Indian Journal of Chemistry
Vol. 52A, October 2013, pp. 1251-1256

Papers

Spectroscopic and electrochemical monitoring on the binding of idarubicin as a chemotherapy drug with ds-DNA

Reza Hajian[a, b, *] & Farkhondeh Panahi[c]

[a]Institute of Advanced Technology, University Putra Malaysia, 43400 Serdang, Selangor, Malaysia

[b]Young Researchers Club, Gachsaran Branch, Islamic Azad University, 75818-63876, Gachsaran, Iran

[c]Department of Chemistry, Gachsaran Branch, Islamic Azad University, 75818-63876, Gachsaran, Iran

Email: hajian@iaug.ac.ir

Received 01 April 2013; revised and accepted 18 September 2013

The interaction between idarubicin, a heterocyclic anticancer drug, and fish sperm DNA has been investigated in physiological buffer (***p***H 7.4) using methylene blue dye as a spectral probe by UV–vis absorption, cyclic voltammetry, fluorescence spectroscopy, as well as viscosity measurements. The hypochromicity effect on the spectra of idarubicin in the presence of DNA with a partial red shift is observed. The results indicate that idarubicin molecule can bind to DNA and the major binding mode is intercalative binding in high ionic strength while the electrostatic binding is dominant in low ionic strength. The binding constants and the number of binding sites of idarubicin molecule with DNA are obtained by UV-vis spectrophotometry and fluorescence quenching methods. The thermodynamic parameters, $\Delta \boldsymbol{H}$, $\Delta \boldsymbol{S}$ and $\Delta \boldsymbol{G}$ are calculated as 1.95×10^{4} J mol^{-1}, 1.52×10^{2} J mol^{-1} K^{-1} and -2.51×10^{4} J mol^{-1} respectively, showing that the binding of idarubicin to DNA is driven mainly by hydrophobic interaction. The diffusion coefficient for the free drug is found to be 2.6×10^{-3} cm^{2} s^{-1}, whereas in the presence of DNA, it is 1.2×10^{-3} cm^{2} s^{-1}.

Keywords: Idarubicin, Fish sperm DNA, Spectroscopy, Cyclic voltammetry, Fluorescence, Viscosity, Hypochromicity, DNA binding, Intercalative binding, Hydrophobic interaction

DNA plays a major role in the life process because it carries heritage information and instructions for the biological synthesis of proteins and enzyme through the process of replication and transcription of genetic information. DNA is quite often the main cellular target for studies with smaller molecules of biological importance like carcinogens, steroids and several classes of drugs. The investigation of drug–DNA interactions is of current general interest and importance[1-3], especially for the designing of new DNA-targeted drugs and there ***in vitro*** screening. Generally, the interactions of small molecules with DNA involve three binding modes, via., intercalation, groove binding and long-range assembly on the molecular surfaces of nucleic acids[4]. The intercalative binding is stronger than the other two binding modes because the surface of intercalative molecule is sandwiched between the aromatic, heterocyclic base pairs of DNA[5,6].

Many techniques have been applied for investigation of the interaction of DNA with small molecules, including UV-vis spectrophotometry[7-10], fluorescence spectroscopy[11], circular dichroism spectroscopy[12], voltammetry[13], X-ray diffraction[14] and dynamic viscosity measurements[15].

Idarubicin or 4-demethoxydaunorubicin is an anthracycline antileukemic drug. It inserts itself into the DNA and prevents DNA from unwinding by interfering with the enzyme topoisomerase II. It is an analog of daunorubicin, but the absence of a methoxy group increases its fat solubility and cellular uptake.

Herein, the binding reactions of idarubicin and DNA have been studied in detail by UV-vis spectrophotometry, cyclic voltammetry, fluorescence spectrometry and viscosity measurements because of their high sensitivity, rapidity and ease of implementation. The binding constant has been measured by spectrophotometry and fluorescence quenching method. The binding mode, diffusion coefficient as well as the quenching mode between idarubicin and DNA are also described.

Materials and Methods

Fish sperm DNA was purchased from Sigma biological Co. and used as received. Methylene blue dye was purchased from Fluka, while idarubicin

hydrochloride solution (1000 mg L^{-1}) was purchased from Pharmaceutical Partners of Canada Inc. A phosphate buffer (***p***H 7.40, examined by acidometer) was used to control the ***p***H of the system. All of the samples were dissolved in the phosphate buffer. Other reagents were at least analytical grade. All the reagents were freshly prepared immediately before use.

The absorption spectra were recorded on a Lamda 25 spectrophotometer (Perkin Elmer) and the fluorescence spectra were recorded on a Cary Eclipse spectrofluorometer from Varian, USA. The electrochemical studies were recorded using an Autolab instrument (model PGSTAT 30 processor) with three electrodes consisting of a glassy carbon as a working electrode, an Ag/AgCl (3.0 ***M*** KCl) reference electrode and a carbon counter electrode that linked to a computer (Pentium IV, 2.0 GHz).

Viscosity measurements were made on an Ancontaar viscometer (model SVM 3000), which was immersed in a thermostat water-bath at room temperature. Varying amounts of DNA was then added into the viscometer while keeping idarubicin concentration constant. The flow times of the samples were repeatedly measured with an accuracy of ±0.20 s by using a digital stopwatch. The data are presented as $(\eta/\eta_0)^{1/3}$ versus $\boldsymbol{C}_{DNA}$, where η and η_0 are the viscosity of idarubicin in the presence and absence of fish sperm DNA, respectively.

Results and Discussion

Absorption spectra: Idarubicin-DNA interaction

UV–vis absorption spectra were obtained by titration of a 1.0×10^{-5} mol L^{-1} idarubicin hydrochloride solution with increasing concentration of DNA (Fig. 1). In the absence of DNA, the spectrum of idarubicin hydrochloride (curve 1) was characterized by three peaks at 253, 290 and 485 nm, respectively. With the addition of DNA, the intensity at 253 nm increased significantly but the intensity at 293 and 485 nm decreased with the increasing concentration of DNA and a partial red shift was observed from 485 to 486 nm. This shift is attributed to the overlap of the decreasing idarubicin bands and the progressively growing 293 nm band from the free DNA in solution. An isobestic point at around 265 nm provides evidence that a new species is forming during the interaction with DNA and that the reaction is homogeneous. These observations are consistent with the partial intercalation of idarubicin hydrochloride into the double helix of DNA[16-18]. Firstly, the overall negative charge of idarubicin hydrochloride indicates that the molecule was unlikely to interact with the negatively charged phosphates of the backbone of the DNA. Secondly, the spectral change observed was a decrease in absorbances at 293 and 485 nm with a partial peak shift. This may be attributed to the decrease in the number of chromophores in solution as the drug was removed by DNA, i.e., intercalated. Thirdly, this intercalation would have to be partial because idarubicin molecule is quite large, and would be unlikely to fit into the cavity completely. Given the probable intercalation mode of binding with the DNA, the following linear relationship should hold and yield the binding constant [19,20] ***K***,

$$\frac{\boldsymbol{A}_0}{\boldsymbol{A}-\boldsymbol{A}_0}=\frac{\boldsymbol{G}}{\boldsymbol{H}-\boldsymbol{G}-\boldsymbol{G}}+\frac{\boldsymbol{G}}{\boldsymbol{H}-\boldsymbol{G}-\boldsymbol{G}}\times\frac{1}{\boldsymbol{K}[\boldsymbol{DNA}]} \qquad \ldots(1)$$

where '$\boldsymbol{A}_0$' and '$\boldsymbol{A}$' are the absorbances of idarubicin hydrochloride in the absence and presence of DNA respectively and ε_G and $\varepsilon_{H\text{-}G}$ are apparent extinction coefficients of free idarubicin, and idarubicin complex in the bound form with DNA, respectively.

The double reciprocal plot of $\frac{\boldsymbol{A}_0}{\boldsymbol{A}-\boldsymbol{A}_0}$ versus $\frac{1}{[\text{DNA}]}$ was linear and the binding constant was calculated as 3.03×10^4 L mol^{-1} from the ratio of the intercept to the slope equation at 298 K.

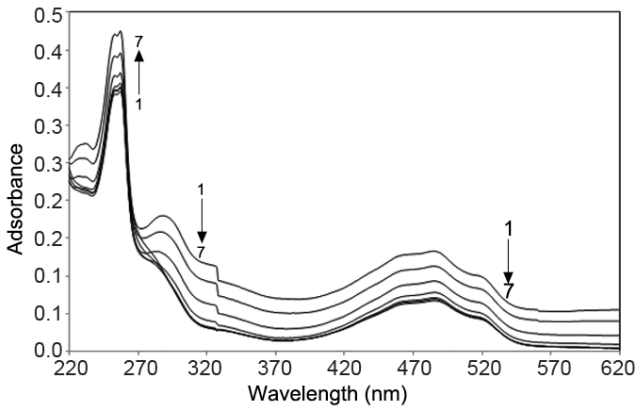

Fig. 1—Absorption spectra of idarubicin in the presence of varying concentrations of DNA [$\boldsymbol{C}_{DNA}$=0.0, 3.5, 6.9, 10.4, 13.8, 17.3 and 20.7 μmol L^{-1} for curves 1-7; $\boldsymbol{C}_{Idarubicin}$=10.0 μmol L^{-1} in phosphate buffer (0.1 mol L^{-1}, ***p***H 7.4)].

Thermodynamic parameters and nature of the binding forces

Small molecules are bound to macromolecules by four possible binding modes: hydrogen bonding, van der Waals, electrostatic and hydrophobic interactions[21]. In order to clarify the interaction of Idarubicin with DNA, thermodynamic parameters, ΔH, ΔG and ΔS, were calculated. If the temperature does not vary significantly, then the enthalpy change (ΔH) can be regarded as a constant and its value can be evaluated from the Clausius-Clapeyron equation.

At 293 K, the values of binding constant K, ΔH, ΔG and ΔS were found to be 3.03×10^4 L mol^{-1}, 1.95×10^4 J mol^{-1}, -2.51×10^4 J mol^{-1} and 1.52×10^2 J mol^{-1} K^{-1} respectively, while the binding constant at 313 K calculated as 8.49×10^4 L mol^{-1}.

The negative sign for ΔG indicates that the interaction process is spontaneous The change of entropy is positive, which indicates that the interaction causes an increase in transitional and rotational degrees of freedom of the molecule[22].

Absorption spectra: Methylene blue dye-DNA interaction

Absorption spectra of methylene blue (MB) dye in the absence of DNA (*p*H 7.4) showed two spectra at maximum wavelengths of 290 and 662 nm (Fig. 2). The absorptions at both wavelengths gradually decreased with the increasing concentration of DNA; this red shift is usually associated with molecular intercalation into the base stack of DNA[23]. The two observed spectral effects are attributed to a strong interaction between the electronic state of the intercalating chromophore and that of the DNA bases. The strength of the electronic interaction relates to the third-order of the distance between the target molecule and DNA bases[24]. The large hypochromism observed during the interaction of the MB dye with DNA strongly suggests that the distance between the intercalated MB dye and DNA bases is small. Also, an isobestic point at 281 nm demonstrates formation of a new adduct between MB and DNA. The binding constant between MB-DNA at 298K was determined as 2.80×10^5 L mol^{-1} using the double-reciprocal equation.

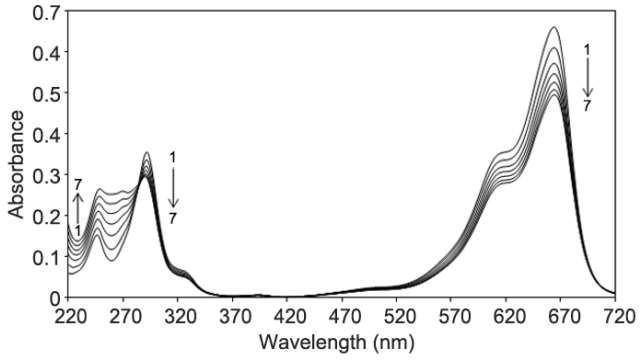

Fig. 2—Absorption spectra of methylene blue in the presence of varying concentrations of DNA. [C_{DNA}=0.0, 5.3, 10.0, 14.8, 19.1, 23.0 and 26.7 μmol L^{-1} for curves 1-7; C_{MB}=10.0 μmol L^{-1} in phosphate buffer (0.1 mol L^{-1}, *p*H 7.4)].

Absorption spectra: Competitive interaction of idarubicin with MB-DNA

The absorbance of MB–DNA complex at 665 nm gradually decreased in intensity with the increasing concentration of idarubicin. This band is shifted towards the red end of the spectrum with the appearance of a new peak at 484 nm, which increased progressively in intensity (Fig. 3). An isosbestic point at around 556 nm provides evidence that a new species was forming during the competitive interaction. The observed changes in intensity and position of the bands with increasing amounts of Idarubicin molecule added to MB-DNA solution, suggests that idarubicin hydrochloride intercalates into the double helix of DNA without exchanging with the MB fluorophoric dye probe. Therefore, the decreasing in absorbance of MB at 665 nm is due to the decrease in the concentration of MB-DNA and matrix effect[25].

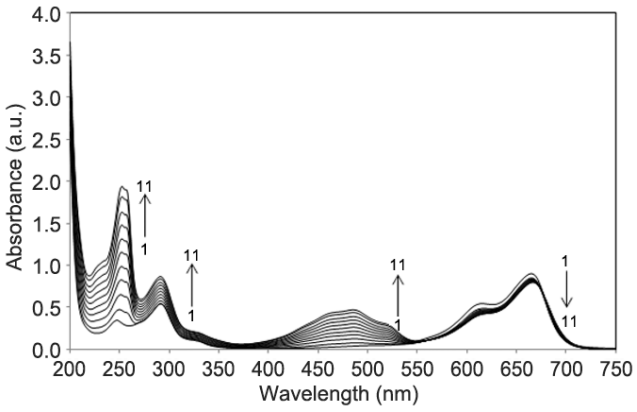

Fig. 3—Absorption spectra of the competitive interaction between idarubicin and methylene blue bonded to DNA. [$C_{Idarubicin}$=0.0, 2.5, 5.0, 7.5 10.0, 12.5, 15.0, 17.5, 20.0, 22.5 and 25.0 μmol L^{-1} for curves 1-11; C_{MB}=10.0 μmol L^{-1}; C_{DNA}=10.0 μmol L^{-1} in phosphate buffer (0.1 mol L^{-1}, *p*H 7.4)].

Synchronous fluorescence spectra: idarubicin hydrochloride-DNA interaction

The fluorescence of idarubicin is at 570 nm in excitation wavelength of 482 nm. The fluorescence intensity decreased significantly in the presence of DNA. Figure 4 shows the fluorescence spectra of idarubicin in the presence and absence of fish sperm DNA. The quenching of fluorescence intensity may be largely due to the diffusion of drug molecule between DNA base pairs and decrease in its effective concentration in the solution. The binding constant and the number of binding sites were determined to 6.7×10^4 L mol^{-1} and 1.2 respectively from the plot of $\log\left(\frac{F_0 - F}{F}\right)$ versus $\log[DNA]$ (refs 26, 27).

Cyclic voltammetric studies

The electrochemical behavior of idarubicin in the absence and presence of DNA at bare GCE was studied by cyclic voltammetry. One reduction peak at -0.45 V (vs. Ag/AgCl) and one oxidation peak at ***ca***. +0.43 V (vs. Ag/AgCl) reveals the irreversibility of the system. The reduction peak current for idarubicin decreased significantly in the presence of DNA after successive addition up to 5.0 mole ratio ([Idarubicin]/[DNA]) and the reduction peak potential shifted in the negative direction (Fig. 5). The binding of drug to DNA should lead to a significant decrease in peak current due to the formation of drug-DNA adduct with smaller diffusion coefficient[28-30]. This can be observed in the decrease in slope of the equations $\boldsymbol{I}_p$ (A) = 6.0×10^{-6} $^{0.5}$(V s^{-1}) + 2.0×10^{-7} and $\boldsymbol{I}_p$ (A) = 5.0×10^{-6} $^{0.5}$ (V s^{-1}) + 2.0×10^{-7}, in the absence and presence of DNA respectively.

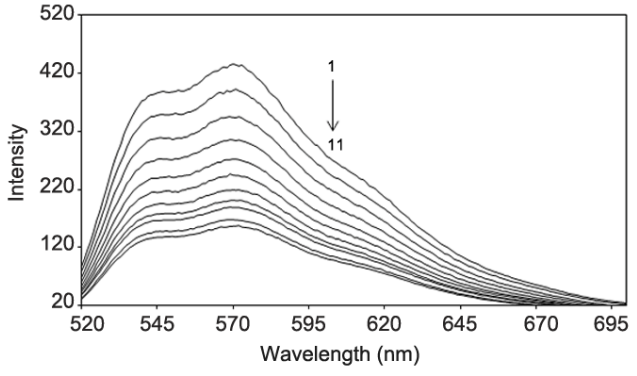

Fig. 4—Fluorescence spectra of idarubicin after addition of varying concentrations of DNA. [$\boldsymbol{C}_{DNA}$=0.0, 10.0, 19.8, 29.4, 39.0, 48.0, 57.6, 66.8, 75.8, 84.7 and 93.4 μmol L^{-1} for curves 1-11; $\boldsymbol{C}_{Idarubicin}$=20.0 μmol L^{-1} in phosphate buffer (0.1 mol L^{-1}, ***p***H 7.4) at excitation wavelength of 482 nm].

In order to demonstrate that the decrease in current was not due to the increase in viscosity of the solution or the blockage of the electrode surface by ds-DNA adsorption, cyclic voltammetry experiment was designed in a $K_4Fe(CN)_6$ solution with or without DNA. In this solution, there is repulsion between the $Fe(CN)_6^{4-}$ ion and DNA backbone because of the same negative charges. The change in the oxidation and reduction currents of $K_4Fe(CN)_6$ is not significantly and also no shift is observed in the peak potential in the absence and presence of DNA. Therefore, there is no change on the viscosity and diffusion coefficients due to $Fe(CN)_6^{4-}$ ion and also no significant obstruction due to DNA on the surface of electrode.

For the irreversible redox reaction of idarubicin, the n_a value (the electron transfer coefficient, $\boldsymbol{n}_a$ the number of electrons involved in the rate-determining step) was calculated as 0.25 and 0.22 for free drug and drug-DNA adduct respectively based on the slopes of Tafel plots. From these values, the diffusion coefficients for the free drug ($\boldsymbol{D}_f$) and drug-DNA adduct (D_b) were calculated as 2.6×10^{-3} and 1.2×10^{-3} cm^2 s^{-1} respectively.

Effect of ionic strength

DNA-small molecule interaction is strongly dependent on the ionic strength of the medium[30], i.e., at low ionic strength values the electrostatic interaction mode between a molecule and the negative phosphate backbone of DNA is predominant, and conversely, at high ionic strength values the negative

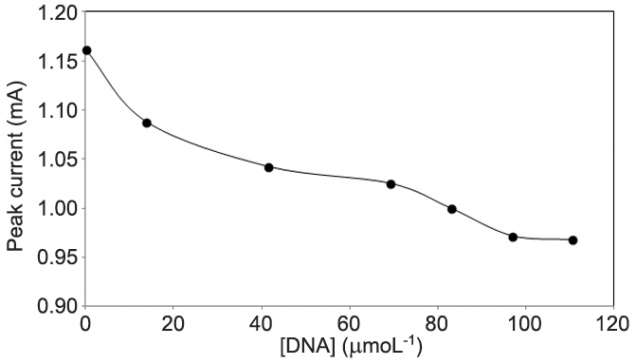

Fig. 5—Dependence of reduction peak current of idarubicin in the presence of varying concentrations of DNA. [C_{DNA}=0.0, 13.8, 27.6, 41.5, 55.3, 69.1, 82.9 and 96.7 μmol L^{-1}; $\boldsymbol{C}_{Idarubicin}$=20.0 μmol L^{-1}, = 100 mV s^{-1}].

charges of DNA phosphates are shielded, which favors the intercalation interaction mode. Thus, in the present study, 50.0 mmol L^{-1} KCl was added to the reaction media before starting the interaction time between idarubicin and ds-DNA. Then the reduction current of idarubicin was determined at both low (0.0 mmol L^{-1} KCl) and high (50.0 mmol L^{-1} KCl) ionic strengths. The normalized current observed after 5 min of interaction with DNA was not affected by addition of KCl in the reaction media, suggesting that the molecule can interact with ds-DNA by electrostatic mode at low ionic strength and by the intercalative mode at high ionic strength. This fact suggests that idarubicin can interact electrostatically with DNA and when the charges of phosphate groups present in DNA are shielded, there is intercalation mode of interaction.

Determination of viscosity

One indication of DNA binding mode is the change in viscosity when a small molecule associates with DNA. Intercalative binding increases the length of DNA and hence the viscosity significantly, whereas groove binding typically has a smaller effect on viscosity[31]. The plot of $(/ _0)^{1/3}$ versus ([Idarubicin]/[DNA]) reveals that the viscosity of ds-DNA increases considerably on the addition of idarubicin (up to 8.0 times that of DNA) and then levels off at higher mole ratios. This supports the proposal that in the interaction between idarubicin and DNA intercalation dominates at low mole ratios and electrostatic is dominant at higher mole ratios (>8.0).

Conclusions

In this work, the interaction of idarubicin, a chemotherapy drug, with ds-DNA has been studied by cyclic voltammetry, viscometery and by various spectroscopic methods. The binding of idarubicin with DNA resulted in a series of changes in the electrochemical behavior and spectral characteristics. Upon binding to DNA, the adsorption spectra of the drug showed peculiar hypochromic effect and the fluorescence emission was efficiently decreased due to the quenching and decrease in the effective concentration of the drug. Also, the viscosity of DNA increased in the presence of idarubicin. The present study affirms that the interaction of idarubicin with ds-DNA is intercalative and electrostatic in the high ionic strength and low ionic strength respectively. Moreover, the large binding constant (3.03×10^4 L mol^{-1}) indicates a high affinity of drug for the DNA base pairs and the intercalation mechanism appears to be critical for this high affinity. These investigations showed that electrochemistry coupled with spectroscopy techniques could provide a convenient way to characterize both the binding mode and the interaction mechanism of some intercalators binding to DNA, which is important for the design of new effective drugs.

Acknowledgement

The authors gratefully acknowledge the support of this work by research council of Islamic Azad University of Gachsaran located in Kohgilooye & Boyerahmad Province of Iran, Iran

References

1 Sahoo B K, Ghosh K S, Bera R & Dasgupta S, ***Chem Phys***, 351 (2008) 163.
2 Sun Y, Bi S Y, Song D Q, Qiao C Y, Mu D & Zhang H Q, ***Sensors Actuators B***: Chem, 129 (2008) 799.
3 Ling X, Zhong W Y, Huang Q & Ni K Y, ***J Photochem Photobiol B: Biol***, 93 (2008) 172.
4 Tjahjono D H, Akutsu T, Yoshioka N & Inoue H, ***Biochim Biophys Acta,*** 1472 (1999) 333.
5 Ling L S, He Z K, Chen F & Zeng Y E, ***Talanta,*** 59 (2003) 269.
6 He X Q, Lin Q Y, Hu R D & Lu X H, ***Spectrochim Acta A,*** 68 (2007) 184.
7 Modukuru N K, Snow K J, Perrin B S J, Bhambhani A, Duff M & Kumar C V, ***J Photochem Photobiol A***: Chem, 177 (2006) 43.
8 Hajian R, Shams N & Parvin A, ***Chinese J Chem*** 27 (2009) 1055.
9 Hajian R, Shams N & Mohagheghian M, ***J Brazil Chem Soc***, 20 (2009) 1399.
10 Hajian R, Shams N & Azarinezhad N, ***Asian J Chem***, 22 (2010) 8007.
11 Li J F & Dong C, ***Spectrochim Acta A,*** 71 (2009) 1938.
12 Mahadevan S & Palaniandavar M, ***Inorg Chim Acta,*** 254 (1997) 291.
13 Liu S Q, Cao M L & Dong S L, ***Bioelectrochem,*** 74 (2008) 164.
14 Carcelli M, Mazza P, Pelizzi C, Pelizzi G & Zani F, ***J Inorg Biochem,*** 57 (1995) 43.
15 Bi S Y, Qiao C Y, Song D Q, Tian Y, Gao D J, Sun Y & Zhang H Q, ***Sensors Actuat B,*** 119 (2006) 199.
16 Plaper A, Golob M, Hafner I, Oblak M, Solmajer T & Jerala R, ***Biochem Biophys Res Commun***, 306 (2003) 530.
17 Rahman A, Shahabuddin S, Hadi S M & Parish J H**,** ***Carcinogenesis,*** 11 (1990) 2001.
18 Takenaka S, Ihara T & Takagi M, ***J Chem Soc Chem Commun,*** 1990 (1990) 1485.
19 Mudasir N & Yoshioka H, ***J Inorg Biochem,*** 77 (1999) 239.
20 Purcell M, Neault J F & Riahi T, ***Biochem Biophys Acta***, 1478 (2000) 61.
21 Barbero N, Barni E, Barolo C, Quagliotto P, Viscardi G, Napione L, Pavan S & Bussolino F, ***Dyes Pig,*** 307 (2009) 80.
22 Temerk Y M & Ibrahim M K, ***Microchim Acta A***, 71 (2009) 1830.

23 Long E C & Barton J K, ***Acc Chem Res,*** 23 (1990) 271.
24 Huang C Z, Li Y F & Feng P, ***Talanta,*** 55 (2001) 321.
25 Ensafi A A, Hajian R & Ebrahimi S, ***J Brazil Chem Soc,*** 20 (2009) 266.
26 Hu Y J, Liu Y, Wang J B, Xiao X H & Qu S S, ***J Pharm Biomed Anal*** 36 (2004) 915.
27 Yantao S, Shuyun B, Daqian S, Chunyu Q, Dan M & Hanqi Z, ***Sensors Actuators B***: ***Chem***, 129 (2009) 799.
28 Shehatta I S & Ibrahim M S, ***Can J Chem,*** 79 (2001) 1431.
29 Aslanoglu M & Ayne G, ***Anal Bioanal Chem,*** 380 (2004) 658.
30 Pang D W & Abruna H D, Anal Chem, 70 (1998) 3162.
31 Breslin D T, Coury J E, Anderson J R, McFail-Isom L, Kan Y Z, Williams L D, Bottomley L A & Schuster G B, ***J Am Chem Soc*** 119 (1997) 5043.