Asian Journal of Chemistry; Vol. 25, No. 7 (2013), 3692-3696

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2013.13724

Study of the Interaction Between S-Ovalbumin and Resveratrol with Spectroscopic Techniques

QUN HUANG[1,2,†], FANG GENG[1,†], MEIHU MA[1,*], YONGGUO JIN[1], JUN FANG[1] and SHUGUO SUN[1,2]

[1]College of Food Science and Technology, Huazhong Agricultural University, Wuhan, P.R. China
[2]Institute of Food Science, Jishou University, Jishou, P.R. China

*Corresponding author: Fax: +86 27 87283177; Tel: +86 27 87283177; E-mail: mameihuhn@yahoo.com.cn
†These authors contributed equally to this work

(*Received*: 12 March 2012; *Accepted*: 9 January 2013) AJC-12676

The interaction between resveratrol and S-ovalbumin was studied by using fluorescence quenching spectra, synchronous fluorescence spectra and ultraviolet spectra. The results indicated that resveratrol has the ability to quench the intrinsic fluorescence of S-ovalbumin because of a complex formed and the quenching was a static event. Furthermore, the quenching efficiency decreased with the rise in temperature. The binding constants were 3.49×10^6 and 4.42×10^4 L mol^{-1} and binding site were 1.39 and 1.04 at 303 and 318 K, respectively. Thermodynamic analysis results suggested that both hydrogen bonds and van der Waals forces played a key role in the interaction. According to the Förster theory of non-radiation energy transfer, the distance between S-ovalbumin and resveratrol and the energy transfer efficiency were calculated as 3.94 nm and 0.11, respectively. The conformational alteration of S-ovalbumin in the presence of resveratrol was also verified by means of synchronous fluorescence spectra and UV-visible absorption spectra.

Key Words: Resveratrol, S-Ovalbumin, Interaction, Fluorescence.

INTRODUCTION

Ovalbumin is one kind of monomer and globular phosphor-glycoprotein, with the content of which in albumen accounts for 54-63 %[1]. During storage, ovalbumin usually exists as a thermal stable form of N-ovalbumin[2]. S-Ovalbumin, a conformational isomer of N-ovalbumin, can be prepared artificially under alkaline condition and heat treatment[3,4]. Resveratrol, a kind of phenolic compound, is mostly contained in polygonum cuspidatum, peanut, grape and mulberry and has many physiological functions including antioxidation, antiinflammation, antiinfection, lowering total cholesterol, reducing the risk of suffering from coronary artery disease, restraining the life cycle of cancer cell and so on[5,6]. As a endogenous compounds, protein can combine with many drug molecules to form a complex and spectroscopy emerges as an important method to study the interaction among them[7-9]. In order to thoroughly understand the transportation and metabolic process of the drug and to clarify the chemical nature of the interaction between protein and drug molecules, study of the interaction between them from different perspective is of great importance[10,11]. In this paper, the quenching of fluorescence of S-ovalbumin by resveratrol under different conditions was studied by measuring the binding constant, binding site, thermodynamic parameter and the energy transfer during binding process and the effects of resveratrol on the conformation of S-ovalbumin were also studied using the synchronous fluorescence spectra and UV-visible absorption spectra.

EXPERIMENTAL

S-Ovalbumin with the concentration of 98.32 % was prepared according to the reported method[1,4]. Resveratrol was purchased from Sigma, St. Louis, MO, USA. Buffer solutions including phosphate buffer solution (pH 5.8 and 7.4) and *Tris*-HCl buffer solution (pH 8.4) were prepared. All of the other reagents used in this experiment were of analytical grade and double distilled water was used as aqueous medium in this work.

Fluorescence spectroscopy: A certain amount of resveratrol solution of ethyl alcohol was added into 10 mL volumetric flask and 5 mL of S-ovalbumin solution was added after the ethyl alcohol was completely volatilized. The mixture was then reacted for 2 h in constant temperature water-bath. The fluorescence measurements were performed using a Hitachi RF-5301pc spectrofluorimeter, with which the excitation wavelength was set at 280 nm and the emission was monitored in the range of 300-500 nm with the fixed slit width of 3 nm.

Ultraviolet-visible absorption spectroscopy: S-Ovalbumin solution and resveratrol solution were added into 10 mL volumetric flask at a certain rate. To this mixed solution, freshly prepared phosphate buffer solution (pH 7.4) was added till

the volume of the mixed solution was 10 mL. The solution was then kept mixing for 2 h at room temperature. UV-visible absorption spectra was scanned using a DU700 UV-VIS spectrophotometer (Beckman Kurt Co., Ltd., America) in the range of 260-320 nm.

RESULTS AND DISCUSSION

Fluorescence quenching mechanism: Aromatic amino acids, including tyrosine (Tyr), tryptophan (Trp) and phenylalanine (Phe) residues in protein, can emit intrinsic fluorescence, the strength of which will be quenched when interacted with quencher. Fig. 1 shows the fluorescence quenching of S-ovalbumin by resveratrol at 303 K and pH 7.4, from which it can be seen that the fluorescence spectra of S-ovalbumin gradually reduced when increasing the concentration of resveratrol. It also revealed that there was an obvious red shift of the fluorescence emission peak, which meant the hydrophilicity of tryptophan increased[12].

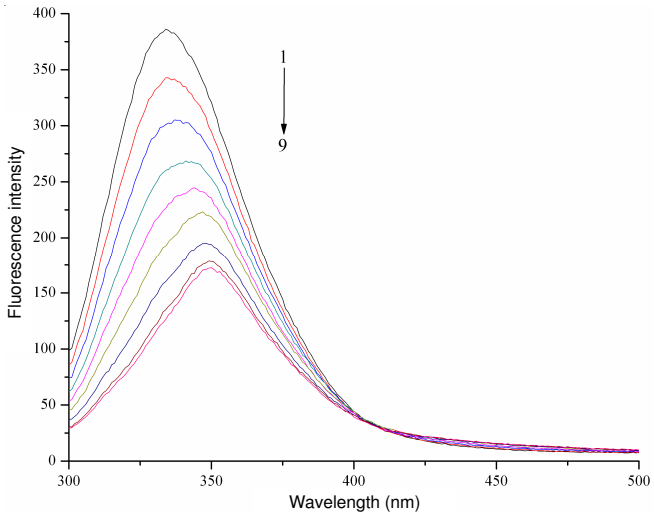

Fig. 1. Fluorescence spectra of S-ovalbumin at different resveratrol concentration. $C_{S\text{-ovalbumin}} = 0.5 \times 10^{-5}$ mol L^{-1}; $C_{Resveratrol}/(10^{-5}$ mol $L^{-1})$, 1-9: 0, 0.4, 0.8, 1.2, 1.6, 2.0, 2.4, 2.8, 3.2

According to the mechanism, fluorescence quenching can be divided into dynamic fluorescence quenching and static fluorescence quenching. Dynamic fluorescence quenching happened when there was diffusion and crash between fluorescence molecules and quenchants, while static fluorescence quenching occurred when there was a stable compound generated after they were mixed[13].

For dynamic quenching, the fluorescence data at ambient temperature was analyzed by the well known Stern-Volmer equation[14]:

$$\frac{F_0}{F} = 1 + K_q \tau_0 [Q] = 1 + K_{SV}[Q] \quad (1)$$

where F_0 and F represent the fluorescence intensities of S-ovalbumin in absence and in the presence of the quencher, respectively. K_q is the quenching rate constant and τ_0 is the average lifetime of the fluorophore without quencher. As for biological macromolecule, τ_0 is 10^{-8} s[14]. [Q] is the concentration of quencher and K_{SV} is the Stern-Volmer dynamic quenching constant. Different K_q under different temperatures can be calculated according to the Stern-Volmer equation, such as K_q is 3.93×10^{12} L mol^{-1} s^{-1} at 303 K and 2.87×10^{12} L mol^{-1} s^{-1} at 318 K, while the maximum diffusion and crash quenching rate constant between fluorescence molecule and quenchant is 2.0×10^{10} L mol^{-1} s^{-1}, which indicated the quenching was not a dynamic but a static mechanism[15].

Effect of temperature on fluorescence quenching: It is well known that the variation of K_{SV} at different temperatures was chosen as another index to distinguish whether the fluorescence quenching is static or dynamic and it got the conclusion that static fluorescence quenching would happen if K_{SV} was found decreased on increasing the temperature, because high temperature could reduce the stability of the compound[8,10]. Fig. 2 displayed the fluorescence quenching spectra of S-ovalbumin by resveratrol at 303 and 318 K, from which the slope of the line at 318 K was found decreased compared with that at 303 K. The result also revealed the existence of static quenching mechanism, which well coincided with the conclusion mentioned above.

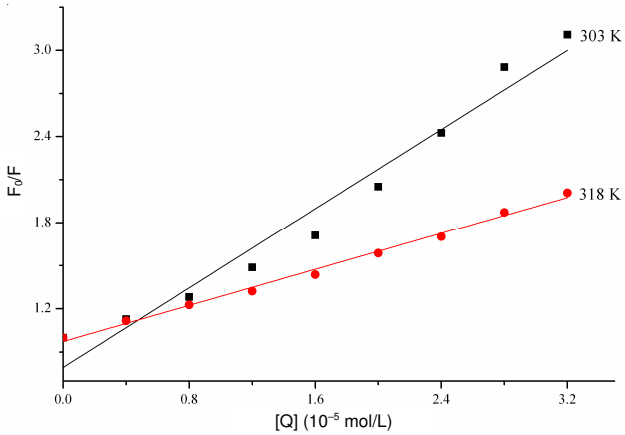

Fig. 2. Stern-Volmer plot of resveratrol *versus* S-ovalbumin at different temperature

Effect of pH on fluorescence quenching: The fluorescence quenching spectra of S-ovalbumin by resveratrol at pH 5.8, 7.4 and 8.4 was measured. Results were presented in Fig. 3, which showed that there was no significant difference between the slopes of the lines under pH 5.8 and 7.4, indicating that the binding of resveratrol to S-ovalbumin was more likely to happen under the approach acid and nearly neutral environment.

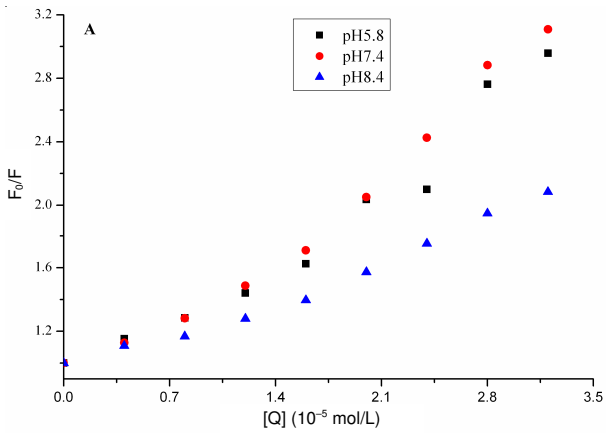

Fig. 3. Stern-Volmer plot of resveratrol *versus* S-ovalbumin at different pH

Binding constant and binding sites: As for static quenching, when ligand molecules bind independently to a set of equivalent sites of a macromolecule, then the equilibrium between free and bound molecules is given by the following equation[16,17]:

$$\log\left[\frac{(F_0 - F)}{F}\right] = \log K_a + n \log[Q] \quad (2)$$

where K_a is the binding constant and n is the number of binding sites, respectively. It could be figured out from Fig. 4 that the K_a of S-ovalbumin and resveratrol is 3.49×10^6 L mol^{-1} at 303 K and the n is 1.39; while at 318 K, the K_a is 4.42×10^4 L mol^{-1} and the n is 1.04. This result showed both of the binding constant and the number of binding sites decreased with increasing of the temperature, indicating high temperature goes against with the binding of S-ovalbumin and resveratrol. This further demonstrated that the fluorescence quenching of S-ovalbumin by resveratrol is static quenching mechanism.

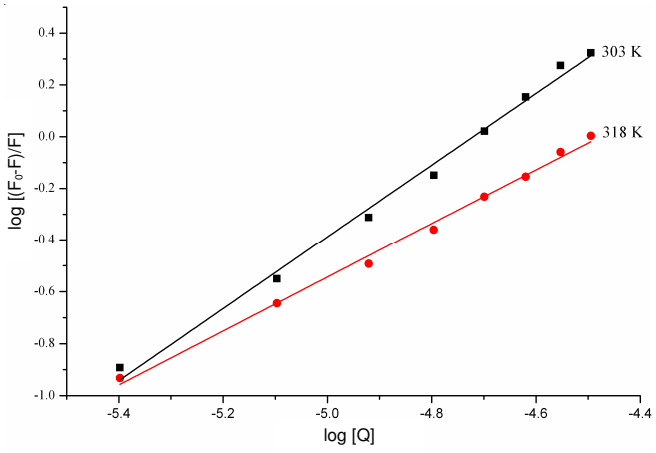

Fig. 4. Plot of log [(F_0 – F)/F] *versus* log [Q]

Thermodynamic parameters and nature of the binding forces: Generally, there are essentially four types of non-covalent interaction, which play a key role in ligand binding to proteins. These are van der Waals forces, electrostatic interactions, hydrogen bonds and hydrophobic forces[18]. To evaluate the forces involved in the binding process, the information that thermodynamic parameters including ΔH and ΔS were analyzed. The enthalpy and entropy changes can be calculated by the following van't Hoff equation[19]:

$$\ln K_a = -\left(\frac{1}{T}\right)\left(\frac{\Delta H}{R}\right) + \left(\frac{\Delta S}{R}\right) \quad (3)$$

where K_a is the binding constant of S-ovalbumin and resveratrol at the corresponding temperature and R = 8.314472 J mol^{-1} K^{-1} is the gas constant. ΔH can be obtained from the slope of the van't Hoff plot, ΔS is calculated from its interception and the free energy change (ΔG) can be obtained from the following expression:

$$\Delta G = \Delta H - T\Delta S \quad (4)$$

As a rule, hydrophobic force exists when the values of ΔH and ΔS are positive, while when they are simultaneously negative, there are both van der Waals forces and hydrogen bonds exists and the existence of electrostatic interaction can be judged if the value of ΔH is equal approximately to zero and ΔS is positive[20]. According to the experimental data, it could be calculated that at 303 K, ΔH = -233.36 kJ mol^{-1}, ΔG = -37.96 kJ mol^{-1} and ΔS = -644.91 J mol^{-1} K^{-1}; while at 318 K, ΔH = -233.36 kJ mol^{-1}, ΔG = -28.28 kJ mol^{-1} and ΔS = -644.91 J mol^{-1} K^{-1}. The negative values obtained for ΔH and ΔS at different temperatures strongly suggested that the participation of van der Waals forces and hydrogen bonds in the binding of resveratrol to S-ovalbumin and the negative value of ΔG illustrated that this binding process was spontaneous.

Energy transfer: According to the Förster theory of non-radiation energy transfer, the energy transfer efficiency (E) depends on the distance (r) between a fluorophore acceptor and a fluorescence donor with the following relation[21,22]:

$$E = 1 - \frac{F}{F_0} = \frac{R_0^6}{(R_0^6 + r^6)} \quad (5)$$

where R_0 is the critical distance, when the transfer efficiency is 50 %.

$$R_0^6 + 8.8 \times 10^{-25} k^2 n^{-4} \varphi J \quad (6)$$

where k_2 is the orientation factor, n is the refractive index of the medium and φ is the fluorescence quantum yield of the donor. According to previous study, the values of k^2, n and φ are taken as 2/3, 1.336 and 0.15, respectively[23]. J is the effect of the spectral overlap integral between the emission spectra of the donor and the absorption spectra of the acceptor and can be calculated by the following equation[24]:

$$J = \frac{[\Sigma F(\lambda)\varepsilon(\lambda)\lambda^4 \Delta\lambda]}{[\Sigma F(\lambda)\Delta\lambda]} \quad (7)$$

where F(λ) is the corrected fluorescence intensity of the donor at wavelength λ and ε(λ) is the extinction coefficient of the acceptor at the same wavelength.

Fig. 5 shows the spectral overlap integral between the emission spectra of S-ovalbumin(a) and the absorption spectra of resveratrol(b) when the amount of S-ovalbumin and resveratrol was equal, from which it could be calculated that the value of overlap integral (J) was 2.38×10^{-14} cm^3 L mol^{-1}, the energy transfer efficiency (E) was 0.11, the critical Förster distance (R_0) was 2.75 nm and the distance (r) was 3.94 nm, respectively. The obtained value of r, which was less than 7 nm, indicating the quenching process conformed to the theory of non-radiation energy transfer.

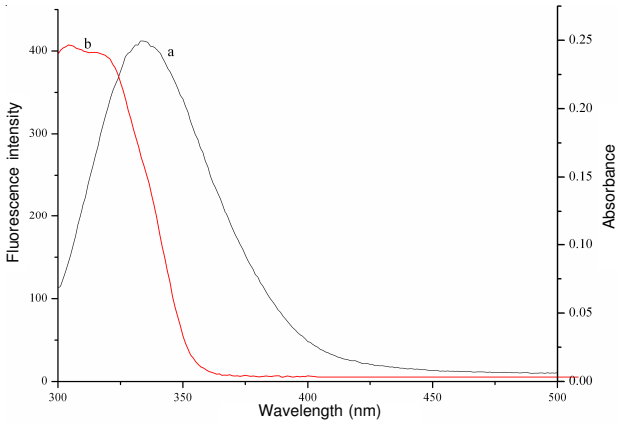

Fig. 5. Overlap spectral of S-ovalbumin's fluorescence spectra (a) and resveratrol's absorption spectra (b)

Synchronous fluorescence spectra: Synchronous fluorescence spectra are used to reflect the changes in the conformation of protein[25]. When the scanning interval $\Delta\lambda$ between excitation and emission wavelength is 15 nm, the synchronous fluorescence only gives the information of tyrosine residues; while that of tryptophan residues can be revealed if $\Delta\lambda$ is 60 nm[26].

Fig. 6 shows the synchronous fluorescence spectra of S-ovalbumin by resveratrol at different values of $\Delta\lambda$. It can be seen that the fluorescence intensity of amino acid residues reduced with increasing the concentration of resveratrol and the fluorescence spectrum of tyrosine residues exhibited a blue shift compared with the red shift exhibited by that of tryptophan residues. The results illustrated an increase in the hydrophobicity of tyrosine residues microenvironment and decrease in that of tryptophan residues. And a marked change in the fluorescence intensity of tryptophan residues can also be observed, disclosing that the fluorescence of S-ovalbumin was most contributed by tryptophan residues and the binding site of resveratrol and S-ovalbumin is closed to tryptophan residues[27].

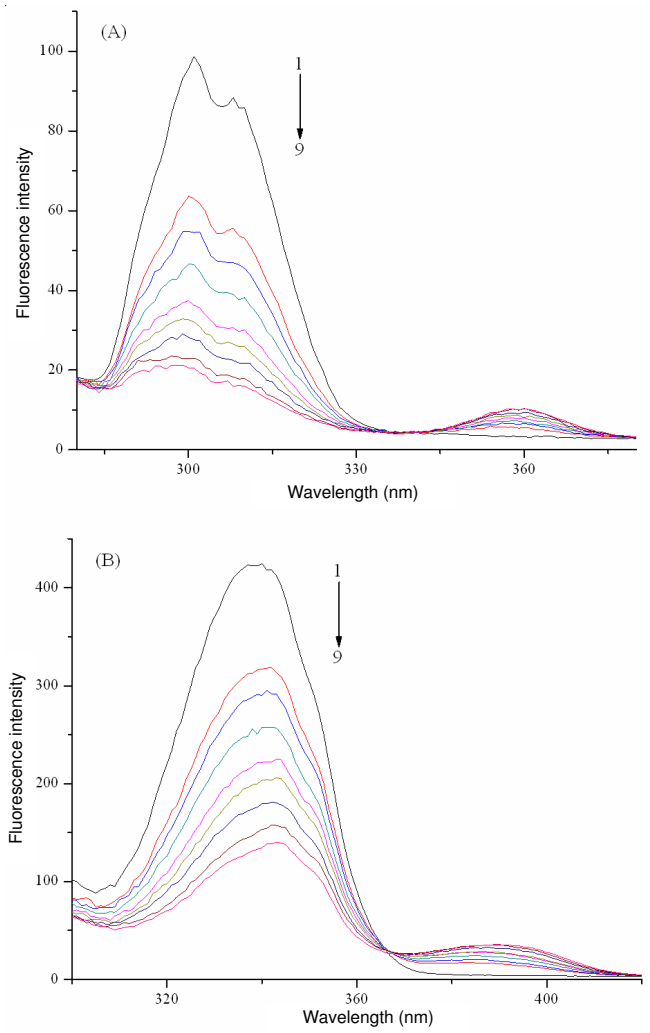

Fig. 6. Synchronous fluorescence spectra of S-ovalbumin-resveratrol. (A) λ = 15 nm; (B) λ = 60 nm; $C_{S\text{-ovalbumin}} = 0.5 \times 10^{-5}$ mol L^{-1}; $C_{Resveratrol}/(10^{-5}$ mol $L^{-1})$, 1-9: 0, 0.4, 0.8, 1.2, 1.6, 2.0, 2.4, 2.8, 3.2; pH 7.5, T = 303 K

Ultraviolet spectra: Ultraviolet spectrometry is a simple and effective method to study the interactions between small organic molecules and proteins, since it can provide information of ultraviolet light absorbed by amino acid residues[28,29]. Ultraviolet spectra effect of resveratrol to S-ovalbumin was measured and results were displayed in Fig. 7. It shows the absorption peak of S-ovalbumin was enhanced by increasing the concentration of resveratrol and a red shift of these absorption peaks could be observed. It revealed that the microenvironment change of aromatic amino acid residues gives rise to the conformational alteration of S-ovalbumin.

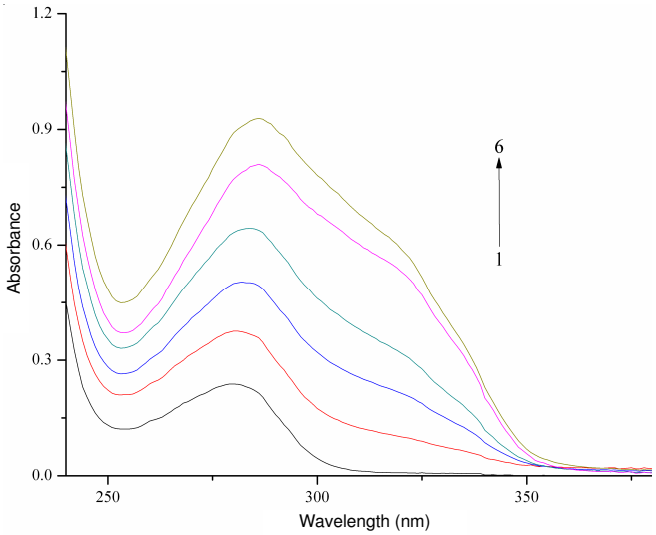

Fig. 7. UV/visible spectra of S-ovalbumin in the presence of resveratrol. $C_{S\text{-ovalbumin}} = 1.0 \times 10^{-5}$ mol L^{-1}; $C_{Resveratrol}/(10^{-5}$ mol $L^{-1})$, 1-6: 0, 1.0, 2.0, 3.0, 4.0, 5.0

Conclusion

A combination of fluorescence quenching spectra, synchronous fluorescence spectra and ultraviolet spectra were applied to study the interaction between resveratrol and S-ovalbumin. The conclusion had been drawn that the fluorescence quenching of S-ovalbumin by resveratrol was a static process. The quenching efficiency decreased with the rise in temperature and pH and hydrogen bonds and van der Waals forces played a key role in the binding of resveratrol to S-ovalbumin. In addition, the conformational alteration of S-ovalbumin was mainly caused by the microenvironmental changes of tyrosine residues and tryptophan residues, which is of great importance in the investigation of interaction between small molecule drug and protein.

ACKNOWLEDGEMENTS

This research was supported by the earmarked fund for Modern Agro-industry Technology Research System. Project code No: CARS-41-K23.

REFERENCES

1. M. Yamasaki, N. Takahashi and M. Hirose, *J. Biol. Chem.*, **278**, 35524 (2003).
2. J.A. Huntington, P.G.W. Gettins and P. Patston, *Protein Sci.*, **4**, 613 (1995).
3. R. Nakamura and M. Ishimaru, *Agric. Biol. Chem.*, **45**, 2775 (1981).
4. M.B. Smith and J.F. Back, *Aust. J. Biol. Sci.*, **18**, 365 (1965).
5. M.I. Fernández-Mar, R. Mateos, M.C. García-Parrilla, B. Puertas and E. Cantos-Villar, *Food Chem.*, **130**, 797 (2012).

6. X.Y. Yu, S.Y. Lu, Y. Yang, X.F. Li and P.G. Yi, *Spectrochim. Acta A*, **83**, 609 (2011).
7. P. Mandal, M. Bardhan and T. Ganguly, *J. Photochem. Photobiol. B*, **99**, 78 (2010).
8. H. Xu, S.L. Gao, J.B. Lv, Q.W. Liu, Y. Zuo and X. Wang, *J. Mol. Struct*., **919**, 334 (2009).
9. D.D. Wu, Z. Chen and X.G. Liu, *Spectrochim. Acta A*, **84**, 178 (2011).
10. T.T. Chen, S.J. Zhu, H. Cao, Y.F. Shang, M. Wang, G.Q. Jiang, Y.J. Shi and T.H. Lu, *Spectrochim. Acta A*, **78**, 1295 (2011).
11. S.Y. Bi, L.L. Yan, B.B. Wang, J.Y. Bian and Y.T. Sun, *J. Lumin*., **131**, 866 (2011).
12. J.G. Xu and Z.B. Wang, The Methods of Fluorescence Analysis, Science Press, Beijing, edn. 3 (2006).
13. J.R. Lakowicz, Principles of Fluorescence Spectroscopy, Springer Science + Business Media, New York, edn. 3 (2006).
14. Y.P. Wang, Y.L. Wei and C. Dong, *J. Photochem. Photobiol. A*, **177**, 6 (2006).
15. T. Pérez-Ruiz, C. Martínez-Lozano, V. Tomás and J. Fenoll, *Analyst*, **125**, 507 (2000).
16. J.Q. Liu, J.N. Tian and J.Y. Zhang, *Anal. Bioanal. Chem*., **376**, 864 (2003).
17. J.N. Tian, J.Q. Liu and J.Y. Zhang, *Chem. Pharm. Bull*., **51**, 579 (2003).
18. Q. Yue, L. Niu, X. Li, X. Shao, X. Xie and Z. Song, *J. Fluoresc*., **18**, 11 (2008).
19. P.D. Ross and S. Subramanian, *Biochemistry*, **20**, 3096 (1981).
20. Y.Z. Zhang, B. Zhou, Y.X. Liu, C.X. Zhou, X.L. Ding and Y. Liu, *J. Fluoresc*., **18**, 109 (2008).
21. X.Y. Yu, Y. Yang, S.Y. Lu, Q. Yao, H.T. Liu, X.F. Li and P.G. Yi, *Spectrochim. Acta A*, **83**, 322 (2011).
22. S.M.T. Saikh, J. Seetharamappa, P.B. Kandagal, D.H. Manjunatha and S. Ashoka, *Dyes Pigm.*, **74**, 665 (2007).
23. A. Ramalingam, B.M. Sivaram, P.K. Palanisamy and V. Masilamani, *Spectrochim. Acta A*, **56**, 1205 (2000).
24. S. Bi, D. Song and Y. Tian, *Spectrochim. Acta A*, **61**, 629 (2005).
25. J.Q. Xi and R. Guo, *Int. J. Biol. Macromol*., **40**, 305 (2007).
26. X.J. Yang, J. Chou, G.Q. Sun, H. Yang and T.H. Lu, *Microchem. J*., **60**, 210 (1998).
27. B. Klajnert and M. Bryszewska, *Bioelectrochemistry*, **55**, 33 (2002).
28. H. Gao, L. Lei, K. Qin, X. Chen and Z. Hu, *J. Photochem. Photobiol. A*, **167**, 213 (2004).
29. Z.Q. Wang, J.Q. Gao, J. Wang, X.D. Jin, M.M. Zou, K. Li and P.L. Kang, *Spectrochim. Acta A*, **83**, 511 (2011).