suitable for the DNA microarray technology. It could be emphasized that both electrochemical genosensors could be used for detection of DNA interactions with some molecules as toxic or anticancer drugs like MC, or chemical war toxicants.

5. Conclusions

The utility of these electrochemical genosensors for interaction between DNA and MC is simple, cost-effective and they provide rapid detection. The DNA modified CSPE or CPE were used in combination with SWV and DPV to obtain the information about the interaction of mitomycin C with ds/ssDNA, based on the changes at guanine signal.

These two voltammetric methods are experimentally convenient and sensitive so that they requires only small amounts of materials.

DNA biosensors also eliminate the need for some difficult analyze techniques, such as radioisotopes, require easy detection and provide short detection time. About the DNA biosensors reported to date, it seems clear that electrochemical biosensors for medical and environmental monitoring, have a very promising future.

The determination of interaction between DNA and DNA-targetted molecules would be valuable in the design of the molecule-specific electrochemical biosensor for application in diagnosis tests and in the development of drugs for the chemotherapy.

The results have shown that these studies can play an important role in developing newly produced chemotherapotic compounds, also the usage of voltammetric techniques for drug–DNA interactions will provide to discover unknown drug–DNA interaction mechanisms.

Acknowledgements

D.O. acknowledges a scholarship from the Organisation for the Prohibition of Chemical Weapons (OPCW) for their financial support and acknowledges to Prof. Marco Mascini and his Biosensor group in Firenze University, Department of Chemistry, Sesto Fiorentino, Italy. A.E. acknowledges Turkish Academy of Sciences, in the framework of the Young Scientist Award Program (KAE/TUBA-GEBIP/2001-2-8). H.K. acknowledges a scholarship for graduate students from Scientific and Technical Research Council of Turkey (TUBITAK).

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