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A label-free Electrochemical DNA Biosensor for the Determination of Low Concentrations of Mitoxantrone in Serum Samples

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Herein, a sensitive, accurate and precise DNA biosensor for the quantification of low levels of mitoxantrone in serum samples was presented. This approach is based on the interaction of the drug with double-stranded DNA and subsequent electrochemical transduction. Chitosan as a positive polymer was used for the immobilization of the DNA on the surface of a carbon paste electrode (working electrode). After optimization of different experimental parameters, the proposed biosensor showed a wide concentration linear range of 0.030-3.50 mg L^{-1} and 3.50-120.0 mg L^{-1} with a detection limit of 0.0013 mg L^{-1}. It was found that the optimized approach could be applied to the determination of mitoxantrone in serum samples with acceptable recovery values.

Keywords: Mitoxantrone, DNA biosensor, Voltammetry, Chitosan

1. INTRODUCTION

Mitoxantrone (MTX) is a synthetic anthracycline with the chemical structure given in Fig. 1. As one can see, it has a planar anthraquinone ring which can intercalate between the base pairs of DNA helix and two nitrogen-containing side chains which can electrostatically bind to the negatively charged phosphate backbone of DNA. These interactions have been demonstrated to inhibit the nucleic acid synthesis [1]. So MTX is used as an antitumor drug against breast and prostate cancers, acute leukemia and non-Hodgkins lymphoma [2, 3]. Due to higher efficacy and lower side effects, MTX is

used widely. On the other hand, since MTX is present in low concentrations in body fluids [4], development of highly sensitive and precise methods for a clinical assay that allows accurate quantification of low concentrations of MTX in biological samples be of great importance. Several analytical methods have been reported for the determination of MTX, including resonance Rayleigh scattering [5], high-performance liquid chromatography [HPLC] [6-10], chemiluminescence [11, 12] and capillary electrophoresis [13]. However, the current methods are not sensitive enough, require expensive equipment, their sample processing is complicated and time-consuming, as well as, their analytical conditions are difficult. However, electrochemical approaches may offer certain advantages, such as requiring easier sample preparation, being less time-consuming and offering detection limits and dynamic ranges comparable to or even better than the mentioned methods. Also, these methods have proven to be useful for the determination of organic molecules including drugs [14-16]. Biosensors are small devices, which utilize biological reactions for detecting different analytes [17]. A typical biosensor consists of a biorecognition element which interacts specifically with the analyte of interest and a physicochemical transducer which relays the resultant signal from this biorecognition event [18]. Electrochemical biosensors using DNA as the recognition element produce affinity biosensors in which the analyte of interest is exposed to the electrode surface after interaction with the DNA immobilized on the electrode surface [19]. The DNA-trapped compounds can either be detected directly if they are electroactive molecules [20, 21] or indirectly via changes in the electrochemical signal of DNA made after interaction with the analyte [22, 23]. Electroactivity of MTX [24], makes it possible to directly determine the concentration of MTX through measuring its electrochemical current as the analytical signal. Direct determination of the analyte benefits the selectivity of the electrochemical method whereas measurement of the changes made in the oxidation signal of DNA possesses no such selectivity [21]. Herein, an electrochemical DNA biosensor is proposed and evaluated for the quantification of MTX in serum samples. The biosensor is fabricated based on the immobilization of DNA on the surface of a chitosan-modified carbon paste electrode. The electrochemical behavior of MTX at the as-prepared biosensor is investigated using differential pulse voltammetry [DPV] due to its excellent speed of analysis, low consumption of the electroactive species and few problems with blocking the electrode surface [25].

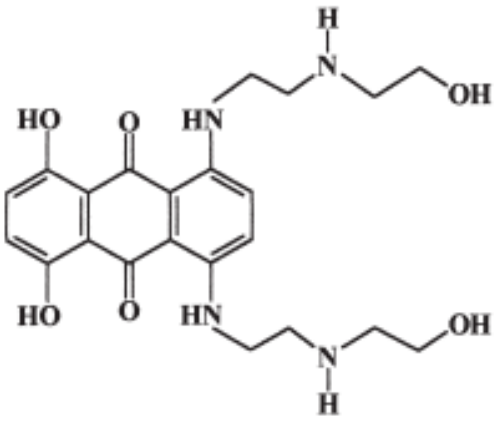

Figure 1. Structure of Mitoxantrone

2. EXPERIMENTAL

2.1. Apparatus

All voltammetric experiments were performed with an Autolab electrochemical system from Metrohm (Herisau, Switzerland) interfaced with a personal computer for data acquisition and potential control. A three-electrode system consisted of a platinum wire as the auxiliary electrode, Ag/AgCl/KCl (saturated) as the reference electrode and a carbon paste electrode (CPE) as the working electrode, was used. All pH measurements were carried out using a Metrohm 827 pH meter supplied with a combined glass reference electrode.

2.2. Reagents and materials

Chitosan was purchased from Acros (Geel, Belgium). Pure graphite powder was obtained from Merck [Darmstadt, Germany]. Two mg mL^{-1} mitoxantrone hydrochloride solution as a synthetic sample solution of Novantrone was supplied by Lederle Laboratories (Gosport, UK) and kept in a refrigerator away from light to avoid photodecomposition. To prepare different concentrations of MTX, a certain amount of stock solution is diluted with 0.025 M phosphate buffer [pH 8.0]. The sodium salt of salmon sperm DNA was purchased from Acros, and its stock solution was prepared by dissolving solid DNA in distilled water and kept frozen.

2.3. Preparation of serum samples

During the first hour of collection, the human blood samples were centrifuged for 10 min at 1500 rpm for the separation of serum. The obtained serum samples were stored in a refrigerator at 4°C until analysis. One mL of each serum sample was transferred into the centrifuge tubes containing different known amounts of MTX and then mixed well with one mL methanol to precipitate the blood proteins. After centrifugation for 15 min at 6000 rpm, supernatants were collected and then diluted to 10 mL with 0.025 M phosphate buffer (pH 8.0).

2.4. Electrode preparation

Chitosan-modified carbon paste electrode (CHIT/CPE) was prepared by mixing graphite powder and chitosan [90/10 W/W%] and wetting with paraffin oil. The paste was carefully hand mixed in a mortar for 20 min to ensure uniform dispersion and then packed into on end of a glass tube (3.4 mm inner diameter). Electrical contact was established via a copper wire connected to the paste. The resulting electrode was designated as CHIT/CPE. The electrode surface was gently smoothed by rubbing it on a piece of filter paper just before use. When necessary, a new surface was obtained by pushing a bit of the paste out of the tube. Bare electrode designated as bare CPE was prepared in the same way without adding chitosan. The electrodes were stored at room temperature when not in use.

2.5. DNA immobilization on the surface of CHIT/CPE

CHIT/CPE was immersed into 20 mL 0.50 M acetate buffer solution (pH 4.8) containing 10 mg L^{-1} DNA and then 0.50 V was applied to the electrode for 400 s while stirring the solution. The electrode was then washed thoroughly with acetate buffer solution. Thus a DNA-coated CHIT/CPE was obtained. The DNA was similarly immobilized on the surface of bare CPE to prepare DNA-coated CPE.

2.6. Cyclic voltammetry of $K_3[Fe[CN]_6]$

Cyclic voltammogram of 0.05 mol L^{-1} $K_3Fe[CN]_6$ in 0.50 mol L^{-1} KCl was recorded from -200 to 700 mV at a scan rate of 100 mV s^{-1}.

2.7. Electrochemical measurement

Differential pulse voltammetry (DPV) was used for recording the electrochemical signal of MTX. For this purpose, DNA-coated CHIT/CPE was immersed in 0.025 M phosphate buffer solution (pH 8.0) containing given concentration of MTX or into the prepared serum sample where the accumulation of MTX on the electrode surface was performed at an open circuit system for 7.0 min. DP voltammogram of the accumulated MTX was then recorded using a pulse amplitude of 0.05 V and a scan rate of 100 mV s^{-1}. The current of the oxidation peak of MTX at about +0.5 V was taken as analytical signal due to its correlation with the MTX concentration.

3. RESULTS AND DISCUSSION

3.1. Cyclic voltammetry studies on the immobilization of DNA on CHIT/CPE

Cyclic voltammograms of $Fe[CN]_6^{3-/4-}$ redox couple as an indicator for DNA immobilization were investigated at the surface of bare CPE, CHIT/CPE, and DNA-coated CHIT/CPE. According to the obtained results shown in Fig. 2, the current of MTX peaks at CHIT/CPE increased in comparison to the unmodified electrode (bare CPE). Also, reversibility was also improved. After immobilization of DNA at the surface of CITH/CPE, both anodic and cathodic peak currents decreased considerably accompanied with increasing the peak-to-peak potential separation. The negatively charged phosphate groups of the immobilized DNA resisted the access of the redox couple to the electrode surface due to electrostatic repulsion which in turn resulted in the less active surface area for the redox process of $Fe[CN]_6^{3-/4-}$ couple. These observations confirmed the immobilization of DNA on the surface of CHIT/CPE.

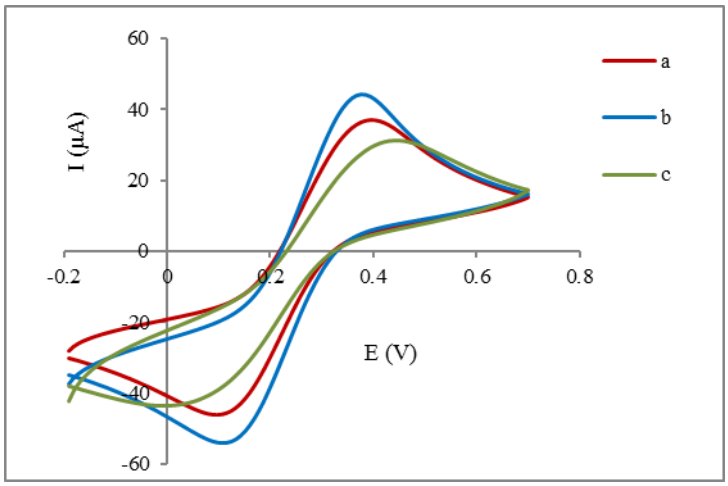

Figure 2. Cyclic voltammograms of 0.050 mol L^{-1} $K_3Fe(CN)_6$ in 0.5 mol L^{-1} KCl at the surface of (a) bare CPE (b) CHIT/CPE (c) DNA-coated CHIT/ CPE at the scan rate of 100 mV s^{-1}.

3.2. Electrochemical behavior of MTX at the surface of DNA-coated CHIT/CPE

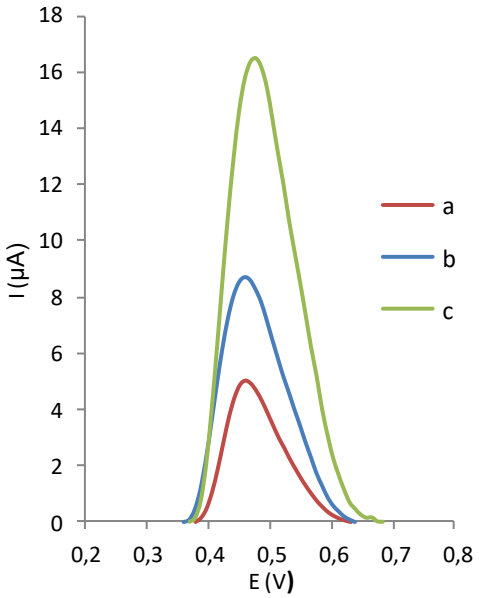

Figure 3. DP voltammograms obtained after the accumulation of MTX on the surface of bare CPE (a), CHIT/CPE (b) and DNA-coated CHIT/CPE (c). Conditions: 60 mg L^{-1} MTX, 0.025 mol L-1 phosphate buffer pH 8.0, accumulation time 7 min, immobilization time 400 s, chitosan percentage 10%.

Fig. 3 represents the DP voltammograms of MTX at the surface of bare CPE, CHIT/CPE and DNA-coated CHIT/CPE at the scan rate of 100 mV s^{-1}. As can be seen, at bare CPE, MTX shows a sharp oxidation peak at about 0.46 V which increases noticeably at CHIT/CPE. At DNA-coated CHIT/CPE, the anodic current increases even more accompanied by a positive shift in the oxidation potential. The improvement made in the current can be as a result of the accumulation and

preconcentration of MTX on the DNA-coated CHIT/CPE surface through a strong interaction with the immobilized DNA layer led to the enhanced sensitivity [20]. The positive shift observed in the peak potential of MTX may propose an intercalative mode of interaction between MTX and the DNA [21].

3.3. Optimization of the experimental parameters

3.3.1. Chitosan percentage

Chitosan is a cationic polymer which can electrostatically attract negatively charged DNA strands and form polyelectrolyte complexes with DNA. In the present study, chitosan was used to immobilize DNA on the electrode surface. So, the amount of DNA immobilized depends on some chitosan presents in the carbon paste which in turn influences on the level of the accumulated MTX and subsequently, the oxidation current. To optimize the amount of chitosan, DNA-coated CHIT/CPEs containing different percentages of chitosan (5-20%) were prepared and used for the determination of 60 mg L^{-1} MTX. Fig. 4 revealed that the electrode modified with 10% chitosan was greatly enhanced the oxidation signal. At the percentages lower than 10% sufficient DNA amounts were not immobilized and on the other hand, at higher percentages, the amount of DNA immobilized was too high that prevented the effective electron transfer between the electrode surface and MTX. Therefore, 10% chitosan was used for the biosensor construction in subsequent experiments.

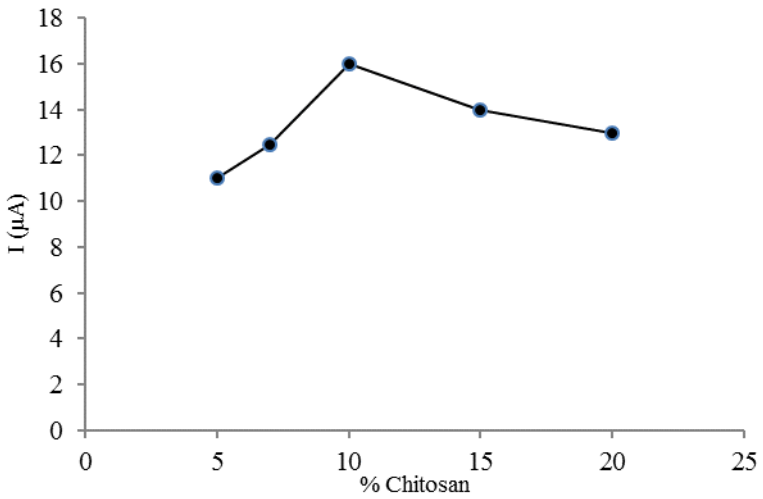

Figure 4. Effect of chitosan percentage on the oxidation peak current of MTX. Conditions were the same as given in Fig. 3 except chitosan percentage.

3.3.2. pH of the supporting electrode

The electrode reaction might be affected by pH of the medium in which the electrochemical signal was recorded. So, the electrooxidation of 80.0 mg L^{-1} MTX in 0.025 mol L^{-1} phosphate buffer solution with different pHs in the range of 4.0–10 was recorded using DP voltammetry [Fig. 5A]. According to the obtained results, the highest electrochemical current was observed at pH 8. Therefore,

this pH was selected as optimum for further experiments. In addition, the anodic peak of MTX shifted towards more negative potentials with increasing pH indicating that the oxidation of MTX was a pH-dependent reaction, as can be seen in Fig. 5A. Fig. 5B illustrates the linear relationship between the peak potential and pH with the linear regression equation of E/V = -0.0588 pH + 0.9325 and the correlation coefficient [R2] of 0.9836.

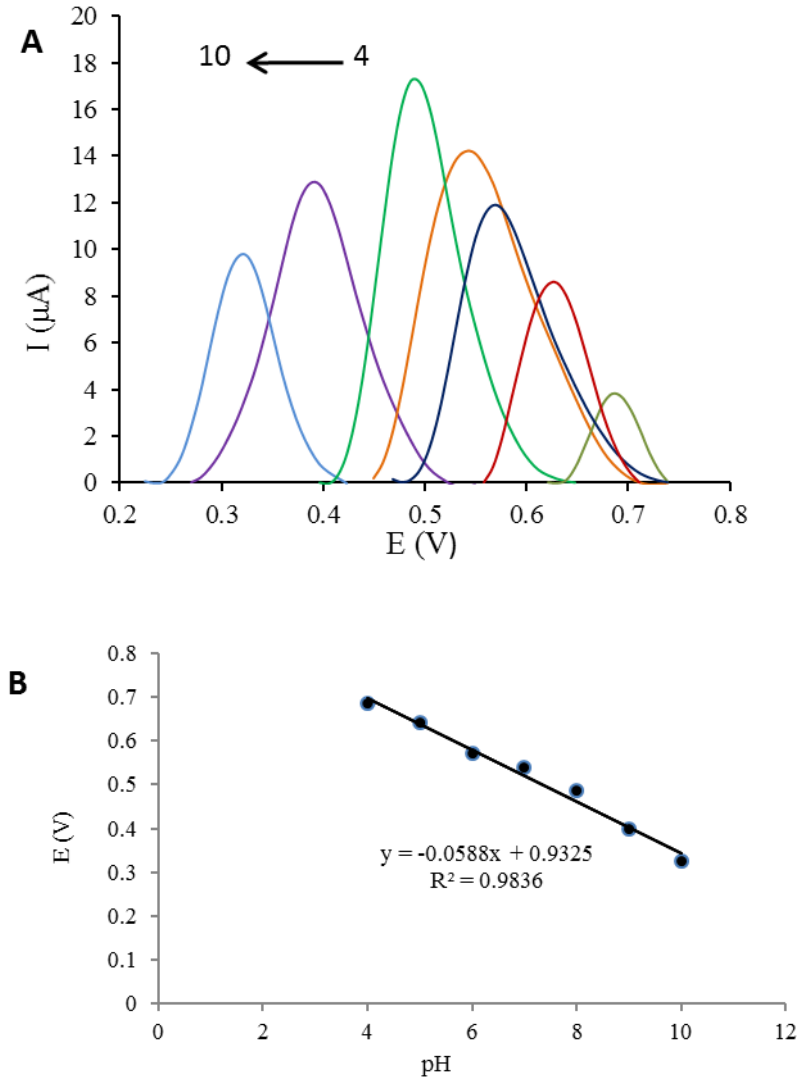

Figure 5. (A) DP voltammograms of 80 mg L^{-1} MTX at the surface of DNA-coated CHIT/CPE at different pHs of 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 and 10.0. Conditions were the same as given in Fig. 3 except pH (B) Plot of pH versus E.

The value of the slope is close to the theoretical value of −0.059 V/pH, demonstrating that the electrode process is an equal proton-electron transfer [26]. The obtained data is in close agreement with a reversible reaction involving two protons and two electrons, as previously reported for the oxidation of MTX [27-29]. That is, there are two electrons and two protons associated with catechol group, involved in the electrode process of MTX. It has been reported that MTX is oxidized in two

anodic processes; oxidation of 5,8-hydroxyl substituents on MTX occurs at less positive potentials, and oxidation of the aminoalkyl substituent in MTX is observed as an anodic peak at higher potentials about 0.5 V [27]. Fig. 6 depicts these two peaks observed at the surface of DNA-coated CHIT/CPE. In the second process considered here due to the higher sensitivity, two –OH groups are oxidized to produce the quinone which is in agreement with the electrochemical mechanism of two-proton and two-electron transfer as understood from the slope of the potential-pH diagram. During the second oxidation process, the stable metabolite cyclic hexahydronaphthol-[2,3-f]-quinoxaline-7,12-dione is formed and the reaction proceeds via formation of free radicals leading to further conversion to an unstable, fully oxidized diimino compound [24].

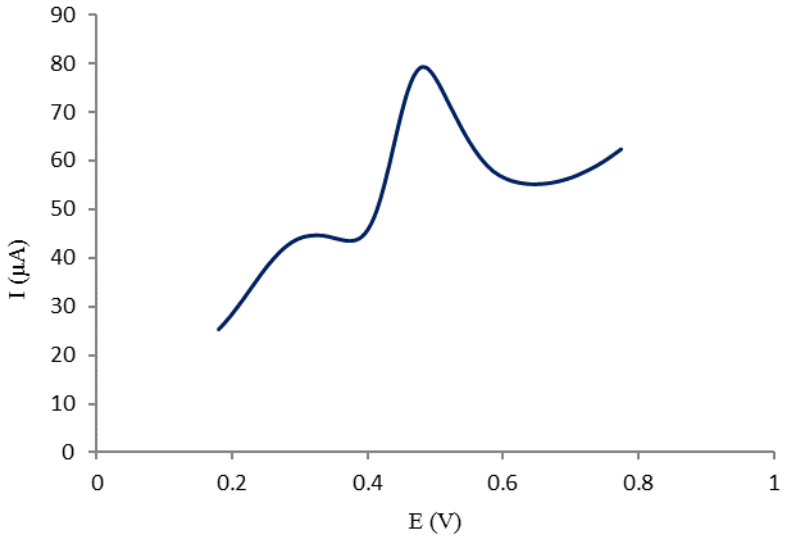

Figure 6. Electrochemical Oxidation of 60 mg L^{-1} MTX on DNA-coated CHIT/CPE. Conditions were the same as given in Fig. 3.

3.3.3. Concentration of the supporting electrolyte

The changes in the oxidation signal of MTX accumulated on the surface of the DNA-coated CHIT/CPE from different concentrations of the phosphate buffer [0.006-0.20 mol L^{-1}] were studied. The obtained results revealed that increasing the buffer concentration to more than 0.025 mol L^{-1} led to a decline in the oxidation current of MTX. Thus, 0.025 mol L^{-1} was chosen as the optimal concentration of the supporting electrolyte.

3.3.4. Accumulation time

Accumulation time is defined as the interval time in which MTX interacts with the DNA immobilized on the CHIT/CPE surface and subsequently, adsorbs on the surface resulting in MTX preconcentration. Therefore, the value of the oxidation signal of MTX is highly influenced by the accumulation time. The experiments on the effect of accumulation time on the electrochemical current

showed that 7 min is the optimal time for the accumulation of the maximum amount of MTX on the electrode surface (Fig. 7).

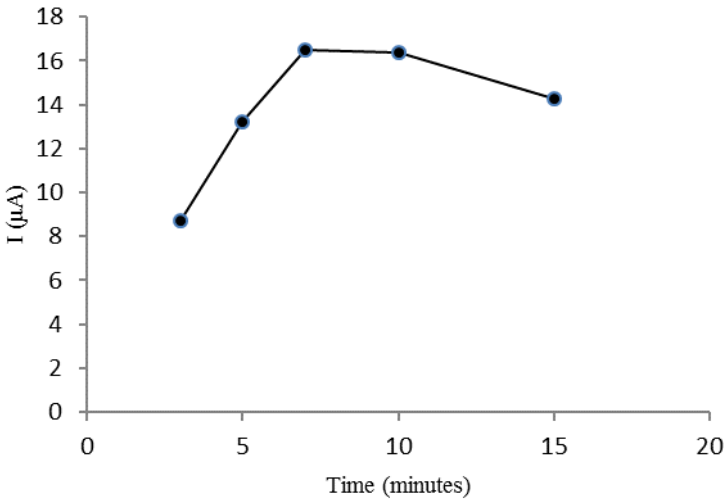

Figure 7. Effect of accumulation time on the electrochemical current of mtx. Conditions were the same as given in Fig. 3 except accumulation time.

3.3.5. Immobilization potential

The electrical potential applied to the surface of CHIT/CPE in the DNA immobilization step influence the amount of the DNA immobilized, the MTX accumulated and hence, the oxidation current. For the optimization of the immobilization potential, different potentials within the range of 0 to 0.7 V were applied to the CHIT/CPE in the DNA solution and then, the oxidation signal of MTX on the obtained DNA-coated electrodes. According to the results obtained, the potential around 0.5 V vs. Ag/Ag CL was obviously favorable for obtaining the maximum accumulation of MTX resulted in the maximum peak current. Therefore, the potential of 0.50 V was selected as optimal immobilization potential.

3.3.6. Immobilization time

We also investigated the signal intensities of the MTX biosensors fabricated under different times of DNA immobilization. It was found that a maximum anodic signal of MTX was achieved when the optimal potential of 0.5 V was applied to the DNA solution for 400 s. Consequently, 400 s is considered the optimal time for the DNA immobilization.

3.3.7. DNA concentration

Since the amount of the DNA immobilized on the electrode surface plays an important role on the peak current of the biosensor, the effect of the DNA concentration in the solution from which the

DNA was immobilized on the CHIT/CPE surface, was investigated. It was observed that the peak current increased with increasing the DNA concentration up to 10 mg L^{-1} and then tended to be stable. Accordingly, in subsequent experiments, the immobilization of DNA was performed using 10 mg L^{-1} DNA.

3.4. Calibration curve and detection limit

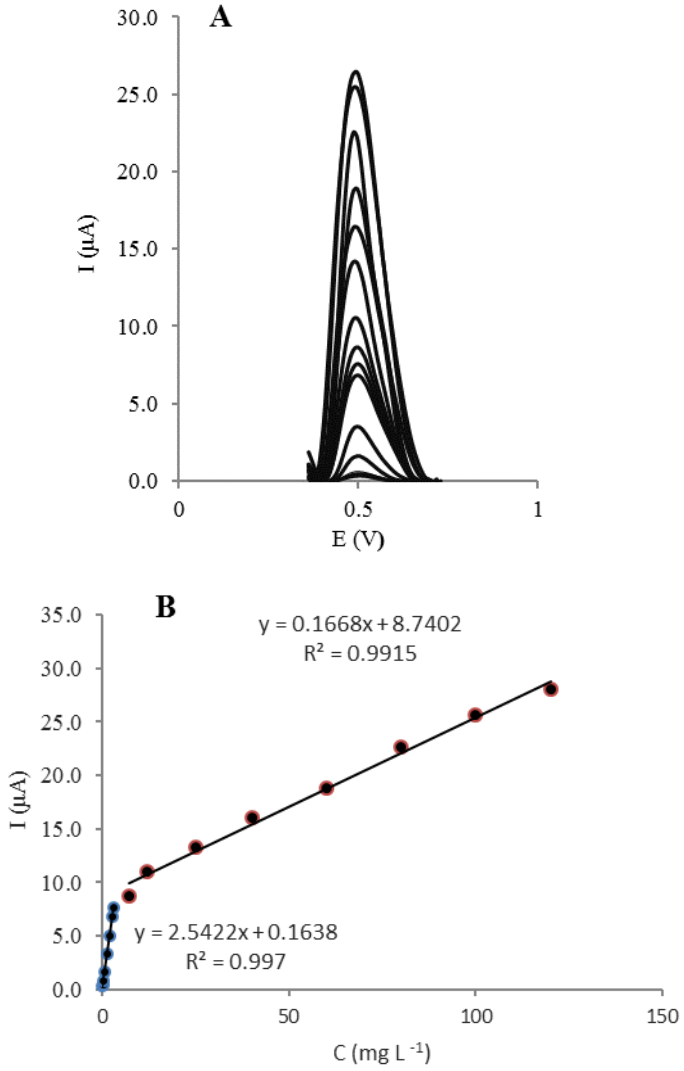

Figure 8. (A) DP voltammograms of different concentrations of MTX (from down to up: 0.030, 0.10, 0.30, 0.60, 1.0, 2.0, 3.0, 7.0, 12.0, 20.0, 40.0, 60.0, 80.0, 100.0 and 120.0 mg L^{-1}) obtained at the surface of DNA-coated CHIT/CPE. (B) Calibration curve for MTX determination extracted from the voltammograms given in Fig. 8A.

Under the optimized parameters, differential pulse voltammograms of increasing concentrations of MTX were recorded indicating that the peak current increased with increasing the MTX concentration, as depicted in Fig. 8A. The plot of peak current vs. the concentration of MTX

(Fig. 8B) consisted of two linear segments in the concentration ranges of 0.03–3.5 mg L^{-1} and 3.5–120 mg L^{-1} following the equations of y = 2.5422x + 0.1638 and y = 0.1668x + 8.7402, respectively. The difference in the slopes obtained for the calibration segments is due to the different activities of the biosensor surface in low- and high-concentration solutions of MTX. In low concentrations, [the first segment], a large number of binding sites (in the DNA) relative to MTX molecules exists at the sensor surface resulted in a larger slope of the calibration curve compared with the slope of the second segment corresponding to high concentrations of MTX [30]. Limit of detection (LOD) calculated as 3sb/m (sb is the standard deviation of blank measurements, and m is the slope of the calibration curve) was found to be 0.0013 mg L^{-1}. To ascertain the repeatability of the analysis, five measurements on 2.0 mg L^{-1} and 40.0 mg L^{-1} MTX solution were carried out using DNA-coated CHIT/CPE. Relative standard deviation [RSD] of the obtained peak currents was found to be 6.3% and 2.4%, respectively for 2.0 mg L^{-1} and 40.0 mg L^{-1} MTX.

3.5. Effect of coexisting compounds on the signal of the proposed biosensor

The effects of some possible interfering compounds such as glucose, urea, uric acid and ascorbic acid on the determination of MTX with DNA-coated CHIT/DNA were investigated. The tolerance limit was defined as the maximum amount of the interfering compounds that created an error of less than ±5% for the measurement of MTX [12]. A species that cause a variation of more than 5% in the biosensor response to MTX was considered as an interfering substance. According to the results given in Table 1, no significant interference from commonly encountered matrix components was observed. It can be concluded that the proposed biosensor is applicable to the analysis of MTX in biological samples.

Table 1. Effect of interfering of coexisting compounds on the determination of 3.0 mg L^{-1} MTX under the optimal conditions.

Substance	Tolerance concentration ratio
Glucose	1000
Urea	500
Ascorbic acid	300
Uric acid	250

3.6. Detection of MTX in human serum sample

In order to verify the accuracy of the approach, the standard addition method was applied for the determination of MTX in a human serum sample. The serum sample prepared as mentioned previously was analyzed for MTX using the DNA-coated CHIT/CPE, and the corresponding DP voltammograms were recorded. The obtained results are reported in Table 2. As may be seen, the recoveries of the spiked samples are satisfactory indicating the acceptable accuracy of the approach. Thus, the designed biosensor is reliable for the determination of MTX in serum samples.

Table 2. Determination of MTX in a serum sample using the proposed method.

Sample	Added (mg L^{-1})	Found[a] (mg L^{-1})	Recovery (%)
Human serum	0	-	-
	0.5	0.49±0.01	98 %
	15	15.3±0.6	102 %

[a] Mean ± standard deviation (n = 3).

3.7. Comparison

The electrochemical biosensor presented here was compared with some other approaches previously reported for the MTX determination [28, 31, 32, 33]. As shown in Table 3, DNA-coated CHIT/CPE has a wide linear range (4 orders of magnitude), which is greater than the other sensors except one of them [31]. For LOD, a similar situation is observed so that the proposed biosensor presents a LOD lower than the other sensors except for Ref. 31. These characteristics along with the excellent repeatability of the proposed approach show its applicability to the quantification of MTX at low concentrations.

Table 3. Comparison of the presented method with some other electrochemical sensors

Electrode	Linear range (mg L^{-1})	LOD (mg L^{-1})	Ref
CS-dispersed graphene modified glassy carbon electrodes	0.00027- 0.44	8.9×10^{-5}	[31]
Co–carbon fiber ion implantation modified ultramicroelectrode	0.089-2.7	0.019	[28]
Pt/C Implanted Modified Microelectrode	0.031-11	0.018	[32]
DNA-coated CPE	---	0.036	[33]
DNA-coated CHIT/CPE	0.03 - 120	0.0013	This work

4. CONCLUSION

In this work, an electrochemical DNA-biosensor was designed and fabricated for the determination of MTX based on its interaction with the DNA immobilized on CHIT/CPE. This interaction resulted in an effective accumulation and preconcentration of MTX on the electrode surface. Additionally, DNA immobilization was performed using chitosan which is a biocompatible, biodegradable and non-toxic cationic polymer that forms polyelectrolyte complexes with DNA. So, the proposed approach is green in addition to its sensitivity, rapidity, and repeatability. The DNA-coated CHIT/DNA was found to be applied successfully for analysis of biological samples.

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References

1. N. Li, Y. Ma, C. Yang, L. Guo and X. Yang, *Biophys. Chem.*, 116 (2005)199.
2. R. Tietze, E. Schreiber, S. Lyer and C. Alexiou, *Biomed. Res. Int.*, 2010(2010)8.
3. G. An and M. E. Morris, *J. Pharm. Biomed. Anal*., 51 (2010) 750.
4. P. Zhang, G. Ling, J. Sun, Y. Sun, X. Pu, Z. Wan and Z. He, *J. Chromgra*. B, 878 (2010) 2260.
5. S. Liu, F. Wang, Z. Liu, X. Hu, A. Yi and H. Duan, *Anal. Chim. Acta*., 601 (2007) 101.
6. K. M. Rentsch, R. A. Schwendener and E. Hanseler, *J. Chromatogr*. B, 679 (1996) 185.
7. K. E. Choi, J. A. Sinkule, S. C. McGrath, K. M. Daly and R. A. Larson, *J. Chromatogr. B: Biomed. Sci. Appl.*, 420 (1987) 81.
8. S. L. Law and T. F. Jang, *J. Chromatogr.* A, 234 (1994) 670.
9. B. Payet, Ph. Arnoux, J. Catalin and J. P. Cano, *J. Chromatogr. B: Biomed. Sci. Appl*., 424 (1988) 337.
10. L. Slørda, A. Andersen and D. J. Warren, Ther. *Drug. Monit*., 15 (1993) 328.
11. H. Yao, M. Zhang, W. Zeng, X. Zeng and Z. Zhang, *Spectrochim. Acta A: Mol. Biomol. Spectrosc.*, 117 (2014) 645.
12. F. Yu, L. Chen and F. Chen. *Microchim. Acta*, 161 (2008) 185.
13. S. Han and H. Wang, *J. Chromatogra*. B, 878 (2010) 2901.
14. C. Radovan, D. Cinghita, F. Manea, M. Mincea, C. Cofan and V. Ostafe, *Sensors*, 8 (2008) 4330.
15. C. D. Souza, O. C. Braga, L. C. Vieira and A. Spinelli, *Sens. Actuat. B: Chem*., 135 (2008) 66.
16. S. A. Ozkan, B. Uslu and H.Y. Aboul-Enein, *Crit. Rev. Anal. Chem*., 33 (2003) 155.
17. A. F. Collings and F. Caruso, *Rep. Prog. Phys*., 60 (1997) 1397.
18. J. P. Chambers, B. P. Arulanandam, L. L. Matta, A. Weis and J. J. Valdes, *Curr. Issues Mol. Biol*., 10 (2008) 1.
19. A. M. Oliveira-Brett and V.C. Diculescu, *Bioelectrochem*., 64 (2004) 143.
20. M. Mohamadi, A. Mostafavi and M. Torkzadeh-Mahani, *J. Electrochem. Soc*., 162 (2015) B344.
21. M. Mohamadi, A. Mostafavi and M. Torkzadeh-Mahani, *Bioelectrochem*., 101 (2015) 52.
22. L. Mirmoghtadaie, A. A. Ensafi, M. Kadivar and P. Norouzi, *Mater. Sci. Eng*., 33 (2013) 1753.
23. M. H. Banitaba, S. S. Davarani and A. Mehdinia, *Anal. Biochem*., 411 (2011) 218.
24. A. M. Oliveira-Bretta, T. R. A. Macedob, D. Raimundo, M. H. Marquesb and S. H. P. Serrano, *Anal. Chim. Acta*., 385 (1999) 401.
25. K. M. Naik, C. R. Ashi and S. T. Nandibewoor, *J. Electroanal. Chem*., 755 (2015) 109.

26. A. J. Bard and L. R. Faulkner, Electrochemical Methods: Fundamentals and Applications, Wiley, (1980), New York.
27. J. C. C. Villar, A. C. García and P. T. Blanco, *Talanta*, 40 (1993) 325.
28. Y. Mao, J. Hu, Q. Li and P. Xue, *Analyst*, 125 (2000) 2299.
29. B. Bozal-Palabiyik, S. Kurbanoglu, M. Gumustas, B. Uslu and S. A. Ozkan, *Rev. Roum. Chim.*, 58 (2013) 647.
30. A. J. Bard and L. R. Faulkner, Electrochemical methods: principles and applications. Electrochemical Methods: Principles and Applications, (2001).
31. B. Hong and Q. Cheng, *Advances in Chemical Engineering and Science*, 2 (2012) 453.
32. Y. G. Qian, Z. J. Sun, J. B. Hu, Y. Q. Lu and Q. L. Li, *Chem. Res. Chin. Univ*., 20 (2004) 103.
33. A. ERDEM and M. ÖZSÖZ, *Turk. J. Chem*., 25 (2001) 469.