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Interaction Force Between Alizarin Red and Bovine Serum Albumin

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The interaction of Alizarin red and bovine serum albumin has been investigated by fluorescence spectroscopy. A 1:1 complex were found to form and the distance between the Trp-212 residues of bovine serum albumin and Alizarin red binding site was calculated to be 1.82 nm. The interaction force between Alizarin red and bovine serum albumin was suggested to be mainly van der Waals force and hydrogen bond through the calculated thermodynamic parameters of the reaction. Also ion strength effects, titration experiment of bovine serum albumin to Alizarin red were used to determine the force. Autodock research further proved the interaction mode. These experimental and theoretical data are of potential importance in understanding the mechanism of interaction between Alizarin red and bovine serum albumin.

Keywords: Interaction, Alizarin red, Bovine serum albumin, Autodock.

INTRODUCTION

Alizarin red (AR) or (sodium 3,4-dihydroxy anthraqinone-2-sulfonate), is an organic substance from nature plant. Because of its superior performance as dye, it was used widely in many fields including mineral identification, organism dye, complexometric titration, acid base indication. It was early reported to form a complex of bovine serum albumin-AR by UV-visible spectra detection[1-3]. Whereas the interaction force between bovine serum albumin to Alizarin red have not yet been reported so far.

It is possible that Alizarin red with aromatic ring react to bovine serum albumin by indulging in hydrophobic domain. And it could also be coordinated to bovine serum albumin by its active groups[4,5]. Are there other forces involved in the interaction? What play a key role in the reaction of bovine serum albumin to Alizarin red? All was explored in the paper by experimental and theoretical data.

EXPERIMENTAL

Bovine serum albumin (BSA) was purchased from Sigma (USA). Sodium 3,4-dihydroxy anthraqinone-2-sulfonate *i.e.*, Alizarin red (AR) was obtained from Tianjin Guangfu Co. All other chemicals were of analytical grade and used as received.

Spectra measurement: Fluorescence spectra were recorded on an F-2500 fluorescence spectrometer. To avoid contamination from tyrosine emission in the energy transfer experiment, protein samples were excited at 295 nm. To correct the dilution, the fluorescence intensity was converted to molar fluorescence intensity by dividing the fluorescence intensity *via* the analytical concentration of bovine serum albumin. For titration process of bovine serum albumin to Alizarin red, 440 nm was selected to be excitation wavelength. The absorption spectra of Alizarin red were recorded on a 2250 UV-visible spectrophotometer.

Docking experiment: The crystal structure of bovine serum albumin (SP: P02769) is obtained from Protein Data Bank. Molecular docking was carried out using the AutoDock4.2 software package[6,7].

One hundred of independent docking runs were carried out for the Alizarin red. Results were clustered according to the root-mean square deviation (RMSD) criterion. The lowest energy of docked conformations of the Alizarin red was chosen.

The PyMOL[8] molecular viewer and the MGLTools are used to render the output and to calculate the distance between nearest atoms.

RESULTS AND DISCUSSION

Fluorescence spectra of bovine serum albumin quenched by Alizarin red: Firstly, the interaction of Alizarin red and bovine serum albumin was evaluated by monitoring the intrinsic fluorescence of bovine serum albumin upon addition of Alizarin red. Fluorescence quenching spectra of bovine serum albumin in the presence of different concentrations of Alizarin red are observed. Alizarin red led to a concentration-dependent quenching of bovine serum albumin intrinsic fluorescence. In order to discern static quenching model from

collision quenching, data was analyzed according to Stern-Volmer quenching equation:

$$F_0/F = 1 + k_q\tau_0[Q] = 1 + K_{sv}[Q] \quad (1)$$

In the equation, F_0 and F are the fluorescence intensities of bovine serum albumin in the absence and presence of quencher (Alizarin red), respectively. k_q is the bimolecular quenching constant, t_0 is lifetime of the fluorophore in the absence of quencher. K_{sv} and [Q] are the Stern-Volmer dynamic quenching constant and the concentration of quencher, respectively. Quenching of bovine serum albumin by Alizarin red at different temperatures is shown in Fig. 1.

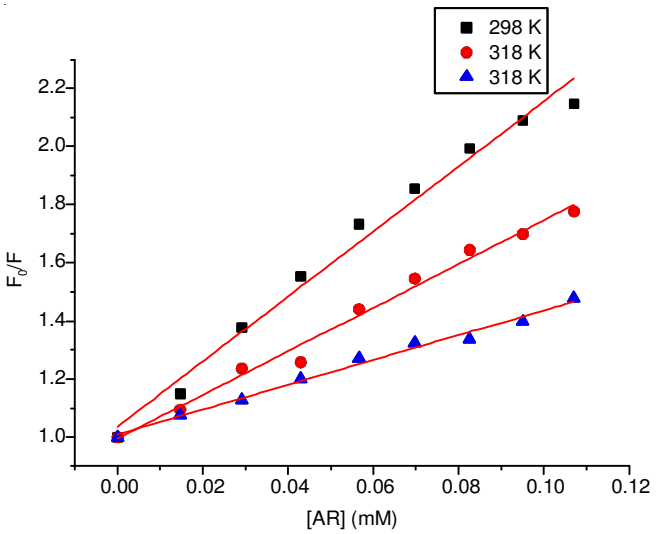


Fig. 1. Stern-Volmer plots at different temperatures

From the plot, the slope of 5.05×10^6 M^{-1} at 298 K was obtained. Since the lifetime of the fluorophore is near 10^{-8} s, k_q of 5.05×10^{14} M^{-1} s^{-1} could be drawn. Usually the bimolecular quenching constant, which can reflect the efficiency of quenching or accessibility of the fluorophore to the quencher, typically results in maximal values of K_q near 2×10^{10} M^{-1} s^{-1}. Obviously $k_q = 5.05 \times 10^{14}$ was the value unexpected for the diffusion and the result of static quenching. Besides, Stern-Volmer plots at different temperatures showed the quenching decline with increasing temperature, distinguishing the static quenching of bovine serum albumin by Alizarin red.

Binding constant and sites: It is given that there are *n* Alizarin red-binding sites and they are independent and identical in Alizarin red-bovine serum albumin complex[6]. Equilibrium between free and bound molecules can be given by Eqn. (2)[9,10]

$$\log (F_0\text{-}F)/F = \log K + n \log[Q] \quad (2)$$

where K is the binding constant for AR-BSA complex and can be determined from the intercept by plotting log $(F_0\text{-}F)/F$ *versus* log[Q].

The number of binding sites *n* equal to a slope is obtained (Fig. 2). The value of K is 1.12×10^7 and the value of n is 0.98, indicating a strong binding site of Alizarin red on the macromolecule.

Distance from tryptophan residue to Alizarin red: Foster's theory of non-radioactive energy transfer was used as determining the distance between the tryptophan residue and

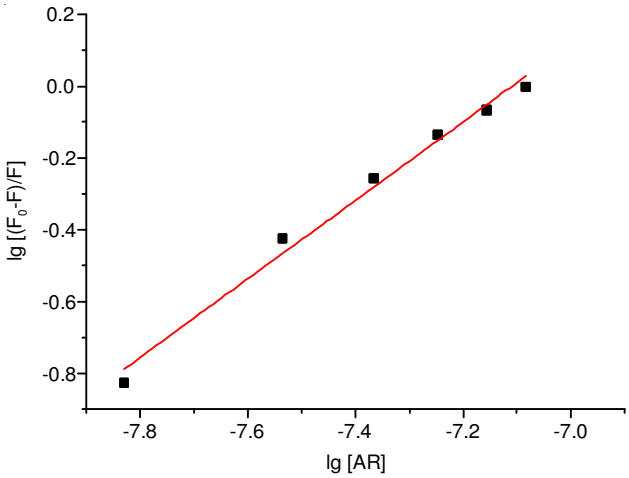


Fig. 2 lg[$(F_0$-F/F)] *vs.* lg [SAR]

Alizarin red binding site. According to the Foster's theory, the energy transfer effect is not only related to the distance between the donor (tryptophan residue) and acceptor, but also influenced by the critical energy transfer distance R_0, that is:

$$E = \frac{1}{1+(r/R_0)^6} \quad (3)$$

where r is the distance between the acceptor and the donor, and R_0 is the critical energy transfer distance, at which 50 % of the excitation energy is transferred to the acceptor is defined by the following equation:

$$R_0^6 = 8.79 \times 10^{-15}\, k^2 Q_{Trp} n^{-4} J(\lambda) \quad (4)$$

In Eqn.(4), k^2 is the orientation factor related to the geometry of the donor-acceptor of dipole, n the refractive index of medium, and Q_{Trp} is the fluorescence quantum yield of the donor. Spectra overlap integral J between the donor emission spectrum and the acceptor absorbance spectrum is then calculated by the equation:

$$J(\lambda) = \frac{\int F(\lambda)\varepsilon(\lambda)\lambda^4 d\lambda}{\int F(\lambda)d\lambda} \quad (5)$$

where, $F(\lambda)$ is the fluorescence intensity of fluorescence donor at wavelength of and $\varepsilon(\lambda)$ is molar absorbance coefficient of the acceptor when wavelength is λ. Under experimental conditions, it had been noted that $k^2 = 2/3$, n = 1.336 and Q_{Trp} = 0.15 for bovine serum albumin[11]. The spectral overlap of UV absorption spectrum of Alizarin red and fluorescence emission spectrum of bovine serum albumin is illustrated in Fig. 3. It was then calculated that $J = 5.39 \times 10^{13}$ $(mol/L)^{-1}$ cm^{-1} $(nm)^4$, $R_0^6 = 1.17 \times 10^7$ $Å^6$, E = 0.24, and r = 1.82 nm.

Interacting forces between bovine serum albumin and Alizarin red determined by thermodynamic parameters: The acting forces include hydrophobic interaction, electrostatic force, hydrogen bond, van der Waals force during a small molecule binding to protein. the binding mode could be discern from thermodynamic parameters relying on temperatures according to reports[12,13]. when the temperature changes were very little, the enthalpy change and entropy change are often be regarded as a constant. ΔG can be obtained from the following Eqn. (6), further ΔH and ΔS can be calculated by vant-Hoff Eqn. (7).

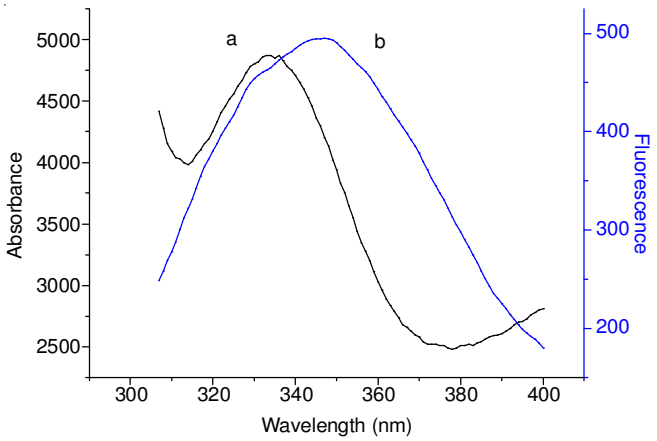


Fig. 3. Overlap of absorption spectra of AR-BSA (a) and the fluorescence emission spectra of bovine serum albumin (b), pH 6.8

$$\Delta G = -RT \ln K \quad (6)$$

$$\Delta G = \Delta H - T\Delta S \quad (7)$$

In eqn. (6), K corresponds to the binding constant at specific temperature.

As is shown in Table-1, the parameters values of ΔG, ΔH and ΔS are all negative. ΔG < 0, suggests that the reaction process is spontaneous. The negative value of ΔH and ΔS shows that the reaction is primarily determined by the enthalpy change and it can also be deduced that the acting forces for the binding reaction between Alizarin red and bovine serum albumin are mainly the hydrogen bonds and van der Waals force based on the previous report[14]. However, it is probably that there are the forces else contributing to the BSA-AR stability. In order to further obtain an affirmation, the following experiments were employed.

TABLE-1
BINDING CONSTANTS AND THERMODYNAMIC PARAMETERS AT DIFFERENT TEMPERATURE

T/K	K (10^6 M)	ΔG (kJ/mol)	ΔH (kJ/mol)	ΔS (J/mol)
298	11.2	-40.87	-76.07	-115.93
313	7.49	-40.51		
318	4.27	-39.71		

Titration experiment of bovine serum albumin to Alizarin red: The bovine serum albumin titration experiments to Alizarin red were performed to further analyze the interaction mode. The fluorescence peak of Alizarin red appears at 560 nm at pH 6.8 in Britton-Robison buffer (Fig. 4). Adding bovine serum albumin decreases the fluorescence intensities of Alizarin red and shifts the peaks towards short wavelength to 545 nm.

Owing to forming the Alizarin red-H_3BO_3 complex, the fluorescence intensities of Alizarin red increase in Britton-Robison buffer according to report[15]. Thus, the decreasing fluorescence in the experiment should attribute to Alizarin red freed from H_3BO_3 binding to bovine serum albumin, being a sign of the formation of the Alizarin red-protein complex. The blue shift might result from Alizarin red combining with bovine serum albumin so that the molecule changes environment surrounding them from aqueous solution into hydrophobic domain or locating on the surface of the protein, partially exposed to water.

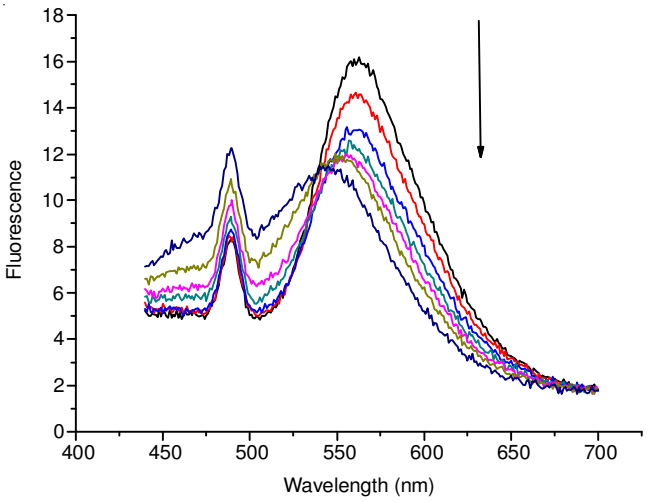


Fig. 4. Alizarin red fluorescence spectra at different concentration of bovine serum albumin

Influence of the ionic strength on the binding: The influence of the ionic strength on the fluorescence intensities of Alizarin red in the presence of bovine serum albumin had been studied, in which KCl was used to control the ionic strength. First, the fluorescence intensities increased with the increase concentration of KCl in the range of 0-120 mM; second, decreased when KCl concentration is up to 120 mM. The addition of salt (KCl) disturbs the binding of Alizarin red and bovine serum albumin. The Cl^- competed with Alizarin red to bind the bovine serum albumin through ionic interaction in the first phase and reduce the number of Alizarin red molecules binding to the bovine serum albumin, which resulted in the increase of Alizarin red fluorescence intensities (Fig. 5). This means that the electrostatic forces play a role in binding process. In the second phase, it may be attributed to dynamic quenching for the decrease intensity of Alizarin red fluorescence at the high concentration of KCl (120 mM).

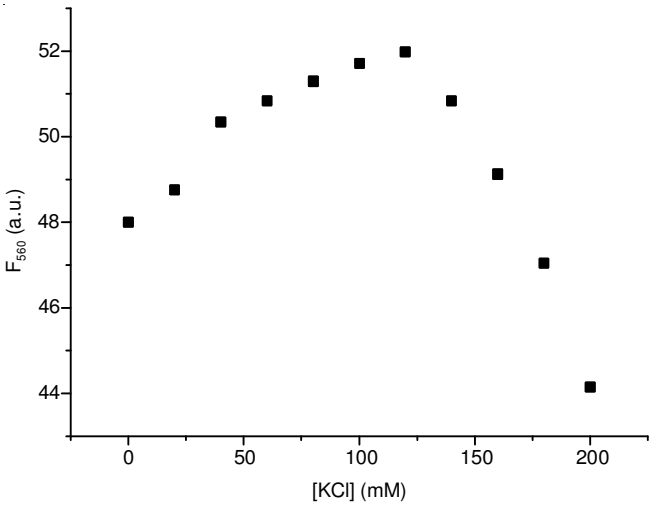


Fig. 5. Ionic strength effect on emission of Alizarin red (AR) in bovine serum albumin (BSA) solution cAR:cBSA = 1:1

Docking research: The one hundred docking conformations for Alizarin red were divided into groups by using the Cluster module in ADT. Cluster conformation analysis is to compare the docking result to the experimentally obtained

values.The lowest energy conformations cluster is chosen. AutoDock also uses the RMSD evaluation to find the best binding mode of the lowest energy conformations. The interaction modes of the Alizarin red and bovine serum albumin are depicted in Fig. 6, and the docking information is listed in Table-2.

TABLE-2
COMPARED DATA FROM DOCK WITH EXPERIMENTAL ONES

	Autodock 4	Experiment
ΔG° (kJ mol^{-1})	-8.34 kKa (35.028 kj)	-40.869
Inhibition constant (mol L^{-1})	7.74*10^{7}	7.29*10^{7}
Distance (Å)	19.38	18.2

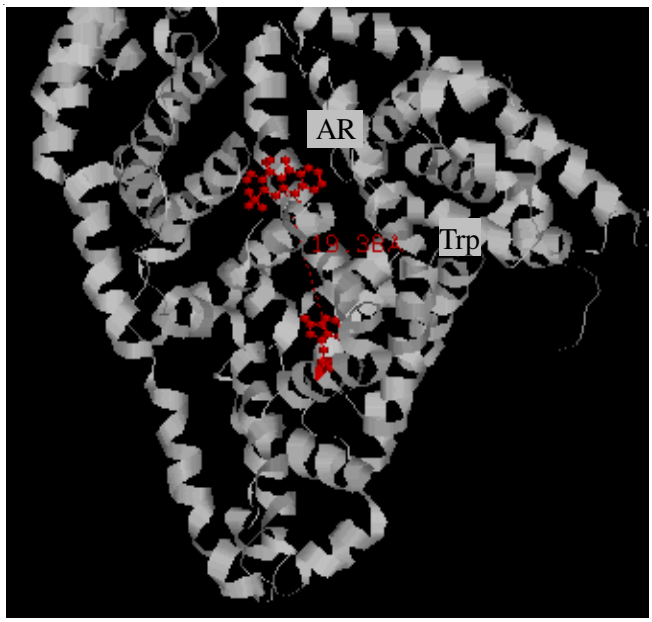
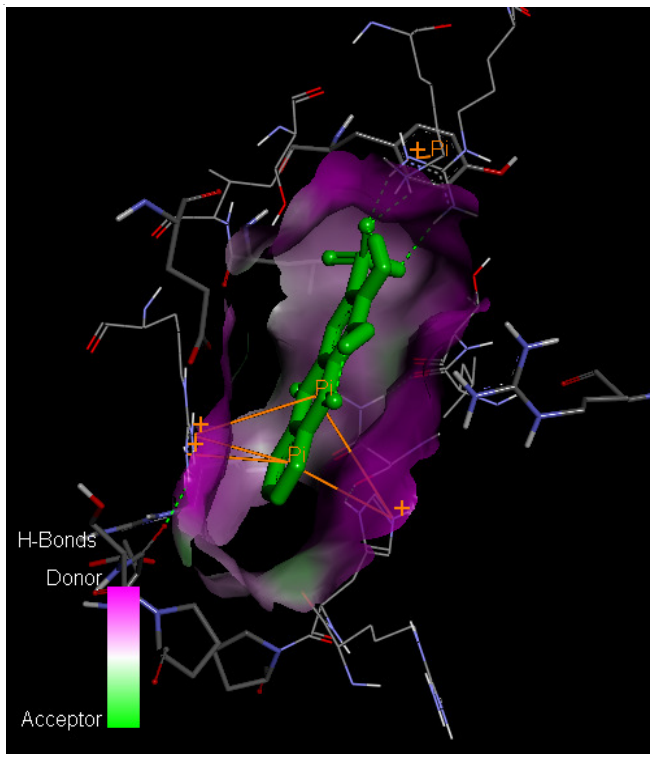


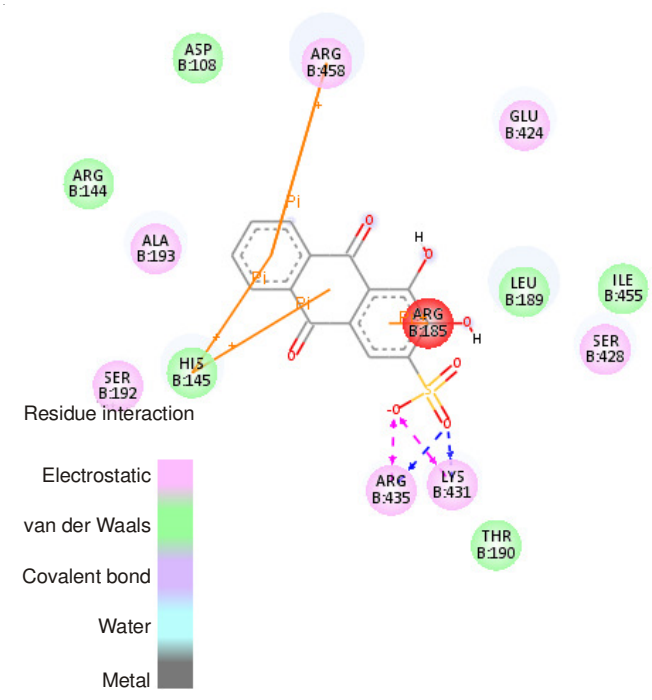
Fig. 6. Alizarin red docked conformation of bovine serum albumin. The distance from Trp to ligand was shown.

The docking results show that the Alizarin red is between the bovine serum albumin subdomains IIIA and IA and is partially exposed to the solvent. The molecule is located within the regions covered by the Förster radius of the Trp-AR pair, in agreement with the distances determined by FRET. The binding constant calculated is close to the experimentally obtained values. The interaction between Alizarin red and bovine serum albumin is shown in Fig.7. Residues interaction was found between LYS431 (ARG435) and sulfonic group by hydrogen bond. Besides, both electrostatic interaction, van der Waals force and π-π weak interaction from His145, Arg185, Arg458 residues and aromatic ring contributes to Alizarin red binding to the protein.

In order to further discern the interaction of Alizarin red to bovine serum albumin, Alizarin red molecule characteristic was also calculated according to ALOGPs methods[16]. ALOGPs and ALOGpS values are used to reveal solubility and lipophilicity of molecules, respectively. Moreover, the molecular flexibility measured by the number of rotatable bonds (nrotb), polar surface area (PSA) or total hydrogen bond count (nHb), are calculated. Results are shown in Table-3. Compared with hydrophobic probe ANS, Alizarin red has low Log Ps and high



(a)



(b)

Fig. 7. (a) Alizarin red docked conformation of the binding sites of bovine serum albumin. LYS (ARG) and sulfonic group by hydrogen bond were shown. (b) 2D diagram

Log Ps, low number of rotatable bonds, high polar surface area or hydrogen bond count, meaning Alizarin red molecule lower hydrophobic and higher hydrophilic property than ANS molecule. Maybe all reveal why Alizarin red is not indulging in hydrophobic domain like ANS but in the domain partially exposed to the solvent.

TABLE-3
CALCULATED PARTITION COEFFICIENTS, PSA, NROTB AND NHB OF ANS AND ANALOGY

	ALOGpS	ALOGPs	nrotb	nHb	PSA
AR	(-2.45)1.2 g/L	1.13	1	9	131.797
1,8-ANS	(-4.76)5.54 mg/L	3.36	3	6	69.226

Conclusion

The interaction of Alizarin red and bovine serum albumin has been investigated by fluorescence spectroscopy. The experimental results showed that Alizarin red and bovine serum albumin formed a 1:1 complex.The distance of the Alizarin red binding site and try-212 in bovine serum albumin was calculated to be 1.82 nm. The interaction between Alizarin red and bovine serum albumin was suggested to be mainly hydrogen bonds and van der Waals force through the calculated thermodynamic parameters of the interaction of Alizarin red and bovine serum albumin, whereas electrostatic force and hydrophobic interaction are also proved to exist by ion strength effect, titration experiment and autodock research. They function cooperatively. These experimental and theoretical data are of potential importance in understanding the mechanism of interaction between Alizarin red and bovine serum albumin and could be a useful guide for fluorescent probe research.

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REFERENCES

1. J. Zhuang, Y.H. Chi, K. Li, N. Li and S.Y. Tong, *Acta Chim. Sin.*, **56**, 827 (1998).
2. Y. Yu and J.H. Ou, *Nat. Sci. J. Hainan. Univ*., **22**, 123 (2004).
3. C.R. Zhao, B.S. Liu and H.Y. Zhang, *Spectrosc. Spect. Anal*., **25**, 92 (2005).
4. X.M. Wang, *Chinese J. Inorg. Chem*., **21**, 315 (2005).
5. W.S. Liao, Y. Wu and F.Y. Wu, *Chin. J. Anal. Lab*., **27**, 99 (2008).
6. M.F. Sanner, *J. Mol. Graph. Model*., **17**, 57 (1999).
7. A. Ray, B. Koley Seth, U. Pal and S. Basu, *Spectrochim. Acta A*, **92**, 164 (2012).
8. W.L. DeLano, The PyMol, Delano Scientific, San Carlos, CA, USA (2004).
9. X.Z. Feng, Z. Lin, L.J. Yang, C. Wang and C. Bai, *Talanta*, **47**, 1223 (1998).
10. H. Gao, L. Lei, J. Liu, Q. Kong, X. Chen and Z. Hu, *J. Photochem. Photobiol. Chem*., **167**, 213 (2004).
11. B. Valeur and J.C. Brochon, New Trends in Fluorescence Spectroscopy, Springer Press, Berlin, edn 6 (1999).
12. L.H. Nie, H.C. Zhao, X.B. Wang and X. Wang, *J. Beijing Normal Univ. Nat. Sci*., **37**, 87 (2001).
13. P.D. Ross and S. Subramanian, *Biochemistry*, **20**, 3096 (1981).
14. K. Flora, J.D. Brennan, G.A. Baker, M.A. Doody and F.V. Bright, *Biophys. J*., **75**, 1084 (1998).
15. N. Chimpalee, D. Chimpalee, B. Boonyanitchayakul and D.T. Burns, *Anal. Chim. Acta*, **282**, 643 (1993).
16. M. Remko, *J. Mol. Struct. THEOCHEM*, **916**, 76 (2009).