Determination of Binding Parameters and Mode of Ferrocenyl Chalcone–DNA Interaction

Afzal Shah, Rumana Qureshi,* Asad Muhammad Khan, Farzana Latif Ansari, and Safeer Ahmad

Department of Chemistry, Quaid-i-Azam University, 45320, Islamabad, Pakistan

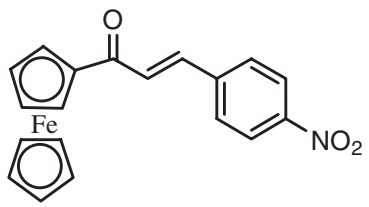
Received September 12, 2008; E-mail: r_qureshy@yahoo.com

This paper reports that how the variation in peak current, absorbance, and viscosity of ferrocenyl chalcone (FC), chemically named as 1-ferrocenyl-3-(4-nitrophenyl)-2-propen-1-one, in 10% aqueous DMF upon addition of DNA can be used to probe the mode of interaction and binding parameters. Binding constant ($K = 5.17\ (\pm 0.25) \times 10^3\ M^{-1}$), binding site size ($s = 1.08 \pm 0.05$ bp) and diffusion coefficient of the free ($D_f = 5.22 \times 10^{-7}\ cm^2\ s^{-1}$) and DNA bound drug ($D_b = 4.39 \times 10^{-8}\ cm^2\ s^{-1}$) were determined from voltammetric data. The binding constant ($K = 4.91\ (\pm 0.20) \times 10^3\ M^{-1}$) was also obtained from UV–vis absorption titration. Gibbs energy change ($\Delta G = -RT \ln K$) of $-21.18\ kJ\ mol^{-1}$ at 25 °C indicated the spontaneity of the binding interaction. The experimental results revealed intercalation of FC into DNA as the dominant mode of interaction. Furthermore, the radii of the free and DNA-bound drug were determined from viscosity measurements.

Ferrocene-based derivatives have drawn utmost attention in various fields of analytical chemistry due to their varied and well-established redox chemistry. These are widely used for medical purposes,[1–3] electrocatalysis,[4] and in the design of new signaling ion sensors.[5,6] The sensitivity of the ferrocenyl groups to covalent or non covalent binding with other molecules and their unique property of retaining simple one electron redox behavior after the introduction of substituents are routinely exploited for the determination of electrochemical parameters.[7–9]

Chalcones (α,β-unsaturated ketones) are promising candidates in the new era of medicines on account of their wide spectrum of antitumor, antibacterial, and anti-inflammatory activities.[10–13] The applications of these compounds in chemotherapy due to their direct interaction with DNA has been reported by previous investigators.[11,12] Their derivatization with ferrocene can enhance their detection by electrochemical methods like cyclic voltammetry (CV), differential pulse voltammetry, and square wave voltammetry. Like an effective chemical sensor, ferrocenyl chalcone has two basic parts: the signaling unit and the binding unit. The interaction of the binding unit with other molecules will be monitored by the tunable redox behavior of the signaling ferrocene moiety. As the study of the electrochemical sensing properties of ferrocenyl chalcones is limited, a ferrocenyl chalcone (FC) was obtained and investigated. Its binding with DNA was monitored by the redox active ferrocenyl group, acting as an intramolecular oxidation antenna.[14]

Drug-DNA interactions have been studied by a variety of techniques such as viscometry, UV–vis spectroscopy, isothermal calorimetry, luminescence, fluorescence, and electroanalytical methods.[15–18] For an active redox species like FC, electrochemical methods could be used to complement the previously used methods of investigation.[15–19] In the present work, the interaction of FC (Scheme 1) with chicken blood DNA (CB-DNA) has been investigated by cyclic voltammetry, UV–vis spectroscopy and viscometry in *N*,*N*-dimethylformamide (DMF) at pH 7.4 and 25 °C.



Scheme 1. Molecular structure of ferrocenyl chalcone (FC) chemically named as 1-ferrocenyl-3-(4-nitrophenyl)-2-propen-1-one.

Experimental

Chemicals. DNA was extracted from chicken blood by the method mentioned in our previous paper.[20] A stock solution was prepared by dissolving an appropriate amount of DNA in doubly distilled water and was stored at 4 °C. The concentration of the stock solution of CB-DNA (0.3 mM in nucleotide phosphate, NP) was determined by UV absorbance at 260 nm using the molar extinction coefficient (ε) of $6600\ M^{-1}\ cm^{-1}$.[21] Ferrocenyl chalcone was prepared according to a literature reported method.[22] DMF (Sigma-Aldrich, 99.93% purity) was used without further purification. Tetrabutylammonium perchlorate (TBAP) (Fluka, 99% purity) was further purified by recrystallization using methanol as the solvent.

The purity (free from bound protein) of DNA was assessed from the ratio of absorbances at 260 and 280 nm. A ratio of 1.85 ($A_{260}/A_{280} = 1.85$) was taken as evidence for protein free DNA.[23] A stock solution of FC (6 mM) was prepared by dissolving it in 10% aqueous DMF. The solutions were buffered at pH 7.4 using phosphate buffer (10 mM K_2HPO_4 and 10 mM KH_2PO_4). Different aliquots were prepared from stock solution by dilution.

Published on the web April 11, 2009; doi:10.1246/bcsj.82.453

Apparatus. Voltammetric experiments were performed using PGSTAT 302 with Autolab GPES version 4.9 Eco Chemie, Utrecht, the Netherlands. Measurements were carried out in a conventional three electrode cell with Ag/AgCl as reference electrode, a thin Pt wire as counter electrode and a bare glassy carbon electrode (GCE) with a geometric area of 0.071 cm^2 as the working electrode. Prior to experiments, the GCE was polished with 0.25-μm diamond paste on a nylon buffing pad, followed by washing with water. For electrochemical measurements the test solution was kept in an electrochemical cell (model K64 PARC) connected to a circulating thermostat LAUDA model K-4R. Absorption spectra were measured on a UV–vis Spectrometer; Shimadzu 1601 by keeping constant the concentration of the drug while varying the concentration of DNA. The viscosity measurements were carried out with an Anton Paar Stabinger Viscometer SVM 3000.

Procedures. Voltammetric Studies: For CV experiments both the concentration and volume of FC were kept constant while varying the concentration of DNA in solution. The voltammograms were recorded as aliquots of known quantity of DNA were added. The solutions were deoxygenated via purging with argon gas for 10 min before every experiment and were maintained under argon atmosphere throughout the measurements. All experiments were carried out at 25 °C and blood pH (7.4). Prior to every electrochemical assay the GCE was polished for carrying out the electrochemical process on a clean electrode surface.

Spectroscopic Studies: Absorption spectra were measured by adding a small aliquot of DNA solution to a constant concentration of the drug solution. Solutions were allowed to equilibrate for 5 min before experimental assay.

Viscometric Measurements: For viscosity measurements, titrations were performed by the addition of aliquots of the drug solution into a constant concentration of DNA solution in the viscometer. Data are presented as (η/η_o) versus the concentration of DNA, where η is the viscosity of the drug in the presence of DNA and η_o is the viscosity of the drug alone.

Results and Discussion

Voltammetric Studies of the Interaction of FC with DNA. The cyclic voltammetric behavior of 3 mM FC in the absence and presence of 200 μM DNA at bare GCE is shown in Figure 1. The voltammogram without DNA (Figure 1a) featured a couple of well-defined and stable redox peaks in the potential range of −0.6 to −1.6 V. The voltammogram registered an anodic peak at −1.036 V and a cathodic peak at −1.173 V versus Ag/AgCl. By the addition of 200 μM DNA (Figure 1b) both the cathodic and anodic peak potentials shifted by 93 and 97 mV in the positive direction. These positive shifts in peak potentials are indicative of an intercalative mode of binding.[24] Furthermore, a 25% decrease in cathodic and 19.35% decrease in anodic peak current was observed. The greater decrease of I_{pc} as compared to I_{pa} is attributed to the intercalation of FC into the double-stranded DNA, referring to appropriate references.[25,26] The rationale behind the diminution in peak currents is the decrease in free drug concentration due to the formation of macromolecular FC–DNA complex with a smaller diffusion coefficient.[27,28] The values of the diffusion coefficient ($D_f = 5.22 \times 10^{-7}$ cm^2 s^{-1}) of the free and DNA bound drug ($D_b = 4.39 \times 10^{-8}$ cm^2 s^{-1}) were determined by the Randles Sevcik expression:[29,30]

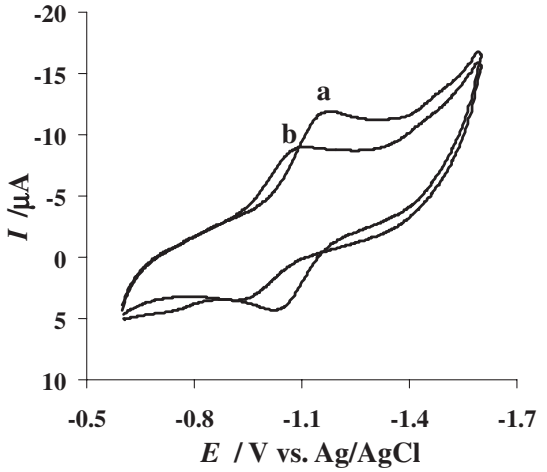


Figure 1. Cyclic voltammograms of 3 mM FC in 10% aqueous DMF with 0.1 M TBAP as supporting electrolyte in the absence (a) and presence of 200 μM DNA (b) at 100 mV s^{-1} scan rate in 0.25 M phosphate buffer at pH 7.4 and 25 °C. Glassy carbon electrode (0.071 cm^2) was used as working electrode and all potentials are reported vs. Ag/AgCl.

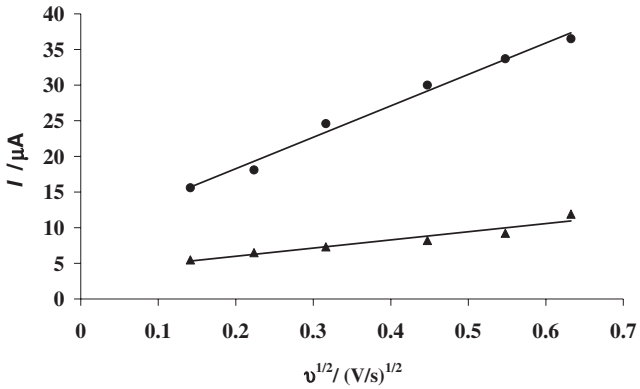


Figure 2. I vs. $v^{1/2}$ plots of 5 mM FC in the absence of DNA (●) and presence of 20 μM DNA (▲) at 20 (a), 50 (b), 100 (c), 200 (d), and 500 mV s^{-1} (e) in 0.25 M phosphate buffer (pH 7.4) at 25 °C.

$$I = 2.69 \times 10^5 n^{3/2} A C D^{1/2} v^{1/2} \quad (1)$$

where I is the peak current (A), A is the surface area of the electrode (cm^2), C is the bulk concentration (mol cm^{-3}) of the electroactive species, D is the diffusion coefficient (cm^2 s^{-1}), v is the scan rate (V s^{-1}), and n is the number of electrons gained or lost by the electroactive species.

The linear dependence of I_p on $v^{1/2}$ (Figure 2) indicates that the redox process of FC in the absence and presence of DNA is diffusion controlled.[31]

It can be seen that the diffusion coefficient of DNA bound drug is an order of magnitude lower than that of the free drug. Similar results have also been obtained by other investigators.[15,32–34] The smaller slope of FC in the presence of DNA could be attributed to its intercalation into DNA resulting in the formation of slowly diffusing supramolecular complex in solution.[35]

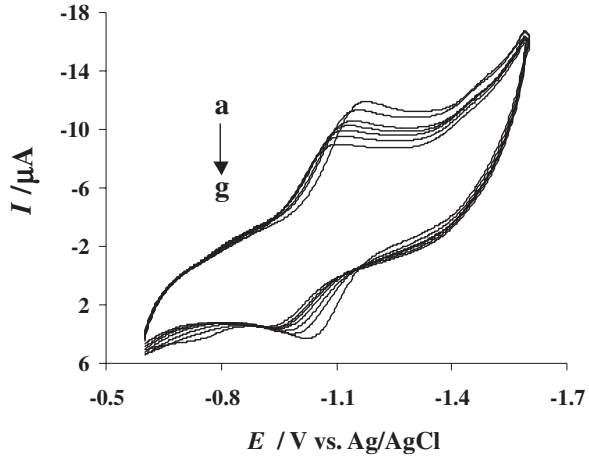


Figure 3. Cyclic voltammograms of 3 mM FC in the absence of DNA (a) and presence of 20 (b), 40 (c), 60 (d), 80 (e), 100 (f), and 200 μM DNA (g).

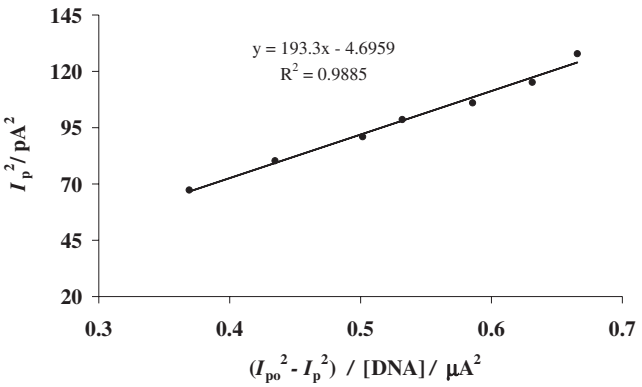


Figure 4. Plot of I_p^2 vs. $(I_{po}^2 - I_p^2)/[DNA]$ for 3 mM FC with varying concentration of DNA ranging from 20 to 200 μM in a medium buffered at pH 7.4, used to calculate the binding constant of FC–DNA adduct.

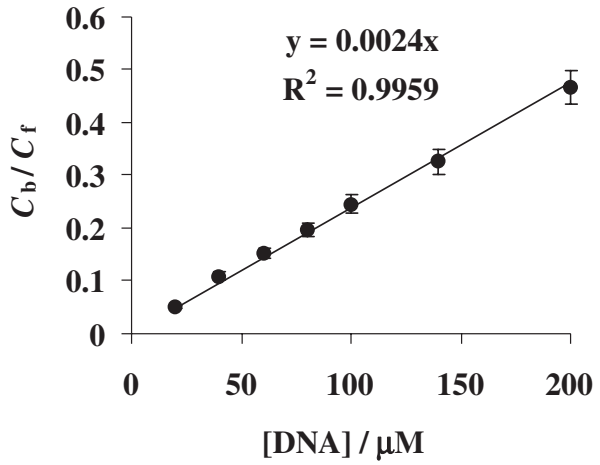


Figure 5. C_b/C_f vs. [DNA] for the determination of binding site size. 5% error is estimated in all the values at y axis.

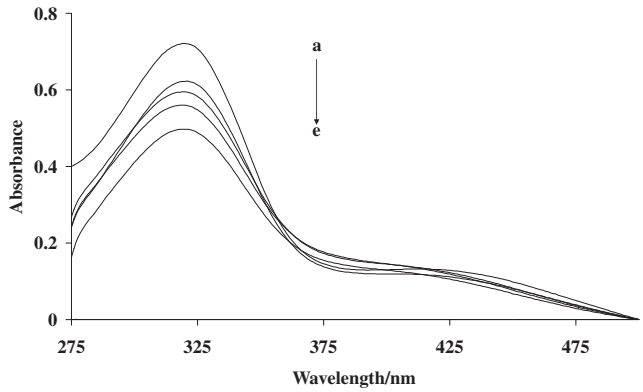


Figure 6. UV–vis absorption spectra of 50 μM FC in the absence of DNA (a), in the presence of 40 (b), 60 (c), 80 (d), and 100 μM DNA (e) at pH 7.4 and 25 °C.

Based on variations in cathodic peak current of FC caused by the addition of increasing concentration of DNA (Figure 3), the binding constant K, was calculated according to the equation:[36]

$$I_p^2 = \frac{1}{K[\text{DNA}]}(I_{po}^2 - I_p^2) + I_{po}^2 - [\text{DNA}] \quad (2)$$

where, I_{po} and I_p are the peak currents of FC in the absence and presence of DNA, respectively. By plotting I_p^2 vs. $(I_{po}^2 - I_p^2)/[DNA]$ a straight line with a binding constant of $5.17\ (\pm 0.25) \times 10^3\ M^{-1}$ was obtained (Figure 4).

For the determination of binding site size the following simple binding model was used:[37]

$$C_b/C_f = K\{[\text{free base pairs}]/s\} \quad (3)$$

where s is the binding site size in terms of base pairs. Measuring the concentration of DNA in terms of [NP], the concentration of base pairs can be expressed as [DNA]/2. So eq 3 can be written as:

$$C_b/C_f = K\{[\text{DNA}]/2s\} \quad (4)$$

C_f and C_b denote the concentrations of free and DNA-bound species respectively.

The C_b/C_f ratio was determined by the equation given below:[38]

$$C_b/C_f = (I - I_{DNA})/I_{DNA} \quad (5)$$

where I_{DNA} and I represent the peak current of the drug with and without DNA.

Putting the value of $K = 5.17\ (\pm 0.25) \times 10^3\ M^{-1}$ as calculated according to eq 2, the binding site size of 1.08 ± 0.05 was obtained from the plot (Figure 5) of C_b/C_f vs. [DNA]. The value of s shows that the drug occupies more than one base pair when intercalated into DNA.[39]

Absorption Studies. The interaction of FC with solution phase CB-DNA, was also characterized by UV–vis absorption titration by keeping the concentration of the drug constant (50 μM) while varying the concentration of DNA from 20 to 200 μM. As shown in Figure 6, the absorption band of FC with the maximum wavelength at 321 nm, resulted in hypochromism (45%), broadening of the envelope and slight red shift of 3 nm by the incremental addition of DNA. The large hypochromism, characteristic of intercalation[40] (in binding mode) is attrib-

uted to the interaction between the electronic states of the intercalative chromophore of FC and that of the DNA bases. However, the lack of pronounced red shift is suggestive of partial intercalation as classical intercalation exhibits appreciable shift in wavelength (red shift ≥ 15 nm).[41] The reason for partial intercalation could be the stereochemical effect of the non-planar ferrocenyl group, which will prevent the whole molecule from intercalating into DNA. The weak bathochromic effect is further correlated to out binding mode,[42] in which the non-intercalating ferrocenyl group of FC is considered more suitable for fitting into the grooves of DNA. The broadening of the envelope can be assigned to the changes in the electronic distribution of FC upon binding to the DNA bases. The non-uniform variation in absorbance can presumably be due to a couple of factors like conformational changes in the structure of either FC and/or nucleic acid upon binding and complex–complex interactions.

The origin of bathochromic and hypochromic effects might lie in the mechanism of interaction of FC with DNA. The introduction of the planar 4-nitrophenyl-2-propen-1-one of FC in DNA is likely to decrease the $\pi \rightarrow \pi^*$ transition due to the coupling of the lowest unoccupied π^*-orbital of the drug molecule with the highest occupied π-orbital of the DNA base pairs. Consequently the partial filling of the empty π^*-orbital by the electrons, the transition probability is expected to be reduced which will lead to hypochromism. The suggested mixed binding mode (intercalation and groove binding) will unwind the DNA helix at the interaction sites which will lead to perturbation in its normal functioning that may culminate in cellular death.

Assuming the two state binding of FC with DNA ("free" and "DNA bound"), the binding constant was calculated from the decay of absorbance according to the following equation:[43–45]

$$\frac{A_0}{A - A_0} = \frac{\varepsilon_G}{\varepsilon_{H-G} - \varepsilon_G} + \frac{\varepsilon_G}{\varepsilon_{H-G} - \varepsilon_G} \cdot \frac{1}{K[\mathrm{DNA}]} \qquad (6)$$

where K is the binding constant, A and A_0 represent the absorbance of the drug with and without DNA, ε_{H-G} and ε_G are the coefficients Fc–DNA adduct and free Fc.

The binding constant K $(4.91\ (\pm 0.20) \times 10^3\ \mathrm{M}^{-1})$ was obtained from the intercept to slope ratio of $A_0/(A - A_0)$ vs. 1/[DNA]. However, it is an order of magnitude greater than the binding constant $(3.45 \times 10^2\ \mathrm{M}^{-1})$ of protonated ferrocene with DNA,[46] due to the presence of planar 4-nitrophenyl-2-propen-1-one, which can effectively intercalate into DNA. However, the value of K is moderate as compared to the high value of K (6.15×10^5), reported for the interaction of 1-(4′-aminophenyl)-3-(4″-*N*,*N*-dimethylaminophenyl)-2-propen-1-one with DNA[20] due to the sandwich-like ferrocenyl group which prevents the whole molecule from intercalating. The interaction of FC with DNA will stop the proliferation of cancerous cell by damaging the DNA transcription machinery.

The value of the binding constant, determined here is comparable to the $K = 5.17\ (\pm 0.25) \times 10^3\ \mathrm{M}^{-1}$ obtained from CV measurements.

Viscosity Measurements. To support the results obtained from CV and UV–vis absorption titrations concerning the mode of binding viscometric titrations were performed by the addition of increasing concentration of DNA (ranging from 20 to 200 μM) into 50 μM constant concentration of the drug buffered at pH 7.4. Data were presented as η/η_o vs. the concentration of DNA, where η is the viscosity of the drug in the presence of DNA and η_o is the viscosity of the drug alone.

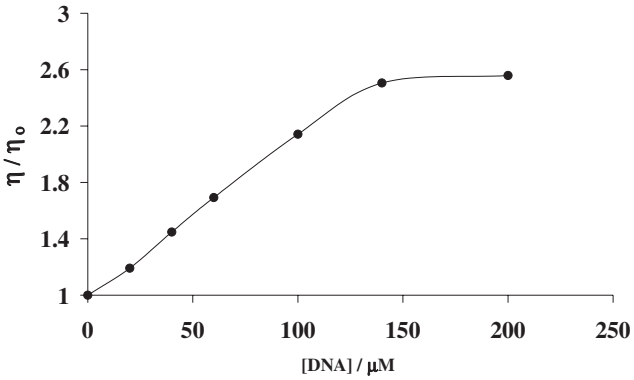


Figure 7. Plot of relative viscosity (η/η_o) vs. concentration of DNA in 0.25 M phosphate buffer (pH 7.4) at 25 °C.

A plot of (η/η_o) against the concentration of DNA is shown in Figure 7. The relative viscosity increases with the increase in concentration of DNA. In general, a classical intercalation mode causes an increase in the viscosity of DNA solution due to the increased separation of base pairs at the intercalation sites, and hence an increase in the overall DNA length.[47] This behavior suggests that FC binds with DNA via an intercalative mode of binding.

The radii of free FC ($r = 4.7$ nm) and its adduct with DNA ($r = 19.5$ nm) were calculated using the following rearranged form of the Stokes–Einstein equation:

$$r = k_B T / 6\pi\eta D \qquad (7)$$

The result shows that the radius of the FC–DNA complex is greater than the free drug. The increase in radius may be linked with the rupture of DNA strands, which will lead to cell apoptosis.

Conclusion

The results demonstrate that electrochemical methods can be successfully employed to evaluate the mode of interaction and binding parameters like binding constant, Gibbs energy of adduct formation and binding site size.

In general, FC shows electrochemically, spectroscopically and viscometrically measurable interactions with DNA at blood pH and ambient temperature of 25 °C. Its CV, UV–vis, and viscometric results reveal intercalation as the dominant mode of interaction. The binding constant with values of $5.17\ (\pm 0.25) \times 10^3$ and $4.91\ (\pm 0.20) \times 10^3\ \mathrm{M}^{-1}$ was obtained from CV and UV–vis spectroscopic techniques. The Gibbs energy change $(\Delta G = -RT \ln K)$ of $-21.18\ \mathrm{kJ\ mol}^{-1}$ at 25 °C indicates the spontaneity of the binding interaction.

These investigations reliably unfold the binding mode and interaction strength as required for the design of effectively specific anticancer drugs.

We are highly grateful to Higher Education Commission Islamabad, Pakistan for supporting this work.

References

1 E. W. Neuse, *J. Inorg. Organomet. Polym.* **2005**, *15*, 3.
2 N. Metzler-Nolte, *Nachr. Chem.* **2006**, *54*, 966.
3 M. J. Clarke, F. Zhu, D. R. Frasca, *Chem. Rev.* **1999**, *99*, 2511.
4 S. C. Barton, H.-H. Kim, G. Binyamin, Y. Zhang, A. Heller, *J. Phys. Chem. B* **2001**, *105*, 11917.
5 F. Otón, A. Tárraga, M. D. Velasco, A. Espinosa, P. Molina, *Chem. Commun.* **2004**, 1658.
6 D. Jimenez, R. Martinez-Manez, F. Sancenon, J. V. Ros-Lis, J. Soto, A. Benito, E. Garcia-Breijo, *Eur. J. Inorg. Chem.* **2005**, 2393.
7 M. Ciszkowska, Z. Stojek, *Anal. Chem.* **2000**, *72*, 754A.
8 P. Hudeczek, F. H. Kohler, P. Bergerat, O. Kahn, *Chem.—Eur. J.* **1999**, *5*, 70.
9 C. B. Hollandsworth, W. G. Hollis, Jr., C. Slebodnick, P. A. Deck, *Organometallics* **1999**, *18*, 3610.
10 T. Sasayama, K. Tanaka, K. Mizukawa, A. Kawamura, T. Kondoh, K. Hosoda, E. Kohmura, *J. Neurooncol.* **2007**, *85*, 123.
11 C.-L. Ye, J.-W. Liu, D.-Z. Wei, Y.-H. Lu, F. Qian, *Pharm. Res.* **2004**, *50*, 505.
12 C.-L. Ye, J.-W. Liu, D.-Z. Wei, Y.-H. Lu, F. Qian, *Cancer Chemother. Pharmacol.* **2005**, *55*, 447.
13 Y. S. Lee, S. S. Lim, K. H. Shin, Y. S. Kim, K. Ohuchi, S. H. Jung, *Biol. Pharm. Bull.* **2006**, *29*, 1028.
14 E. Hillard, A. Vessières, L. Thouin, G. Jaouen, C. Amatore, *Angew. Chem., Int. Ed.* **2006**, *45*, 285.
15 M. T. Carter, M. Rodriguez, A. J. Bard, *J. Am. Chem. Soc.* **1989**, *111*, 8901.
16 E. Palecek, *Talanta* **2002**, *56*, 809.
17 A. Erdem, M. Ozsoz, *Turk. J. Chem.* **2001**, *25*, 469.
18 M. Aslanoglu, C. J. Isaac, A. Houlton, B. R. Horrocks, *Analyst* **2000**, *125*, 1791.
19 S. A. Özkan, Y. Özkan, Z. Şentürk, *J. Pharm. Biomed. Anal.* **1998**, *17*, 299.
20 A. Shah, A. M. Khan, R. Qureshi, F. L. Ansari, M. F. Nazar, S. S. Shah, *Int. J. Mol. Sci.* **2008**, *9*, 1424.
21 M. E. Reichmann, S. A. Rice, C. A. Thomas, P. Doty, *J. Am. Chem. Soc.* **1954**, *76*, 3047.
22 X. Wu, E. R. Tiekink, I. Kostetski, N. Kocherginsky, A. L. Tan, S. B. Khoo, P. Wilairat, M. L. Go, *Eur. J. Pharm. Sci.* **2006**, *27*, 175.
23 J. Marmur, *J. Mol. Biol.* **1961**, *3*, 208.
24 X. Lu, M. Zhang, J. Kang, X. Wang, L. Zhuo, H. Liu, *J. Inorg. Biochem.* **2004**, *98*, 582.
25 D.-W. Pang, H. D. Abruna, *Anal. Chem.* **1998**, *70*, 3162.
26 J. Labuda, M. Bučková, M. Vaníčková, J. Mattusch, R. Wennrich, *Electroanalysis* **1999**, *11*, 101.
27 T. W. Welch, H. H. Thorp, *J. Phys. Chem.* **1996**, *100*, 13829.
28 F. Wang, Y. Xu, J. Zhao, S. Hu, *Bioelectrochemistry* **2007**, *70*, 356.
29 J. E. B. Randles, *Trans. Faraday Soc.* **1948**, *44*, 327.
30 A. Sevcik, *Collect. Czech. Chem. Commun.* **1948**, *13*, 349.
31 M. T. Carter, A. J. Bard, *J. Am. Chem. Soc.* **1987**, *109*, 7528.
32 S. Chanfreau, P. Cognet, S. Camy, J. S. Condoret, *J. Electroanal. Chem.* **2007**, *604*, 33.
33 J. Swiatek, *J. Coord. Chem.* **1994**, *33*, 191.
34 I. S. Shehatta, M. S. Ibrahim, *Can. J. Chem.* **2001**, *79*, 1431.
35 S. Wang, T. Peng, C. Yang, *Biophys. Chem.* **2003**, *104*, 239.
36 J. Niu, G. Cheng, S. Dong, *Electrochim. Acta* **1994**, *39*, 2455.
37 M. T. Carter, M. Rodriguez, A. J. Bard, *J. Am. Chem. Soc.* **1989**, *111*, 8901.
38 M. Aslanoglu, C. J. Isaac, A. Houlton, B. R. Horrocks, *Analyst* **2000**, *125*, 1791.
39 A. J. Bard, L. R. Faulkner, *Electrochemical Methods Fundamentals and Applications,* John Wiley & Sons, Inc., NY, USA, **1986**, p. 256.
40 M. Kožurková, D. Sabolová, L. Janovec, J. Mikeš, J. Koval', J. Ungvarský, M. Štefanišinová, P. Fedoročko, P. Kristian, J. Imrich, *Bioorg. Med. Chem.* **2008**, *16*, 3976.
41 R. F. Pasternack, E. J. Gibbs, J. J. Villafranca, *Biochemistry* **1983**, *22*, 2406.
42 J. Kang, H. Wu, X. Lu, Y. Wang, L. Zhou, *Spectrochim. Acta, Part A* **2005**, *61*, 2041.
43 X.-J. Dang, R. Tong, H.-L. Li, *J. Inclusion Phenom.* **1996**, *24*, 275.
44 M. Y. Nie, Y. Wang, H. L. Li, *Pol. J. Chem.* **1997**, *71*, 816.
45 J. B. Lepecq, C. Paoletti, *J. Mol. Biol.* **1967**, *27*, 87.
46 A. Shah, R. Qureshi, N. K. Janjua, S. Haque, S. Ahmad, *J. Anal. Sci.*, **2008**, *24*, 1437.
47 J. M. Veal, R. L. Rill, *Biochemistry* **1991**, *30*, 1132.