Int. J. Electrochem. Sci., 9 **(2014)** 1771 - 1783

International Journal of
ELECTROCHEMICAL
SCIENCE
www.electrochemsci.org

The Behavior of Ciprofloxacin at a DNA Modified Glassy Carbon Electrodes

Nizam Diab[1,*], *Ibrahim Abu-Shqair*[2], *Radi Salim*[2] *and Mohammad Al-Subu*[2]

[1]Arab American University– Jenin; P.O. Box: 240 Jenin, Palestine
[2]An-najah National University; P.O. Box: 7 Nablus, Palestine
[*]E-mail: ndiab@aauj.edu

Received: 3 December 2013 / *Accepted:* 8 January 2014 / *Published:* 2 February 2014

The voltammetric behavior of ciprofloxacin was investigated using cyclic voltammetry and differential-pulse anodic stripping voltammetry at bare glassy carbon (GC) electrodes and DNA-modified glassy carbon (DNA-GC) electrodes. For both types of electrodes, only one anodic irreversible wave was observed. A comparison between the current responses for the ciprofloxacin at the modified DNA-GC and unmodified GC electrodes, it was showed that the DNA- modified electrode exhibits a significant enhancement of the voltammetric current response with a better peak shape. Also, the interaction of ciprofloxacin with DNA was studied by using cyclic voltammetry technique at (DNA-GC) electrodes, which showed a weak interaction with a binding constant (K) = 2.89 x 10^5 M^{-1}. A linear relationship between the peak current and ciprofloxacin concentrations was observed in the range 1.0–10.0 µM, with a slope a detection limit of 0.117 µM, with r = 0.998, and 1.0 µM.

Keywords: ciprofloxacin, modified electrode, DNA-modified electrode, anodic stripping voltammatry, differential pulse voltammetry

1. INTRODUCTION

In recent years there has been an increase in the use of nucleic acids as a tool in the recognition and monitoring of many compounds of analytical interest by using DNA as a surface-modification element in electrochemical biosensors [1]. DNA electrochemical sensors have a variety of possible applications in detection of small molecules (e.g. drugs, pollutants, carcinogens, etc.) binding to the structure of DNA. One of the most important applications of DNA biosensors is related to the determination and studying the behavior of drugs, dues to the fact that the interaction of drugs with

DNA is one of the most important aspects in drug discovery and development. These interactions are responsible for the desired action of such drugs [2].

Ciprofloxacin, [1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(piperazinyl) -3-quinoline carboxylic acid], (figure 1) which belongs to a group of fluoroquinolones that is used as an antibiotic [3] and widely used in the treatment of the infections caused by Anthrax infections [4]. Different analytical methods have been used for the determination of ciprofloxacin in its pharmaceutical preparations. These methods include spectrophotometric [5,6], chromatographic [7,8], capillary electrophoresis [9,10], conductometric methods [11]. Electrochemical methods have been used for investigation of quinolones [12,13], for example ciprofloxacin was determined by anodic stripping voltammetry technique at mercury and carbon paste electrodes [14], and the presence of a carbonyl group attached to the quinolones nucleus, in conjunction with carboxylic acid group leads to study the electrochemical behavior for this class of drugs [15].

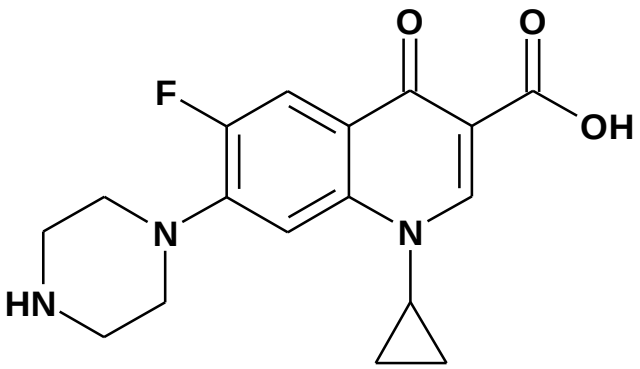

Figure 1. The chemical structure of ciprofloxacin.

It is known that quinolones are active against the DNA-gyrase enzyme, a type II topoisomerase. It is believed that DNA-gyrase introduces negative supercoils in DNA [16], and several structural models have been suggested to account for the action of quinolones. In common, all suggested models require a direct interaction between the drug and either single or double-stranded DNA [17,18]. Although the exact mechanism for the reaction between the drug and the DNA is still unclear, thus, contribution to deeper insight into the mechanism of interaction of this class of antibiotics with DNA is important for a better understanding of their therapeutic efficiency [19]. Modification of the electrode surface with an immobilized layer of DNA was successfully used for the accumulation of drugs [20], therefore in this communication, we aim at the elucidation of the voltammetric behavior of ciprofloxacin in aqueous solution at bare glassy carbon (GC) and DNA-modified glassy-carbon electrodes and evaluating the binding constant of the ciprofloxacin-DNA complexes to investigate the nature of binding of ciprofloxacin drug with DNA.

2. EXPERIMENTAL

2.1. Chemicals

Ciprofloxacin drug was obtained from Sigma. Calf thymus DNA (sodium salt, type 1) was obtained from Sigma-Aldrich Chemie GmbH (Steinheim, Germany) and was used as received without further purification. 0.2 M acetate buffer solution pH 5 was used to prepare and condition the DNA-modified electrode. Single stranded DNA (ssDNA) solution was prepared by a previously reported method [21]. Exactly 3 mg of DNA were dissolved with 0.5 mL of 65% pure perchloric acid, and then 0.5 mL of 9 M NaOH was added to neutralize the solution. The volume was completed to 10 mL with and the solution was kept at 4 ^{o}C. All solutions were prepared using analytical grade reagents and triply distilled water.

2.2. Electrode modification

A glassy carbon electrode (0.071 cm^2) was polished with alumina powder and was then rinsed thoroughly with distilled water to obtain a mirror-like, shiny electrode surface. The dsDNA-modified electrode was prepared by a previously established method [21]. In short, 3 mg of dsDNA were dissolved in 80 μL of acetate buffer solution, and this solution was placed onto the GC electrode surfaces using a pipette and allowed to dry. The electrode was then placed into three electrode electrochemical cell containing acetate buffer (pH 5) and conditioned at +1.4 V for 5 minutes. Afterwards, the electrode was scanned following a differential pulse voltammetry profile in ssDNA solution from 0 and +1.4 V (vs Ag/AgCl) to check that no electrochemical reaction is taking place on the surface of the modified electrode in the supporting electrolyte. The conditioning process was repeated until stable peak currents were obtained for guanine and adenine oxidation. The electrode was then transferred into acetate buffer solution for two minutes, then removed and left to dry. This procedure led to the formation of a relatively thick DNA layer with good conductivity on the electrode surface [22].

2.3. Instrumentation

All voltammetric measurements were carried out using an EG & G polarographic analyzer/stripping voltammeter model 264B coupled with 303A stand, 305 automatic stirrer and RE 0150 X-Y recorder. All experiments were performed using an electrochemical cell of 10 mL with a three electrode system consisting of a bare glassy carbon electrode (GCE) or a dsDNA-modified glassy carbon electrode (dsDNA-GCE) as a working electrode, a platinum wire as an auxiliary electrode and an Ag/AgCl/(3M KCl) as a reference electrode. The pH measurements were carried out using HANNA pH meter model HI 8424.

3. RESULTS AND DISCUSSION

3.1. Interaction of ciprofloxacin with DNA in solution:

The interaction of the ciprofloxacin with DNA in solution was studied by using cyclic voltammetry technique at the surface of a bare glassy carbon electrode.

Figure 2 shows the cyclic voltammograms of the ciprofloxacin drug in the absence and presence of dsDNA at a bare glassy carbon electrode, it is clearly shows a single irreversible anodic peak, which reveals most probably that the oxidation takes place at the piperazine moiety [23,24].

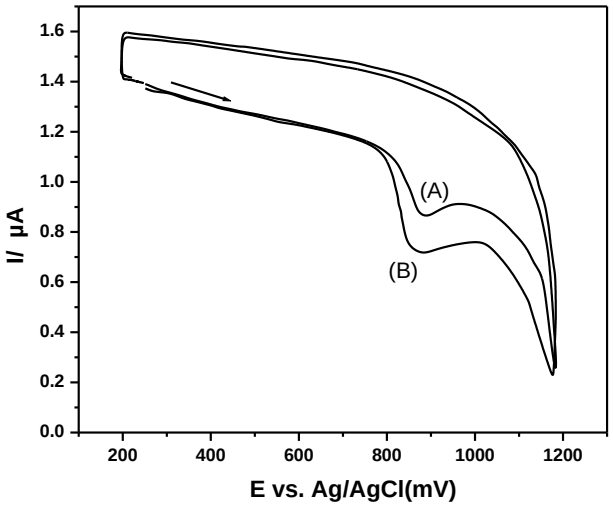

Figure 2. Cyclic voltammograms of 3×10^{-5} M ciprofloxacin in acetate buffer solution (pH 5) in: (A) the absence and (B) presence of 1.2×10^{-3} M DNA (scan rate: 100 mVs^{-1}).

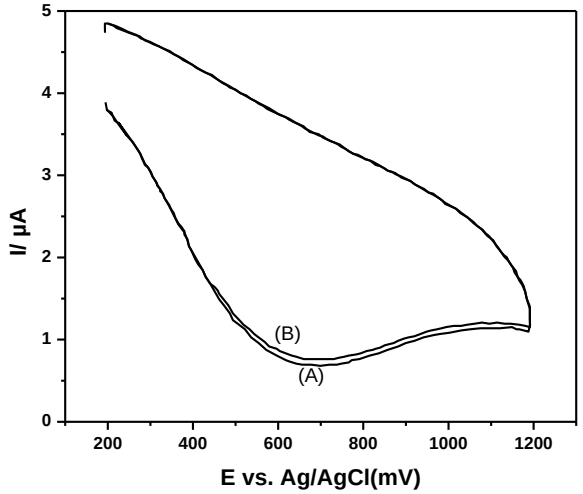

Figure 3. Cyclic voltammograms of $K_4Fe(CN)_6$ in: (A) the absence and (B) the presence of 1.2×10^{-3} M DNA (scan rate: 100 mVs^{-1}).

Addition of calf thymus dsDNA to a solution of the drug caused a marked decrease in the current response and a shift of the peak potential to more positive values as shown in Figure 2. This might be attributed to the binding of the ciprofloxacin to the bulky and slowly diffusing DNA, which results in a considerable decrease in the apparent diffusion coefficient. The shift of peak potential to more positive value indicates that the drug has properties of intercalative binders [25].

In order to demonstrate that the decrease in current response is due to the slow diffusion rate of ciprofloxacin-DNA complex, and not to the increased viscosity of the solution or the blockage of the electrode surface by DNA adsorption, a special cyclic voltammetry experiment was performed in $K_4Fe(CN)_6$ solution in the absence and also in the presence of dsDNA solution. In these solutions, the ions of $Fe(CN)_6^{4-}$ do not interact with DNA because of the coulombic repulsions between their negative charges. Figure 3 shows that the addition of DNA to $Fe(CN)_6^{4-}$ solution affected the current only slightly and no shift of peak potentials was observed. This confirms that there is no obvious effect on diffusion from the changed viscosity of the solution. This indicates also that there is no significant obstruction of the electrode surface from DNA adsorption. Thus the great decrease in current in CV experiments (Figure 2) could be attributed to the diffusion of the drug bound to DNA with large molecular weight.

The change in current and shift in potential upon DNA addition can be used to quantify the binding of the ciprofloxacin to DNA. The association of an electroactive molecule (EM) with a binding site (S) composed of *s* base pairs on a DNA duplex, to form a complex (EM-S) can be expressed as:

$$EM + S \leftrightarrow EM–S \qquad (1)$$

And if the equilibrium constant of this reaction is given by:

$$K = C_b/(C_fC_s) \qquad (2)$$

Where, K is the equilibrium constant of the EM-S complex, and C_b, C_f and C_s are the equilibrium concentrations of EM-S, free EM and free S, respectively.

The total concentration of the electroactive molecule, C_t, is given by the equation:

$$C_t = C_b + C_f \qquad (3)$$

The ratio of the nucleotide phosphate concentration (C_{NP}) to the total concentration of elecroactive molecule (C_t) can be defined as R and C_{NP} was determined by UV spectrometry at 260 nm.

For an irreversible reaction at 25°C, the total anodic current (I_p) with any R can be calculated by [26]:

$$I = B\,[(\alpha n)_f^{1/2} D_f^{1/2} C_f + (\alpha n)_b^{1/2} D_b^{1/2} C_b] \qquad (4)$$

Making appropriate substitutions of C_f and C_b, an equation for I_p is obtained [27]:

$$I_p = B\,\{(\alpha n)_f^{1/2} D_f^{1/2} C_t + [(\alpha n)_b^{1/2} D_b^{1/2} - (\alpha n)_f^{1/2} D_f^{1/2}]\,[b - (b^2 - 2K^2 C_t^2 R/s)^{1/2}]\,/(2K)\} \qquad (5)$$

Where, $B = 2.99 \times 10^5 n A v^{1/2}$, α : electron transfer coefficient, n : number of electrons, A : electrode surface area, s is the binding site size of the electroactive molecule interacting with DNA and $b = 1 + KC_t + KRC_t/(2s)$.

Equation (5) is valid for the assumption of non-cooperative, non-specific binding to DNA with the existence of one type of discreet binding site. The diffusion coefficients of EM and EM-DNA (D_f,

D_b), the binding constant (K) and the binding site size (s) of EM-DNA can be obtained by non-linear fit analysis of the experimental data (I_p and R) according to equation (5).

The peak currents (I_p) of the ciprofloxacin and ciprofloxacin-DNA complexes were examined as a function of scan rate (v). The plot of I_p vs $v^{1/2}$ for both the free and bound ciprofloxacin is shown in Figure 4. The plots were linear for both free and bound ciprofloxacin indicating an irreversible electrode process without surface adsorption. This means that the oxidation process was controlled by of the electroactive species to the electrode surface [26].

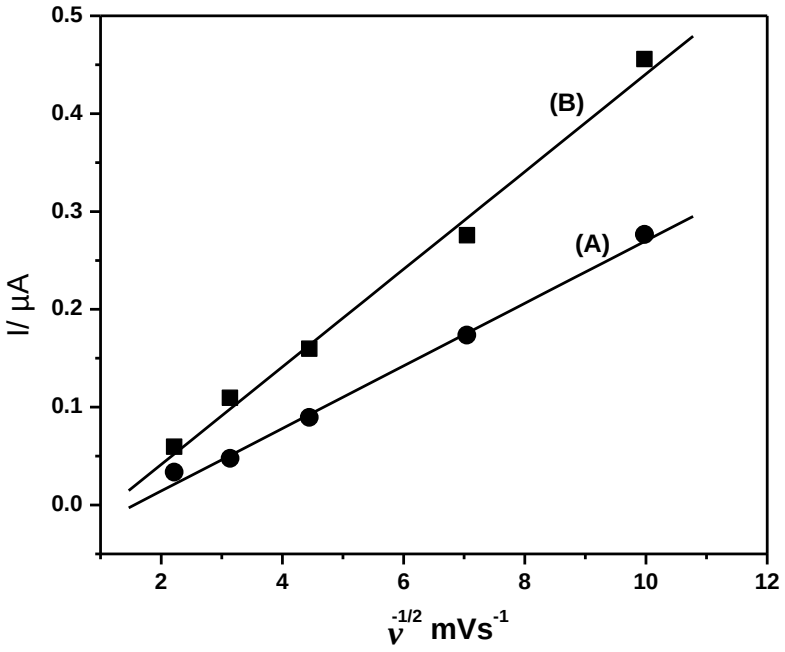

Figure 4. Plot of I_p vs $v^{1/2}$ of acetate buffer solution (pH 5) containing 3×10^{-5} M ciprofloxacin in the (A) absence and (B) presence of 1.6×10^{-4} M DNA.

Furthermore, the smaller linear slope of ciprofloxacin-DNA complex demonstrates that the ciprofloxacin drug can bind with DNA in solution, forming ciprofloxacin-DNA adduct with large molecular weight, resulting in a considerable decrease in the apparent diffusion coefficient [28].

The relationship between E and ln v over the studied range (5 – 100 mV) was studied for the drug and drug-DNA complexes according to the classical equation of peak potential for an irreversible electrode process [29]:

$$E = E^o + RT/(\alpha nF)\ \{0.780 + 0.5 \ln [\alpha nDFv/(RT)] - \ln k^o\} \quad (6)$$

Where;

E^o: the formal electrode potential.

α : electron transfer coefficient.

k^o: the standard heterogeneous rate constant.

R: universal gas constant = 8.3145 J/mol.K

F: Farady = 96485 coulomb.

D: diffusion coefficient.

According to equation (6) the relation between E_p and ln*v* should be linear, and the plots of E_p versus ln*v* are showed in Figure 5. The value of αn can be obtained from the slope of the straight lines in the absence and in the presence of DNA; in the absence of DNA the slope of E_p vs ln*v* for the drug is calculated to be 0.0237, and the value of αn is 0.541. While in the presence of DNA the slope of E_p vs ln*v* is 0.0132, and αn is 0.972.

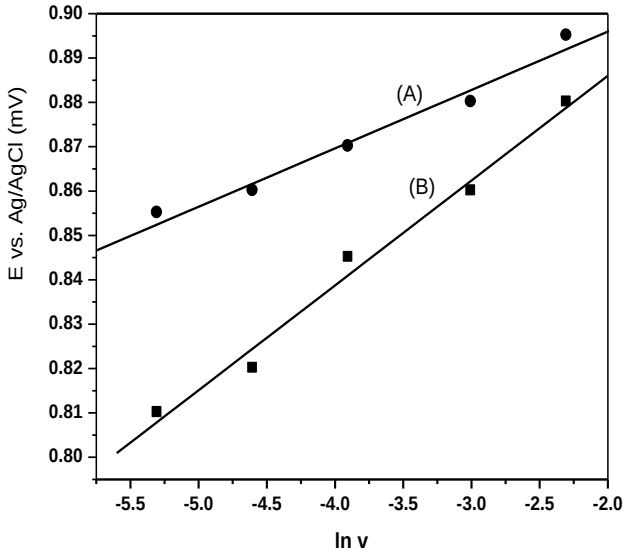

Figure 5. Plot of E_p vs ln*v* of acetate buffer solution (pH 5) containing 3×10^{-5} M ciprofloxacin in (A) the absence and (B) the presence of 1.2×10^{-3} M DNA.

A non linear fit analysis of the data to equation (5) yielded the binding curves shown in Figure 6. The diffusion coefficients of both free and bound ligands (D_f, D_b), the binding constant (*K*) and the binding site size (*s*) were simultaneously obtained by non–linear fit analysis of the electrochemical data.

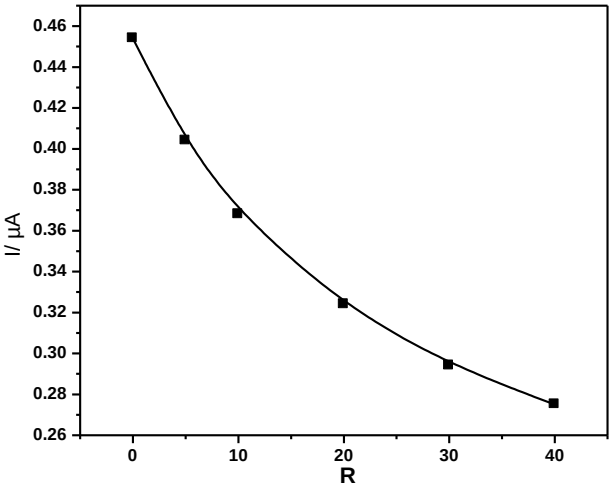

Figure 6. Binding curve of 3 x 10^{-5} M ciprofloxacin with DNA acetate buffer solution (pH 5). Scan rate: 100 mVs^{-1}. ($R= C_{NP}/C_t$)

The results illustrate that the ciprofloxacin binds to dsDNA. However, the binding constant value for ciprofloxacin–DNA complex (2.89 x 10^5) is smaller than those obtained for molecules that are known to bind strongly to DNA such as anti-tumor drugs ($K \geq 10^7$) [25, 27-**Error! Bookmark not defined.**8]. This indicates a weaker binding between ciprofloxacin and DNA. As seen the value of the calculated binding site size (0.10) is a fraction, which indicates that the ciprofloxacin drug cannot be considered as typical intercalator. The positive shift in oxidation potentials for the drug upon binding to DNA, suggests that intercalative properties of ciprofloxacin cannot be ruled out completely. On the other hand, the small value of the binding constant and binding site size require a non–intercalative (electrostatic) mode of interaction with DNA.

3.2. Interaction of ciprofloxacin with DNA at the Electrode Surface

Cyclic voltammetry (CV) and differential-pulse anodic stripping voltammetry (DPASV) techniques were used for investigating the electrochemical oxidation behavior of ciprofloxacin at the DNA-modified glassy carbon electrode, as a potential biosensor for the determination of this drug.

Figure 7 shows the cyclic voltammograms that were obtained after the accumulation of the ciprofloxacin at a bare glassy carbon electrode and dsDNA-modified glassy carbon electrode for 1.0 x 10^{-5} M ciprofloxacin in 0.2 M acetate buffer solution for 1 min at open circuit conditions.

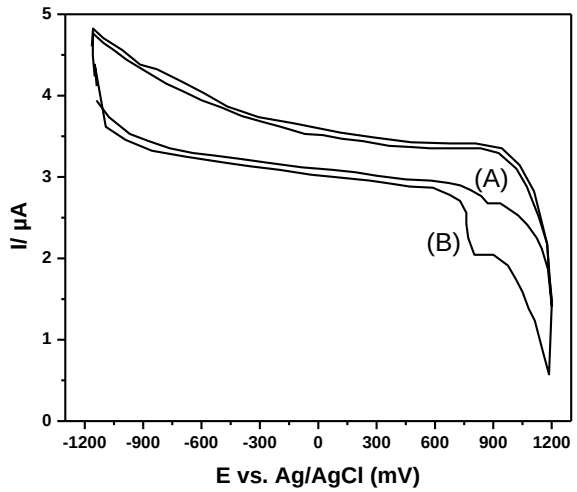

Figure 7. Cyclic voltammograms of 1×10^{-5} M ciprofloxacin in acetate buffer solution (pH 5) at: (A) bare GC electrode and (B) DNA-modified GC electrode. (scan rate: 100 mV/s. Initial potential: +0.2 V, a pre-concentration time: 1 min).

For both types of electrodes, one wave was observed during oxidation of the drug, and no waves were obtained in the reverse direction, which characterizes irreversible electrode processes which agreed with previous studies using carbon paste and graphite electrodes for the oxidation of ciprofloxacin [30-32]. Also, figure 7 shows that the DNA-modified electrode exhibit a larger anodic current signal compared to unmodified glassy carbon electrode. This behavior reflects the binding of

ciprofloxacin drug with surface-confined DNA layer. The presence of the nucleic acid coating the glassy carbon electrode surface greatly enhances the sensitivity. In addition, the DNA-modified glassy carbon electrode showed a shift in the oxidation wave of the studied drug to less positive potentials that agreed with a recently study for interaction of ciprofloxacin with DNA [33]. Also, we have seen similar results for norfloxacin and enrofloxacin drugs, both members of the fluoroquinolones family (unpublished).

3.3.Oxidation of ciprofloxacin using differential pulse Anodic Stripping Voltammetry:

It is obviously seen that the DNA-modified GC electrode has much higher ability to pre-concentrate the ciprofloxacin drug compared to unmodified GC electrode (Figure 8). Similar results for pefloxacin-which is a member of fluoroquinolones family- were shown using carbon paste electrodes modified by dsDNA [34]. Moreover, the DNA modified electrode exhibits a better peak shape.

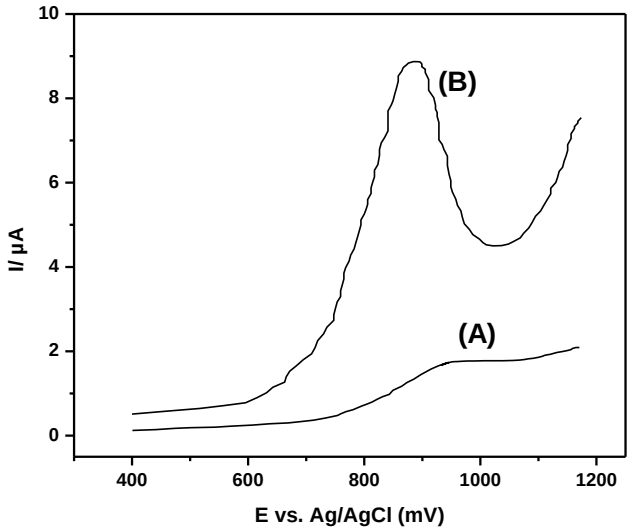

Figure 8. Differential-pulse anodic stripping voltammograms of 1×10^{-5} M ciprofloxacin in acetate buffer solution (pH 5) at (A) bare GC electrode and (B) DNA-modified GC electrode. (Scan rate: 10 mV/s. Initial potential: +0.4 V, a pre-concentration time: 1 min).

The effect of several factors on the electrochemical behavior of ciprofloxacin at DNA-modified glassy carbon electrodes was studied using differential-pulse anodic stripping voltammetry:

3.3.1 Effect of pH

The influence of pH on both: the peak current and peak potential for the oxidation of ciprofloxacin is clearly seen in Figure 9, and agrees with the expected behavior for proton-dependent process coupled to a final irreversible chemical reaction [31]. It is obviously seen that the peak current is maximum in the pH interval 3.5-5.0.

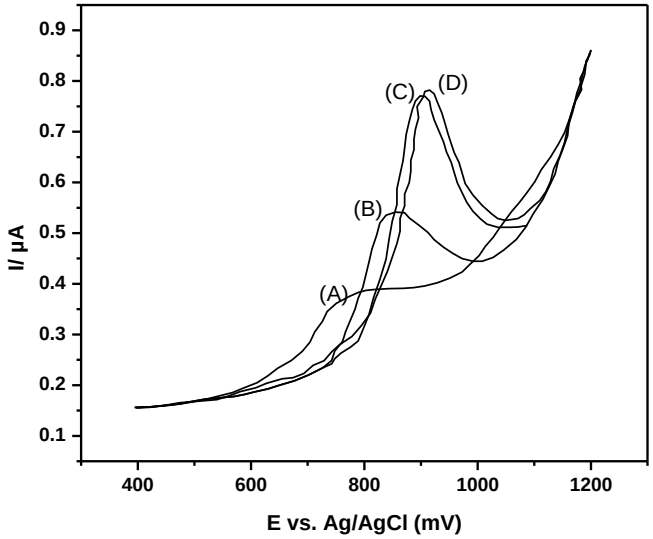

Figure 9. Differential-pulse anodic stripping voltammograms of 1×10^{-5} M ciprofloxacin at DNA-modified GC electrode in acetate buffer solutions of different pH values: (A) pH 9, (B) pH 7, (C) pH 5, (D) pH 4. (Scan rate: 10 mV/s, a pre-concentration time: 1 min, and the initial potential: + 0.4 V).

The ciprofloxacin possess two ionizable functional groups; a carboxylic group and a basic piperazinyl group, and ciprofloxacin can exist in four possible forms; cation, neutral unionized species, zwitterion and anion depending on the given pH. And based on the electrostatic attachment, one can expect that the cationic form that exists at acidic pH binds more strongly than the other forms present at neutral and basic pH values. The peak current is attributed to the irreversible oxidation of the piperazine moiety ciprofloxacin as was seen for similar drugs [23,35], and the number of electrons transferred per molecule was calculated to be two for similar molecules containing the piperazine moiety [36].

3.3.2 Effect of a pre-concentration time

The effect of a pre-concentration time on the current response was examined for a drug concentration of $1.0X10^{-5}$ M in 0.2 M acetate buffer (pH 5) at the dsDNA-modified electrode (figure is not shown). These results show a current increase within 30 s and a leveling off at longer a pre-concentration times. This indicates clearly that with a pre-concentration time of 30 s the modified electrode surface is primarily saturated with the analyte molecules [20].

3.3.3. Effect of a pre-concentration potential

The effect of a pre-concentration potential (E_p) on the oxidation of $1.0X10^{-5}$ M of the drug was also examined at the dsDNA-modified electrode using differential-pulse anodic stripping voltammetry.

The peak currents for ciprofloxacin are affected only slightly within the studied potential range (-600 - +600 mV). Thus, open circuit accumulation was chosen for conducting analysis of the present work.

3.3.4 Effect of Ionic Strength

The interaction of ciprofloxacin with dsDNA immobilized at the glassy carbon electrode was investigated under different ionic strength conditions. The differential pulse peak currents for ciprofloxacin drug at various concentration of NaCl were studied. The peak current response for ciprofloxacin decreased with increasing the ionic strength [37]. This suggests that this compound binds to the double helix of DNA by a combined effect of intercalative and electrostatic interaction with the anionic phosphate moieties [38,39]. It may be assumed that the positively charged drug molecule is electrostatically attached to the negatively charged phosphate backbone of dsDNA at low ionic strength conditions. This interaction overcomes the intercalative interaction. At higher ionic strength, the drug starts to intercalate between the double helix because the ionic shielding of the negative charges on the DNA is established. In solutions of high ionic strength, where electrical neutrality is ensured, the anodic signal decreases, which indicates that the drug molecule associate with dsDNA.

3.3.5 Effect of ciprofloxacin concentration

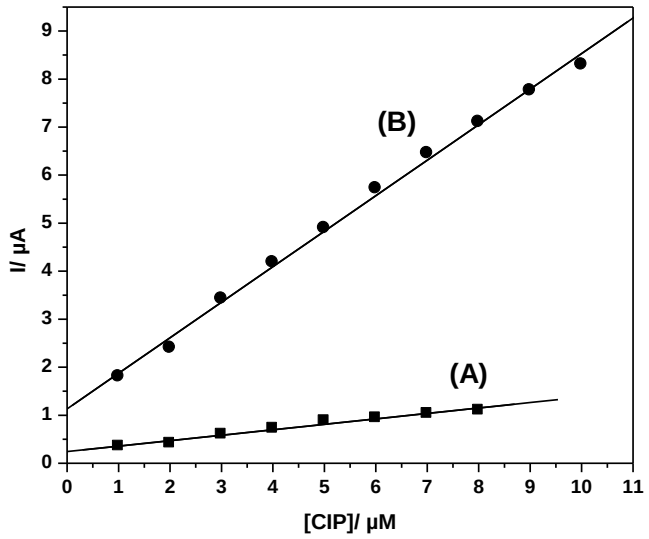

Figure 10. Calibration curves for ciprofloxacin oxidation at: (A) a DNA-modified GC electrode and (B) a bare GC electrode. (Scan rate: 10 mV/s, initial potential: +0.4 V, pre-concentration time: 1 min, pH 4).

The relationship between the peak current and ciprofloxacin concentrations was found to be linear over the range 1.0–10.0 µM, with a slope of 0.716 µA/µM and intercept of 1.26 µA, a detection limit (based on standard deviation) of 1.17×10^{-7}M, and with a percentage relative standard deviation

(%RSD) of 2.05% (n=5, c= $1.0X10^{-6}$ M) for the modified GC electrode. The relation between peak currents and the concentration of the studied drug was also investigated at the bare glassy carbon electrode as shown in figure 10.

4. CONCLUSION

DNA-modified GC electrodes have been used for studying the behavior and the interaction of ciprofloxacin with DNA. The modified electrode was capable of accumulating ciprofloxacin on the surface due to the interaction between the DNA backbone on the electrode surface and ciprofloxacin in solution. This led to an increase in sensitivity using the DNA-modified electrode towards the oxidation of ciprofloxacin as compared with the unmodified electrode. The oxidation wave of ciprofloxacin can be used for its quantification at the DNA-modified electrode. The obtained results revealed that the DNA-modified electrode has the ability to pre-concentrate the drug, leading to an enhanced sensitivity for the drug and a better peak definition. The small value of the binding constant (K) for the drug-DNA complexes, obtained by CV favored non-intercalative or partial intercalative binding to DNA**.**

References

1. A. Erdem. And M. Ozsoz, *Electroanalysis*, 14 (2002) 965.
2. S. Rauf, J. J. Gooding, K. Akhtar, M. A. Ghauri, M. Rahman, M. A. Anwar, and A. M. Khalid, *J. Pharm. Biomed. Anal.*, 37 (2005) 205.
3. S. L. Gorbach, and K. W. Nelson, in: A. P. R. Wilson, R. N. Gruneberg (Eds.), *Ciprofloxacin: 10 Years of Clinical Experience, Maxim Medical*, Oxford, 1997.
4. A. A. J. Torriero, E. Salinas, J. Raba, and J. J. Silber, *Biosens. Bioelectron.*, 22 (2006) 109.
5. A. S. Saglik, and D. Betul, *Journal of AOAC International*, 93 (2010) 510.
6. H. Salem, *Am. J. Appl. Sci.*, 2 (2005) 719.
7. N. M. Kassab, A. K. Singh, E. R. M. Kedor-Hackmam, and M. I. R. M. Santoro, *Braz. J. Pharm. Scien.*, 41 (2005) 507.
8. S. Watabea, Y. Yokoyamaa, K. Nakazawaa, K. Shinozakia, R. Hiraokab, K. Takeshitab, and Y. Suzukib, *Journal of Chromatography B*, 878 (2010) 1555.
9. L. Suntornsuk, *Analytical and Bioanalytical Chemistry*, 398 (2010) 29.
10. J. L. Beltran, E. Jimenez-Lozano, D. Barron, and J. Barbosa, *Anal. Chim. Acta*, 501 (2004) 137.
11. G. H. Ragab, and A. S. Amin, *Spectrochim. Acta A Mol Biomol. Spectrosc.*, 60 (2004) 973.
12. Y. Ni, Y. Wang, and S. Kokot, *Talanta*, 69 (2006) 216.
13. A. A. Ensafi , M. Taei, T. Khayamian, and F. Hasanpour, Anal Sci, 26 (2010) 803.
14. P. Odea, A. C. Garcic, A. J. M. Ordieres, P. T. Blanco, and M. R. Smyth, *Elecroanalysis*, 3 (1991) 337.
15. F. Belal, A. A. Al-Majed, and A. M. Al-Obaid, *Talanta*, 50 (1999) 765.
16. M. Gellert, K. Mizuuchi, M. H. Odea, and H. A. Nash, *Proc. Natl. Acad. Sci. U.S.A.*, 73 (1976) 3872.
17. S. C. Kampranis, and A. Maxwell, *J. Biol. Chem.*, 273 (1998) 22615.
18. G. Palu, S. Valisena, G. Ciarrocchi, B. Gatto, and M. Palumbo, *Proc. Natl. Acad. Sci. U.S.A.*, 89 (1992) 9671.
19. A. Radi, M. A. El-Ries, and S. Kandil, *Anal. Chim. Acta*, 459 (2003) 61.
20. N. Diab, A. Abu-Zuhri, and W. Schuhmann, *Bioelectrochemistry*, 61 (2003) 57.

21. A.M.O. Brett, S.H.P. Serrano, T.A Macedo, D. Raimundo, M.H Marques, and M.A La-Scalea, *Electroanalysis*, 8 (1996) 992.
22. C.M.A. Brett, A.M.O. Brett, and S.H.P. Serrano, *Elec. Chim. Acta*, 44 (1999) 4233.
23. A. Radi, and Z. El-Sharif, Talanta, 58 (2002) 319.
24. S. T. Girousi, I. C. Gherghi, and M. K. Karava, *J. Pharm. Biomed. Anal.*, 36 (2004) 851.
25. J. M. Kauffmann, L. J. Nunez-Vergara, J. C. Vire, J. A. Squella, and G. J. Patriarche, *Electrochem. Acta*, 32 (1987) 1159.
26. A. J. Bard, M. T. Carter, and M. Rodriguez, *J. Am. Chem. Soc.*, 111 (1989) 8901.
27. E. L. Debeer, A. E. Bottone, and E. E. Voest, *Eur. J. Pharmacol.*, 415 (2001) 1.
28. A. J. Bard and L. R. Faulkner, *Electrochemical Methods Fundamentals and Applications,* Chemical Industry Press, Beijing, (1986) 256.
29. S. Wang, T. Peng, and F. C. Yang, *Biophysical. Chemistry*, 104 (2003) 239.
30. M. Warowna-Grześkiewicz, J. Chodkowski, and Z. Fijałek, *Acta Pol Pharm.*, 52 (1995) 187.
31. S. Zhang, and S. Wei, *Bull. Korean Chem. Soc.*, 28 (2007) 543.
32. B. Uslu, B. Bozal and M. Emin Kuscu, *The Open Chemical and Biomedical Methods Journal*, 3 (2010) 108.
33. L. Fotouhi, Z. Atoofi, M. M. Heravi, *Talanta*, 103 (2013) 194.
34. A. Radi, M.A. El Ries, and S. Kandil, *Anal. Bioanal. Chem.*, 381 (2005) 451.
35. S. T. Girousi, I. C. Gherghi, and M. K. Karava, *J. Pharm. Biomed. Anal.*, 36 (2004) 851.
36. H. Berg, G. Horn, and U. Luthardt, *Bioelectrochem. Bioenergy*, 8 (1981) 537.
37. I. D. Vilfan, P. Drevensek, I. Turel, N. Poklar Ulrih, *Biochim Biophys Acta.*, 1628 (2003) 111.
38. H. Nawaz, S. Rauf, K. Akhtar, and A.M. Khalid, *Anal. Biochem.*, 354 (2006) 28.
39. Q. Shi, S. Wang, B. Zhu, M.Ji, *Front. Chem. Chin.*, 3 (2008) 52.