Multi-Walled Carbon Nanotubes (MWCNT)-Ionic Liquid-Modified Carbon Paste Electrode: Probing Furazolidone–DNA Interactions and DNA Determination

by **Lida Fotouhi*** and **Shahrbanoo Arabiyan**

Department of Chemistry, School of Sciences, Alzahra University, P.O. Box 1993891176, Tehran, Iran (phone: +98-21-88044040; fax: +98-21-88035187; e-mail: lfotouhi@alzahra.ac.ir)

The interactions of furazolidone (Fu) with double-stranded calf thymus DNA (dsDNA) on the multi-walled carbon nanotubes-ionic liquid-modified carbon paste electrode (MWCNT-IL-CPE) have been studied by cyclic voltammetry. In the presence of DNA, the cathodic peak current of Fu decreased and the peak potential shifted to a positive potential, indicating the intercalative interaction of Fu with DNA. The binding constant of Fu with DNA and stoichiometric coefficient has been determined according to the *Hill*'s model. This electrochemical method was further applied to the determination of DNA. Two linear calibration curves were obtained for DNA detection in the concentration ranges of 0.03 – 0.10 and 0.10 – 4.0 $\mu g l^{-1}$ with a detection limit of 0.027 $\mu g l^{-1}$. The method was successfully applied to analyze Fu in serum samples.

1. Introduction. – DNA as a molecule of great biological significance carries genetic information in a cell. It is the major target for drug interaction, as it is the origin of most important cellular processes of storage, copying, and transmission of gene messages. Thus, studies on the binding nature of small drug molecules to DNA are very interesting not only for understanding the mechanism of interaction, but also for the design of new drugs [1][2].

On the other hand, ionic liquid (IL)–nanoparticles-modified electrodes have received extensive attention in electroanalysis, due to their high sensitivities and lower detection limits. Biosensors modified with nanoparticles show good performances through increasing the effective area, and enhancing mass transport and catalysis [3]. However, they suffer from less ion-pairing ability. The common carbon paste electrodes (CPEs) usually employ nonpolar pasting liquids, such as paraffin oil, which fulfill some important criteria such as chemical inertness, insulating properties, and water immiscibility [4]. To improve the ionic conductivity of CPEs, they have been modified by using ionic liquid as a pasting binder [5]. ILs have many specific physicochemical properties such as high chemical and thermal stability, high ionic conductivity, and broad potential window [6]. High ionic conductivity and limited miscibility with H_2O led to a considerable current interest in ILs as materials to modify the electrochemical reactivity for supporting biocatalytical processes [7]. Recently, *Safavi et al.* [8] reported the application of ILs in electrochemical analysis. They reported a high-performance carbon composite electrode using ILs as binder for sensitive electrochemical sensing of biomolecules such as NADH, dopamine, and ascorbic acid [9].

Due to the importance of DNA as the primary holder of genetic information for cells, the study and detection of DNA has become an important research area in life sciences. *Liu et al.* [10] suggested that a ferrocenylimine derivative could interact strongly with DNA based on *Langmuir–Blodgett* films-modified electrode. DNA-Based sensors have a variety of possible applications in providing information about the mechanism of the interaction of small molecules with DNA and the determination of a variety of analytes [11]. *Li et al.* [12] prepared a biosensor based on chitosan doped with carbon nanotube (CNT) to detect DNA. *Sun et al.* [13] reported a DNA biosensor using dendritic Au nanoparticles and electrochemical reduced graphene composite-modified carbon IL electrode for determination of *Listeria monocytogenes*.

Furazolidone (Fu), belonging to a group of nitrofurans, is used as antimicrobial and as cellular-sensing drug in therapeutics [14]. Fu is genotoxic in bacteria, as it can be activated by reductive metabolism associated with nitroreductases which could react with DNA through NO_2^+ [15]. Subsequent studies have also shown that Fu preferentially inhibits DNA synthesis in *Vibrio cholerae* cells by producing interstrand cross-link in DNA [16]. Nowadays, Fu is still available for medical and veterinary uses. However, due to scarcity of the data available to assess the clinical diagnostic value of Fu in humans, it is necessary to further clarify the Fu mechanism in humans by investigating the interaction of DNA with Fu. Recently, we reported the electrochemical response of Fu at a multi-walled CNT (MWCNT)-modified glassy carbon electrode [17] and its interaction with DNA [18]. In continuation of our studies [19–21], we investigated the electrocatalytic properties of both MWCNT and 1-butyl-3-methylimidazolium hexafluorophosphate (BMIM-PF_6) toward reduction of Fu in order to investigate the interaction of Fu with DNA. Furthermore, an analytical procedure for the determination of DNA in serum was developed.

2. Experimental. – 2.1. *Apparatus.* Electrochemical measurements were carried out with a *Metrohm* model *746VA* trace analyzer connected to a *747VA* stand. The working electrode was a CPE. Pt Wire and a commercial Ag/AgCl sat. KCl electrode from *Metrohm* were used as auxiliary and ref. electrodes, resp.

2.2. *Chemicals.* Fu was obtained from *Sigma*. All other reagents were of anal. grade and used without further purification. A Fu soln. (0.5 mM) was used in a *Britton–Robinson* (*B–R*) buffer (pH 7.0)/10% DMF soln. A stock *B–R* buffer soln., 0.04M with respect to H_3BO_3, H_2PO_4, and AcOH, was prepared from proanalysis reagents. From this stock, buffer solns. with various pH values were prepared by addition of 1.0M NaOH soln. Double-stranded calf thymus DNA (dsDNA) was purchased from *Sigma*. Denatured single-stranded DNA (ssDNA) was obtained by heating the dsDNA soln. in a water bath at 97° for 5 min, immediately followed by rapid cooling in an ice bath. MWCNTs with purity 95% (10–30 nm diameters and 5 μm length) and IL BMIM-PF 6 were obtained from *IoLiTec* (*Ionic Liquid Technologies*).

2.3. *Preparation of Carbon IL Electrode.* The IL/CPE was prepared as follows: 0.093 g of graphite powder, 0.024 g paraffin oil, and 0.016 g IL were mixed thoroughly in an agate mortar to form a uniform paste. A portion of the resulted carbon paste was filled into one end of a glass tube (1-mm diameter), and a Cu wire was inserted through the opposite end to establish an electrical contact. The traditional CPE was prepared as described in [7] by hand mixing of graphite powder with paraffin oil at a ratio of 70:30 (*w*/*w*).

2.4. *Preparation of MWCNT/IL/CPE.* MWCNT (4.0 mg) was added to 1 ml of DMF. A homogeneous and stable suspension of 4.0 mg ml^{-1} MWCNT was achieved with the aid of ultrasonic agitation for *ca.* 30 min.

The MWCNT/IL/CPE was prepared by casting 4.0 μl of the suspension of MWCNT on the surface of an IL/CPE, which was dried in air for 30 min at r.t.

2.5. *Procedure for Analysis of Real Samples.* Serum sample was obtained from the *Iranian Blood Transfusion Organization* (Tehran, Iran) and stored at −20° prior to use. The frozen serum sample was thawed and allowed to reach r.t. 1 ml of serum sample was diluted in 10 ml *B–R* buffer soln. (0.04M, pH 7.0). The resulting soln. was transferred to the electrochemical cell, and the voltammograms were recorded. To ascertain the validity of the results, the sample was spiked with certain amounts of DNA, and the recovery rates of the spiked samples were determined.

3. Results and Discussion. – 3.1. *Interaction of Fu with DNA. Fig. 1* shows the cyclic voltammograms (CVs) of Fu obtained at MWCNT/IL/CPE in 0.04M *B–R* buffer (pH 7.0) over a potential sweep from −0.20 to −0.55 V. A small irreversible cathodic peak (−0.5 V) could be observed in the CV of Fu at the bare CPE, corresponding to the 4 e^- reduction of the NO_2 group to hydroxylamine (RNHOH), as it was reported for nitroaromatic compounds (*Fig. 1,a*, curve *1*) [22]. The MWCNT exhibited excellent electrocatalytic behavior for reduction of Fu, as evidenced by the enhancement of peak current and the shift in the cathodic peak potential to less negative values in comparison with a bare CPE (*Fig. 1,a*, curve *2*). After modification of the electrode with IL, the cathodic peak increased greatly and became well-defined, indicating that, in the presence of both IL and MWCNT, the electron transfer rate was greatly enhanced (*Fig. 1,a*, curve *3*). The background current clearly increased, when both MWCNT and IL were used as modifier reagents in the electrode, indicating that the use of MWCNT and IL significantly enhanced the electrical conductivity of the electrode [23]. To study the interaction of Fu with DNA, the CV of 2.0 mM Fu in the presence of 4.0 μg ml^{-1} DNA was recorded at MWCNT/IL/CPE (*Fig. 1,a*, curve *4*). The cathodic peak shifted positively (10 mV) with decreasing peak current compared to the CV of Fu, evidencing the interaction of Fu with DNA. The decrease in peak current of Fu in the presence of DNA is attributed to the formation of DNA–Fu_m supramolecular complex because of which the concentration of free drug is lowered [24].

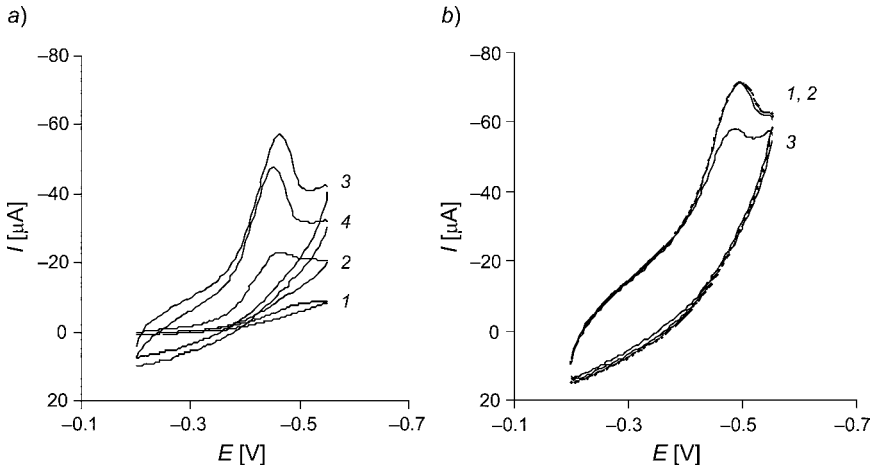

Fig. 1. *Cyclic voltammograms of 2.0 mM Fu* a) *at bare CPE* (1), *at MWCNT/CPE* (2), *at MWCNT/IL/CPE* (3), *in the presence of 4.0 μg ml^{-1} DNA at MWCNT/IL/CPE* (4); b) *in the absence of ssDNA* (1), *in the presence of 4.0 μg ml^{-1} ssDNA* (2), *in the presence of 4.0 μg ml^{-1} dsDNA* (3). *B–R* Buffer solution, 0.04M, pH 7.0; scan rate, 100 mV s^{-1}.

To assess the interaction mode of Fu with DNA, CVs of Fu were recorded in the presence and absence of denatured ssDNA (*Fig. 1,b*). There are two binding models for the binding of small molecules to DNA: *i*) electrostatic interactions with the outer negatively charged DNA phosphates, *ii*) intercalative binding, in which the small molecule or drug intercalates into the relatively nonpolar interior part of the DNA helix. Among the two modes of interactions, intercalation binding mode is dependent on DNA double helix (dsDNA), while the electrostatic binding occurs out of the intercalation of the DNA. If the interaction mode is intercalative, the interaction capability would decrease in the presence of ssDNA. However, the electrostatic interactions may continue to operate even after DNA denaturation. From *Fig. 1,b*, it can be seen that, upon addition of ssDNA (*Fig. 1,b*, curve *2*), no appreciable changes in the CV of Fu (*Fig. 1,b*, curve *1*) were observed compared with dsDNA (*Fig. 1,b*, curve *3*), as ssDNA did not possess the double helix. This ruled out the interaction between Fu and ssDNA [25]. The above results confirm the dominance of intercalative interaction of Fu with dsDNA.

3.2. *The Effect of the Scan Rate. Fig. 2* shows the CVs of Fu at the MWCNT/IL/CPE when the scan rate (υ) varies from 10 to 70 mV s^{-1}. A linear relationship ($y = -318.2x - 19.21$) with a correlation coefficient of $R^2 = 0.975$ was observed between

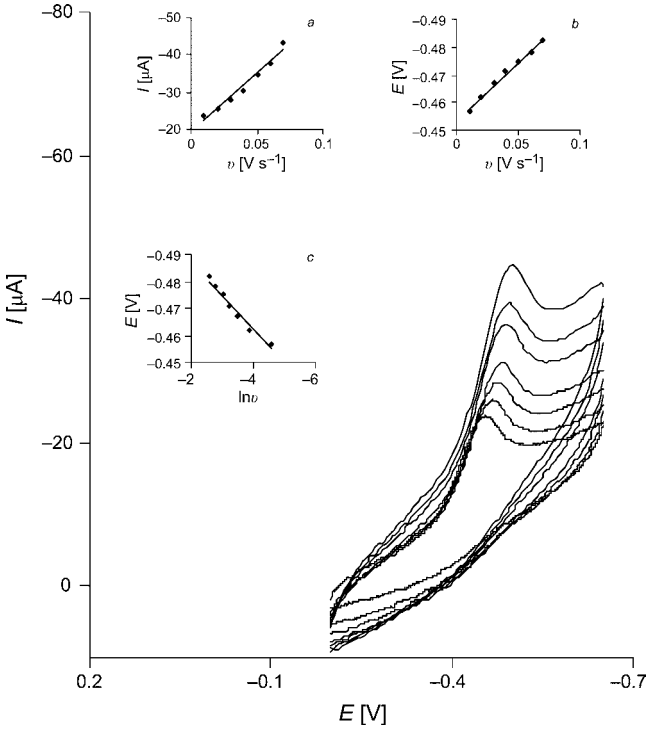

Fig. 2. *Cyclic voltammograms of 2.0 mM at scan rates* (inner to outer) *of 10, 20, 30, 40, 50, 60, 70 mV s^{-1}. a*) Plot of peak currents *vs.* scan rate. *b*) and *c*) variations of peak potential *vs.* υ and lnυ, respectively. *B–R* Buffer solution, 0.04M, pH 7.0; at MWCNT/IL/CPE.

the peak current and the scan rate (*Fig. 2,b*), which indicates that the electrode process is surface-controlled. From the slope of the linear plot of I *vs.* v, the surface concentration of the electroactive species (Γ) can be estimated to be *ca.* $4.2 \cdot 10^{-8}$ mol cm^{-2} according to *Eqn. 1* [26]:

$$i_p = n^2 F^2 v \cdot A\Gamma/4RT \quad (1)$$

As shown by increasing the scan rate, the peak potential was shifted to a more negative potential. Because of the irreversible electrode process of the reduction reaction of Fu, the *Laviron*'s equation [27] was used to estimate αn and k_s values (*Eqn. 2*).

$$E_p = E^0 - (RT/\alpha nF)[\ln(RTk_s/\alpha nF) - \ln v] \quad (2)$$

where α is the electron transfer coefficient, k_s is the standard rate constant of the surface reaction, v is the scan rate, n is the electron transfer number, and E^0 is the formal potential. k_s and αn values can be concluded from the intercept and slope of the linear plot of E_p with respect to $\ln v$, if the value of E^0 is known.

The E^0 value at MWCNT/IL/CPE can be deduced from the intercept of E_p *vs.* v plot on the ordinate by extrapolating the line to $v = 0$ (*Fig. 2,a*). Knowing E^0, and from the graphical representations of E_p *vs.* $\ln v$ for Fu (*Fig. 2,c*), the values $\alpha n = 2.04$ and $k_s = 0.751$ s^{-1} were obtained from the slope and intercept, respectively. Since, for a totally irreversible electron transfer, α was assumed to be 0.50, n was calculated to be 4 indicating that four electrons were involved in the reduction of Fu on the MWCNT/IL/CPE.

3.3. *Determination of Association Constant* (K_a) *and Binding Number between Fu and DNA.* As no new electrochemical signals appeared after DNA–Fu interaction, we assume that only one complex is formed, DNA–Fu$_m$, according to [28] (*Eqn. 3*).

$$\text{DNA} + m\ \text{Fu} \rightleftarrows \text{DNA–Fu}_m \quad (3)$$

If DNA and Fu form a single adduct, the plot $\log[\Delta I/(\Delta I_{max} - \Delta I)]$ *vs.* $\log[\text{Fu}]$ becomes linear with the slope of m and the intercept of K_a according to *Eqn. 4*.

$$\log[\Delta I/(\Delta I_{max} - \Delta I)] = m \log K_a + m \log[\text{Fu}] \quad (4)$$

where ΔI_{max} represents the maximum difference of peak current of Fu in the absence and presence of DNA, ΔI represents the difference of peak current of Fu in the absence and presence of DNA, m is the *Hill* coefficient, and K_a is the association constant. The corresponding experimental data (*Fig. 3*) yielded $m = 1.1$ and $K_a = 2.375 \cdot 10^3$ M^{-1}. The stoichiometry of the cooperative Fu binding was at least one per base pair unit. Thus, the formation of a stable 1:1 complex of DNA–Fu was proposed.

3.4. *Analytical Application.* Under the optimal experimental conditions (pH 7.0 and 4.0 µl of 4.0 mg ml^{-1} MWCNT), the peak current difference of Fu, ΔI, was found to be proportional to DNA concentration over two linear ranges 0.03–0.1 ($y = 52.37x - 0.827$, $R^2 = 0.996$) and 0.1–4.0 µg l^{-1} ($y = 2.677x + 5.363$, $R^2 = 0.973$; *Fig. 4*). The limit of detection (LOD) was obtained as $Y_{LOD} = X_B + 3S_B$, where Y_{LOD} is the signal for the

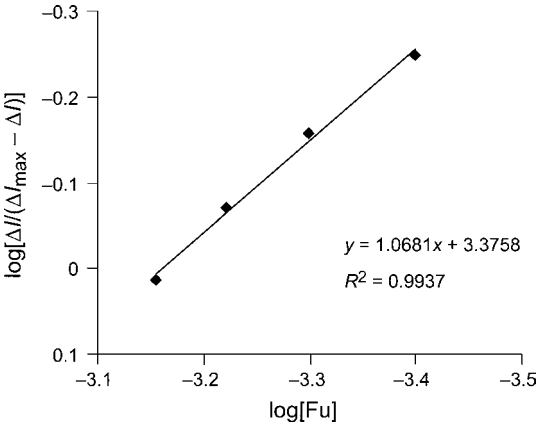

Fig. 3. *Linear plot of log[(ΔI/(ΔI – ΔI$_{max}$)]* vs. *log[Fu]*. 4.0 μg ml^{-1} DNA, Fu concentrations: 0.0001 to 0.0009M. *B–R* buffer solution, 0.04M, pH 7.0; at MWCNT/IL/CPE.

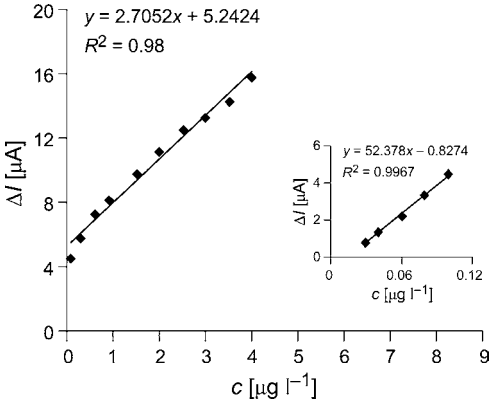

Fig. 4. *Plots of* ΔI vs. *DNA concentration*

limit of detection, X_B and $3S_B$ are the mean and the standard deviation of the blank signal, respectively. The LOD was obtained as 0.027 μg l^{-1}. The reproducibility of the method was checked by successive determinations ($n = 8$) of 2.0 μg ml^{-1} DNA. The relative standard deviation (RSD) was lower than 1.17%.

Under the optimum conditions, the interferences of various foreign ions and other possibly coexisting substances were studied at a DNA concentration of 0.08 μg l^{-1}. The tolerance limit was defined as the concentration ratio of interferences to DNA concentration causing less than ±5.0% relative error. Hundredfold concentration of Na^+, K^+, Ba^{2+}, Mg^{2+}, Ni^{2+}, Pb^{2+}, Sr^{2+}, Co^{2+}, Cr^{2+}, Cu^{2+}, Cd^{2+}, Al^{3+}, NO_3^-, SO_4^{2-}, and Cl^-, and 50-fold concentration of lysine, glutamic acid, arginine, and cysteine had almost no interference on the current response of DNA (signal change below 5%).

3.5. *Recovery Studies and DNA Determination in Serum.* To evaluate the applicability of the present method at real matrices, assays were performed on serum

samples. Recovery experiments were carried out by adding standard solutions of DNA to serum matrices. According to the results, satisfactory recovery for DNA could be obtained. The data obtained for DNA assays performed on the matrices, which were studied using the optimized experimental methodology, are compiled in *Table 1*. The levels of detection limit, linear ranges, and precision listed in *Table 1* are suitable for the routine quality-control analysis of DNA in serum samples.

Table 1. *Results of Analysis of Real Samples*

Sample ($n=3$)	Added [$\mu g l^{-1}$]	Found [$\mu g l^{-1}$]	Recovery [%]	RSD [%]
1	0.04	0.039	95	2.3
2	0.10	0.098	98	1.5
3	2.00	1.956	97	1.7

3.6. *Comparison of the Applied Methods with Other Reported Methods.* The present method was compared with other methods in terms of validation and precision (*Table 2*). The modified electrode here was shown to be effective for determination of DNA in real samples. This electrode has some advantages in comparison with other modified electrodes, including not requiring sample pretreatment, simplicity, stability, and low cost of reagents.

Table 2. *Comparison of the Proposed Methods with Other Reported Methods for Determination of DNA*

Electrode/ sensing element	Detection method	Binding mechanism	Kind of DNA	Detection limit [$\mu g l^{-1}$]	Linearity range [$\mu g l^{-1}$]	RSD [%]	Ref.
GCE/Oxidation of daunomycin	CV	Intercalative	Calf thymus	$2.4 \cdot 10^3$	$0 - 2.8 \cdot 10^4$	–	[29]
DME/Reduction of CTZAMB–Cu^{II}	SSP	Electrostatic	Herring sperm	$1.0 \cdot 10^2$	$3.0 \cdot 10^2 - 7.0 \cdot 10^3$	1.30	[30]
Hg/Reduction of basic brown G	LSV	Intercalative	Herring sperm	$4.0 \cdot 10^1$	$1.0 \cdot 10^2 - 3.6 \cdot 10^4$	3.50	[31]
DME/Reduction of acridine orange	LSV	–	Calf thymus	$5.1 \cdot 10^1$	$2.0 \cdot 10^3 - 2.0 \cdot 10^4$	–	[32]
GCE/Reduction of Victoria Blue B	DPV	Electrostatic	Fish sperm	$7.0 \cdot 10^1$	$1.0 \cdot 10^2 - 8.0 \cdot 10^4$	0.45	[33]
GCE/Reduction of colchicine	DPV	Electrostatic	Calf thymus	$4.0 \cdot 10^1$	$1.0 \cdot 10^2 - 2.2 \cdot 10^2$	–	[34]
GCE/Reduction of furazolidone	LSV	Intercalative	Calf thymus	$2.5 \cdot 10^1$	$4.0 \cdot 10^1 - 1.0 \cdot 10^2$ and $1.0 \cdot 10^2 - 1.0 \cdot 10^3$	0.47	[18]
Au–MPA–Zr SAM	CV	–	Calf thymus	$9.5 \cdot 10^{-2}$	$5.0 \cdot 10^{-1} - 1.0 \cdot 10^2$	2.50	[35]
MWCNT/GCE/ Oxidation of sulfadiazine	CV	–	Calf thymus	$3.0 \cdot 10^{-2}$	$3.0 \cdot 10^{-2} - 1.3 \cdot 10^{-1}$ and $6.0 \cdot 10^{-1} - 3.5$	1.12	[21]
This work	CV	–	Calf thymus	$2.7 \cdot 10^{-2}$	$3.0 \cdot 10^{-2} - 1.0 \cdot 10^{-1}$ and $1.0 \cdot 10^{-1} - 4.0$	1.17	–

High sensitivity and selectivity, and low detection limit, together with the very easy preparation and surface regeneration of the modified electrode and reproducibility of the voltammetric responses, make the prepared modified electrode very useful in construction of simple devices for determination of DNA.

4. Conclusions. – In this study, the interaction of Fu with DNA was investigated on the MWCNT/IL/CPE. Due to the specific functions of both IL and MWCNT present on the electrode surface, the electron transfer rate was greatly enhanced, and a well-defined peak appeared. The voltammetric study revealed a marked decrease in cathodic current of Fu with a positive shift in peak potential at the MWCNT/IL/CPE, which indicates the interaction of Fu with DNA. The electrochemical parameters of Fu in the absence and presence of DNA were calculated. The binding mechanism was discussed by calculating the binding constant and *Hill* coefficient. The interaction of Fu with DNA indicated that Fu was bound to DNA; forming a DNA–Fu complex. The electrocatalytic response for the determination of DNA was used. Under optimized experimental conditions, good analytical performance was obtained, including suitable precision, excellent linear dynamic range, and detection limit. The method is sensitive enough for analysis of lower concentrations of DNA. Furthermore, the proposed method does not require expensive instruments or critical analytical reagents.

The authors express their gratitude for financial support from Alzahra University.

REFERENCES

[1] K. Namoto, J. Gardiner, T. Kimmerlin, D. Seebach, *Helv. Chim. Acta* **2006**, *89*, 3087.
[2] T. Alizadeh, L. Allahyari, *Electrochim. Acta* **2013**, *111*, 663.
[3] J. Liu, J. Qiu, K. Sun, J. Chen, Y. Miao, *Helv. Chim. Acta* **2009**, *92*, 462.
[4] M. E. Rice, Z. Galus, R. N. Adams, *J. Electroanal. Chem.* **1983**, *143*, 89.
[5] M. Mazloum-Ardakani, A. Khoshroo, *Electrochim. Acta* **2013**, *103*, 77.
[6] K. Kalcher, *Electroanalysis* **1990**, *2*, 419.
[7] J. Ping, S. Ru, X. Luo, K. Fan, J. Wu, Y. Ying, *Electrochim. Acta* **2011**, *56*, 4154.
[8] A. Safavi, N. Maleki, F. Sedaghati, F. Tajabadi, *Anal. Biochem.* **2007**, *369*, 149.
[9] A. Safavi, N. Maleki, O. Moradlou, F. Tajabadi, *Anal. Biochem.* **2006**, *359*, 224.
[10] P. Liu, Q. Chen, H. Zhou, B. Ye, T. Li, *Electrochim. Acta* **2014**, *115*, 306.
[11] P. Arias, N. F. Ferreyra, G. A. Rivas, S. Bollo, *J. Electroanal. Chem.* **2009**, *634*, 123.
[12] J. Li, Q. Liu, Y. Liu, Sh. Lu, Sh. Yao, *Anal. Biochem.* **2005**, *346*, 107.
[13] W. Sun, X. Qi, Y. Zhang, H. Yang, H. Gao, Y. Chen, Z. Sun, *Electrochim. Acta* **2012**, *85*, 145.
[14] P. L. Olive, *Cancer Res.* **1979**, *39*, 4512.
[15] H. K. Debnath, C. Hansch, K. H. Kim, Y. C. Martin, *J. Med. Chem.* **1993**, *36*, 1007.
[16] S. N. Chatterjee, S. Ghose, M. Maiti, *Biochem. Pharmacol.* **1977**, *26*, 1453.
[17] L. Fotouhi, M. Nemati, M. M. Heravi, *J. Appl. Electrochem.* **2011**, *41*, 137.
[18] L. Fotouhi, F. Bahmani, *Electroanalysis* **2013**, *25*, 757.
[19] L. Fotouhi, S. Arabiyan, O. Moradlou, *Anal. Bioanal. Electrochem.* **2013**, *5*, 283.
[20] L. Fotouhi, Z. Atoofi, M. M. Heravi, *Talanta* **2013**, *103*, 194.
[21] L. Fotouhi, A. B. Hashkavayi, M. M. Heravi, *Int. J. Biol. Macromol.* **2013**, *53*, 101.
[22] J. H. Tacherz, D. I. Edwards, *Free Radical Res. Commun.* **1992**, *16*, 19.
[23] P. J. Britto, K. S. V. Santhanam, P. M. Ajayan, *Bioelectrochem. Bioenerg.* **1996**, *41*, 121.
[24] A. Shah, M. Zaheer, R. Qureshi, Z. Akhter, M. F. Nazer, *Spectrochim. Acta, Part A* **2010**, *75*, 1082.
[25] S. S. Kalanur, J. Seetharamappa, S. N. Prashanth, *Colloids Surf., B* **2011**, *82*, 438.

[26] A. J. Bard, L. R. Faulkner, in 'Electrochemical Methods: Fundamentals and Applications', John Wiley & Sons, Inc., New York, 2001.
[27] E. Laviron, *J. Electroanal. Chem.* **1979**, *101*, 19.
[28] X. Tian, Y. Song, H. Dong, B. Ye, *Bioelectrochemistry* **2008**, *73*, 18.
[29] X. Chu, G. L. Shen, J. H. Jiang, T. F. Kang, B. Xiong, R. Q. Yu, *Anal. Chim. Acta* **1998**, *373*, 29.
[30] L. R. Wang, R. X. Wang, L. Z. Yang, G. H. Lu, *J. Electroanal. Chem.* **2005**, *585*, 214.
[31] Y. Q. Li, Y. J. Guo, X. F. Li, J. H. Pan, *Talanta* **2007**, *71*, 123.
[32] W. Sun, N. Zhao, X. Yuan, K. Jiao, *J. Serb. Chem. Soc.* **2007**, *72*, 1085.
[33] B. Xu, K. Jiao, W. Sun, X. Zhang, *Int. J. Electrochem. Sci.* **2007**, *2*, 406.
[34] H. Xu, F. Hou, X. Zhang, *Curr. Phys. Chem.* **2011**, *1*, 11.
[35] R. K. Shervedani, S. Pourbeyram, *Bioelectrochemistry* **2010**, *77*, 100.

Received April 29, 2014