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Study of the interaction between baicalin and bovine serum albumin by multi-spectroscopic method

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Abstract

The interaction of baicalin and bovine serum albumin (BSA) was investigated using fluorescence spectroscopy (FS), resonance light scattering spectroscopy (RLS), and ultraviolet spectroscopy (UV). The apparent binding constants (K_a) between baicalin and BSA were 1.67×10^6 (22 °C), 1.98×10^6 (32 °C) and 2.01×10^6 (42 °C), and the binding sites values (n) were 1.33 ± 0.01. According to the Förster theory of nonradiation energy transfer, the binding distances (r) between baicalin and BSA were 1.94, 1.95 and 1.96 nm at 22, 32, and 42 °C, respectively. The experimental results showed that the baicalin could be inserted into the BSA, quenching the inner fluorescence by forming the baicalin–BSA complex. The addition of increasing baicalin to BSA solution leads to the gradual enhancement in RLS intensity, exhibiting the formation of the aggregate in solution. It was found that both static quenching and non-radiation energy transfer were the main reasons for the fluorescence quenching. The entropy change and enthalpy change were positive, which indicated that the interaction of baicalin and BSA was driven mainly by hydrophobic forces. The process of binding was a spontaneous process in which Gibbs free energy change was negative.

Keywords: Baicalin; Bovine serum albumin; Interaction; Fluorescence spectroscopy

1. Introduction

The interactions between bio-macromolecules and drugs have attracted great interest among researchers in recent years [1–3]. Among bio-macromolecules, serum albumin is the major soluble protein constituents of the circulatory system and has many physiological functions. The most outstanding function of albumins is that they serve as a depot protein and a transport protein for many exogenous compounds [4,5]. The drug–protein interaction may result in the formation of a stable protein–drug complex, which has important effect on the distribution, free concentration and the metabolism of a drug in the blood stream. Thus, the drug–albumin complex may be considered as a model for gaining fundamental insights into drug–protein interactions. Therefore, studies on the binding of drug with protein will facilitate interpretation of the metabolism and transporting process of drug, and will help to explain the relationship between structures and functions of the protein. In this regard, bovine serum albumin (BSA) has been studied extensively, partly because of its structural homology with human serum albumin (HSA) [6–10].

Baicalin, 5, 6-dihydroxy-4-oxygen-2-phenyl-4H-1-benzopyran-7-beta-D-glucopyranose acid (Fig. 1.), is a flavonoid compound from *Scutellaria baicalensis* Georgi [11,12]. Baicalin is a strong antioxidant [13] and has a preventive effect against ischemic stroke [14] and antipyretic effects [15]. Baicalin can be used as a prophylactic agent for heatstroke and protect against cerebrovascular dysfunction and brain inflammation in heatstroke [16] and could block polymorphonuclear leukocytes

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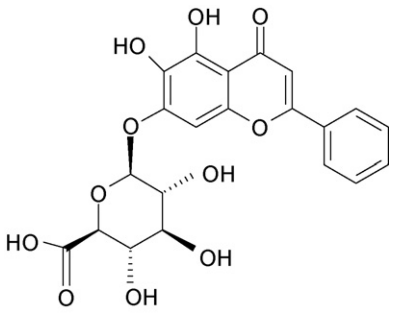

Fig. 1. Structure of baicalin.

(PMNs) degranulation induced by interleukin (IL)-8, a CXC chemokine [17].

Fluorescence spectroscopy is an appropriate method to determine the interaction between a small molecule ligand and a bio-macromolecule. By means of measurement and analysis of the emission peak, the transfer efficiency of energy, the lifetime, and fluorescence polarization, etc., much information may be obtained concerning the structural changes and the microenvironment surrounding the fluorophore in the macromolecule.

Resonance light scattering (RLS), an elastic scattering, occurs when an incident beam is close to an absorption band. Pasternack et al. first established the RLS technique to study the bio-macromolecules on an ordinary fluorescence spectrometer [18,19]. RLS is a sensitive and selective technique for monitoring molecular assemblies. RLS has attracted great interest among researchers. In recent years, RLS has been used to determine pharmaceutical [20,21], bacteria [22], inorganic ion [23] and various bio-macromolecules [24,25]. To gain some insights into the medicinal action of baicalin, the interactions between baicalin and BSA were investigated by means of multi- spectroscopic method. Some important information such as the apparent binding constants and binding sites values were obtained.

2. Experimental

2.1. Apparatus

Fluorescence and resonance light scattering spectra were recorded on a JASCO FP-6500 spectrofluorometer equipped with a thermostated cell compartment using quartz cuvettes (1.0 cm) (Tokyo, Japan). The ultraviolet visible (UV–vis) spectra were recorded on a UV-2450 spectrophotometer using quartz cuvettes (1.0 cm) (Shimadzu, Japan). The pH measurements were carried out on a PHS-3C Exact Digital pH meter equipped with Phoenix Ag-AgCl reference electrode (Cole–Paemer Instrument Co.), which was calibrated with standard pH buffer solutions.

2.2. Reagents

Baicalin was obtained commercially from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). A working solution of baicalin (0.5×10^{-3} mol L^{-1}) was prepared by dissolving baicalin in methanol-water solution (1:1, v/v). Bovine serum albumin (fraction V) was purchased from Sigma Co. (St. Louis, MO, USA). The working solution of BSA (1.0×10^{-5} mol L^{-1}) in the doubly distilled water was prepared and stored in refrigerator prior to use. Aqueous Tween-80 (10.0 mg mL^{-1}) was used as stabilizer. Tris–HCl buffer (0.20 mol L^{-1}, pH 7.40) containing 0.10 mol L^{-1} NaCl was selected to keep the pH value and maintain the ionic strength of the solution. All other reagents and solvents were of analytical reagent grade and used without further purification unless otherwise noted. All aqueous solutions were prepared using newly double-distilled water.

2.3. Fluorescence and ultraviolet spectra

Appropriate quantities of 0.5×10^{-3} mol L^{-1} baicalin solution were transferred to a 10 mL flask, and then 1.0 mL of BSA solution was added and diluted to 10 mL with water. The resultant mixture was subsequently ultrasonicated for 5 min and incubated at 22, 32, or 42 °C for 10 min. The solution was scanned on the fluorophotometer in the range of 290–500 nm. The spectral bandwidths of excitation and emission slits were both kept at 3.0 nm. The fluorescent intensity at 340 nm was recorded under the excitation at wavelength of 280 nm. The operations were carried out at fixed temperature (22, 32, and 42 °C).

The UV spectra were obtained by scanning the solution on the spectrophotometer with the wavelength range of 220–400 nm. The operations were carried out at room temperature.

2.4. Resonance light scattering spectra

An appropriate aliquot of BSA working solution was added to 1.0 mL baicalin working solution and 1.0 mL Tween-80 aqueous solution, and diluted to 10 mL with water. RLS spectra were obtained by synchronous scanning with the wavelength range of 250–750 nm on the spectrofluorophotometer. The spectral bandwidths of the excitation and emission monochromators were both kept at 3.0 nm. The obtained RLS spectrum was recorded and its intensity was measured at 569.5 nm. The operations were carried out at room temperature.

3. Results and discussion

3.1. Characteristics of the fluorescence spectra

The fluorescence spectra of BSA in the presence of different concentrations of baicalin were shown in Fig. 2. With the increasing concentration of baicalin, the fluorescence intensity of BSA decreased remarkably, indicating that interactions between baicalin and BSA occurred and baicalin–BSA complex may form. Fluorescence quenching could proceed via different mechanisms, usually classified as dynamic quenching and static quenching. Dynamic and static quenching can be distinguished by their different dependence on temperature. Higher temperatures will result in faster diffusion and hence larger amounts of collisional quenching, and higher temperatures will typically result in the dissociation of weekly bound complexes and hence

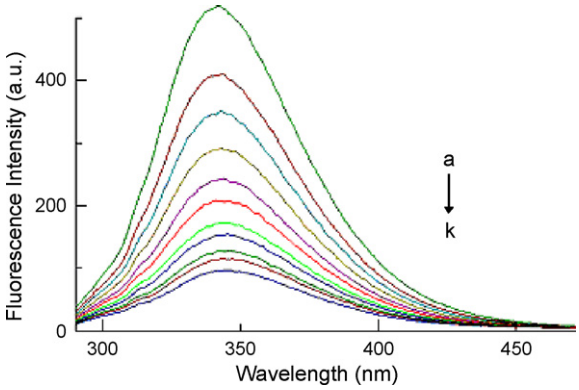

Fig. 2. The quenching effect of baicalin on BSA fluorescence intensity. λ_{ex} = 280 nm, (a–k) BSA, 1.0×10^{-6} mol L^{-1}: 0.00, 0.25, 0.50, 0.75, 1.00, 1.25, 1.50, 1.75, 2.00, 2.25, 2.50 ($\times 10^{-5}$ mol L^{-1}) of baicalin.

smaller amounts of static quenching. For the dynamic quenching, the mechanism can be described by the Stern–Volmer equation [7,26,27]:

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

where F_0 and F represent the fluorescence intensities in the absence and in the presence of quencher, K_q is the quenching rate constant of the bimolecular, K_{SV} is the dynamic quenching constant, τ_0 is the average lifetime of the molecule without quencher and $[Q]$ is the concentration of the quencher.

To clarify the fluorescence quenching mechanism of BSA by baicalin, it was first assumed that the interaction proceeds via a dynamic way. The temperature-dependent fluorescence quenching of BSA by baicalin was then carried out. The Stern–Volmer plots at different temperatures were shown in Fig. 3. From the experimental data, the corresponding dynamic quenching constants for the interaction between baicalin and BSA were $K_{SV} = 1.78 \times 10^5$ (22 °C, $R = 0.9934$), $K_{SV} = 1.73 \times 10^5$ (32 °C, $R = 0.9951$) and $K_{SV} = 1.59 \times 10^5$ (42 °C, $R = 0.9929$), respectively. Because the fluorescence lifetime of the biopolymer is 10^{-8} s [28–30], the quenching constants K_q at 22, 32, and 42 °C were calculated to be 1.78×10^{13}, 1.73×10^{13}, and 1.59×10^{13} L mol^{-1} s^{-1}, respectively.

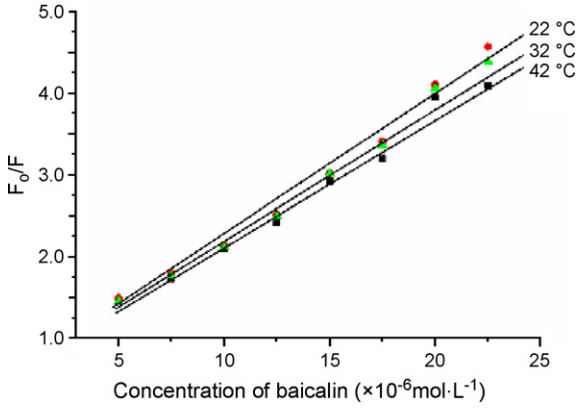

Fig. 3. The Stem–Volmer curves of fluorescence quenching of BSA by baicalin at different temperatures (22, 32 and 42 °C).

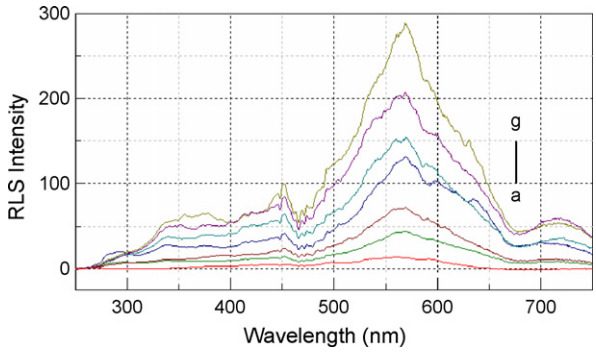

Fig. 4. RLS spectra of five baicalin–BSA systems: (a–g) baicalin solution, 1.0×10^{-4} mol L^{-1} and Tween-80 (1.0 mg mL^{-1}): 0.0, 0.5, 1.0, 1.5, 2.0, 2.5×10^{-6} mol L^{-1} of BSA.

According to the literatures [30,31], for dynamic quenching, the maximum scattering collision-quenching constant of various quenchers with the biopolymer is 2.0×10^{10} L mol^{-1} s^{-1}, and the K_{SV} increases with increasing temperature. Considering that in our experiment the rate constant of the protein quenching procedure initiated by baicalin is much greater than 2.0×10^{10} L mol^{-1} s^{-1} and that the K_{SV} decreased with increasing temperature, it can be concluded that the quenching is not initiated by dynamic quenching, but probably by static quenching resulting from the formation of baicalin–BSA complex.

3.2. Characteristics of the RLS spectra

The RLS spectra of baicalin-BSA in Tris–HCl buffer solution (0.020 mol L^{-1}) were shown in Fig. 4. It can be seen that the RLS intensity of free baicalin is quite weak in the whole scanning wavelength region (Fig. 4a). In contrast, upon addition of trace amount of BSA to baicalin solution, a remarkably enhanced RLS with a maximum peak at 569.5 nm and a secondary one at 452.0 nm was observed, exhibiting a BSA concentration-dependent relationship (Fig. 4b–g). The production of RLS is correlated with the formation of certain aggregate and the RLS intensity is dominated primarily by the particle dimension of the formed aggregate in solution. Bearing these points in mind, it is inferred from the results that the added BSA may interact with baicalin in solution, forming a new baicalin–BSA complex that could be expected to be an aggregate. Moreover, the dimension of the resultant baicalin–BSA particles may be much less than the incident wavelength, and thus the enhanced light-scattering signal occurs under the given conditions.

3.3. Binding constant and binding sites

For static quenching, the relationship between fluorescence quenching intensity and the concentration of quenchers can be described by the binding constant formula [31]:

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

where K_a is the binding constant, and n is the number of binding sites per BSA. After the fluorescence quenching intensities on

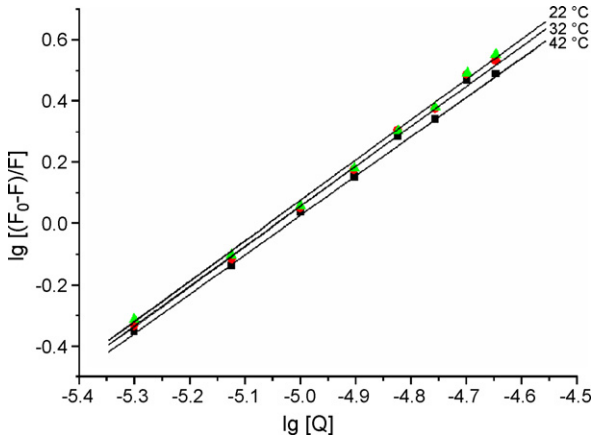

Fig. 5. Double-log plot of baicalin quenching effect on BSA fluorescence at different temperatures (22, 32 and 42 °C).

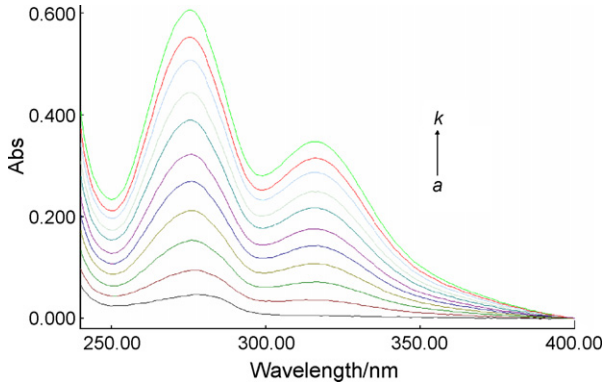

Fig. 6. UV absorption spectra of baicalin–BSA system. (a–k) BSA, 1.0×10^{-6} mol L^{-1}: 0.00, 0.25, 0.50, 0.75, 1.00, 1.25, 1.50, 1.75, 2.00, 2.25, 2.50 ($\times 10^{-5}$ mol L^{-1}) of baicalin.

BSA at 340 nm were measured, the double-logarithm algorithm was assessed by Eq. (2).

Fig. 5. showed the double-logarithm curve and Table 1 gave the corresponding calculated results. The apparent binding constants (K_A) between baicalin and BSA were 1.67×10^6 (22 °C, $R = 0.9988$), 1.98×10^6 (32 °C, $R = 0.9990$) and 2.01×10^6 (42 °C, $R = 0.9982$), and the binding sites values (n) were 1.33 ± 0.01. The data clearly showed that the binding site on BSA for baicalin was independent of temperature from 22 to 42 °C. The result illustrated that there is a strong binding force between baicalin and BSA, and a binding site would be formed. The correlation coefficients are larger than 0.998, indicating that the interaction between baicalin and BSA agrees well with the site-binding model underlying the Eq. (2). However, the binding constant between baicalin and BSA with respect to the increasing temperature was noticed. From the temperature dependence of the binding equilibrium constants, it is possible to calculate values for the thermodynamic functions involved in the binding and dissociation process. The increase in the binding constant with increase in temperature suggested the involvement of covalent interactions and a major role for non-ionic interactions in the binding of baicalin to BSA. The temperature may affect the diffusion coefficient and stability of the baicalin–BSA system. The increasing temperature may result in the increasing diffusion coefficient, but it also leads to the lower stability of the baicalin–BSA system. The competition of the diffusion coefficient and stability of the baicalin–BSA system with increased temperature may induce the above results. The binding constants in 32 and 42 °C were almost the same (1.98×10^6 and 2.01×10^6). The decreased stability of the baicalin–BSA system played a more important role in the interaction between baicalin and BSA with an increase in temperature.

Table 1
The binding parameters for the system of baicalin-BSA

Temperature (°C)	Binding constant (L mol^{-1})	Binding site	R
22	1.67×10^6	1.33	0.9988
32	1.98×10^6	1.34	0.9990
42	2.01×10^6	1.32	0.9982

3.4. Characteristics of the UV spectra

Fig. 6. shows the ultraviolet absorption spectra of BSA in the presence of various concentrations of baicalin. The BSA solution showed weak absorption weak at 281.0 nm. The addition of baicalin to the BSA solution led to two new absorption weak at 276 and 317 nm. The addition of increasing baicalin to the BSA solution led to the gradual enhancement in UV intensity and exhibited a concentration-dependent relationship.

3.5. Binding distance between the drug and the amino acid residues of BSA

According to the Förster non-radiation energy transfer theory [32,33], the energy transfer effect is related not only to the distance between the acceptor and donor, but also to the critical energy transfer distance (R_0).

$$E = \frac{R_0^6}{(R_0^6 + r^6)} \tag{3}$$

$$E = \frac{1 - F}{F_0} \tag{4}$$

where E is the energy transfer efficiency, R_0 is the critical distance when the transfer efficiency is 50%, and r is the binding distance between donor and acceptor.

$$R_0^6 = 8.8 \times 10^{-25} K^2 N^4 \Phi J \tag{5}$$

where K^2 is the spatial orientation factor of the dipole, N the refractive index of the medium, Φ the fluorescence quantum yield of the donor, and J the overlap integral of the fluorescence emission spectrum of the donor and the absorption spectrum of the acceptor [34]. Therefore,

$$J = \frac{(\Sigma I_P(\lambda)\varepsilon_D(\lambda)\,\lambda^4 \Delta\lambda)}{(\Sigma I_P(\lambda)\Delta\lambda)} \tag{6}$$

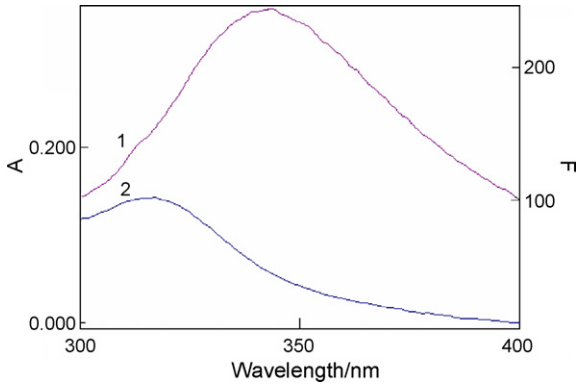

Fig. 7. Overlap spectra of baicalin UV absorption spectra (1) and BSA's fluorescence emission spectra (2): $C_{BSA} = 1.0 \times 10^{-5}$ mol L^{-1}, $C_{baicalin} = 1.0 \times 10^{-5}$ mol L^{-1}.

Here $I_P(\lambda)$ is the fluorescence intensity of the fluorescence donor at wavelength λ and $\varepsilon_D(\lambda)$ the molar absorptivity of the acceptor at wavelength λ.

The overlap of the absorption spectrum of baicalin and the fluorescence emission spectrum of BSA is shown in Fig. 7. The overlap integral was calculated to be 2.81×10^{-15} cm^3 L mol^{-1} at 22 °C by integrating the spectra for 300–400 nm in Fig. 7. The critical distance, R_0, corresponding to 50% energy transfer from BSA to baicalin was calculated to be 1.98 nm from the Eq. (5) when $K^2 = 2/3$, $N = 1.336$ and $\Phi = 0.118$ [32]. The binding distance, r, between baicalin and the amino acid residue in BSA was found to be 1.94, 1.95 and 1.96 nm at 22, 32, and 42 °C, respectively, which are much smaller than 7 nm, a criterion value for energy transfer phenomenon to occur, suggesting that the energy transfer from BSA to baicalin may occur with high possibility.

3.6. Thermodynamic parameters and nature of the binding forces

The interaction forces between a drug and a biomolecule may involve hydrophobic forces, electrostatic interactions, van der Waals interactions, hydrogen bonds, etc. According to the data of enthalpy change (ΔH) and entropy change (ΔS), the model of interaction between a drug and a biomolecule can be concluded [35]:

1) $\Delta H > 0$ and $\Delta S > 0$, hydrophobic forces;
2) $\Delta H < 0$ and $\Delta S < 0$, van der Waals interactions and hydrogen bonds;
3) $\Delta H < 0$ and $\Delta S > 0$, electrostatic interactions.

In order to elucidate the interaction of baicalin with BSA, we calculated the thermodynamic parameters from Eqs. (7)–(9). If the temperature does not vary significantly, the enthalpy change (ΔH) can be regarded as a constant. The free energy change (ΔG) can be estimated from the following equation, based on the binding constants at different temperatures:

$$\Delta G = -RT \ln K \tag{7}$$

Table 2
The thermodynamic parameters of baicalin-BSA binding procedure

Temperature (°C)	ΔH (kJ mol^{-1})	ΔG (kJ mol^{-1})	ΔS (J mol^{-1} K^{-1})
22	13.01	−35.15	163.17
32		−36.79	163.20
42		−38.03	161.95

where R is the gas constant, T is the experimental temperature, and K is the binding constant at the corresponding T. Then the enthalpy change (ΔH) and entropy change (ΔS) can be calculated from the Eqs. (8) and (9):

$$\ln \frac{K_2}{K_1} = \left[\frac{1}{T_1} - \frac{1}{T_2}\right] \frac{\Delta H}{R} \tag{8}$$

where K_1 and K_2 are the binding constant at the experiment temperatures T_1 and T_2, respectively.

$$\Delta G = \Delta H - T\Delta S \tag{9}$$

The thermodynamic parameters for the interaction of baicalin with BSA are shown in Table 2. The negative sign for ΔG means that the interaction process is spontaneous. The free energy of binding of drugs to bovine serum albumin consists of two main contributions: (a) from electrostatic interactions and (b) from hydrophobic forces. The positive ΔH and ΔS values indicated that hydrophobic forces might play a major role in the binding between baicalin and BSA [33].

4. Conclusions

The binding interactions of baicalin with BSA in dilute aqueous solution were studied using fluorescence spectra, resonance light scattering spectra and absorbance spectra. The apparent binding constants (K) between baicalin and BSA were 1.67×10^6 (22 °C), 1.98×10^6 (32 °C) and 2.01×10^6 (42 °C), and the binding sites values (n) were 1.33 ± 0.01. According to the Förster theory of non-radiation energy transfer, the binding distances (r) between baicalin and BSA were 1.94, 1.95 and 1.96 nm at 22, 32, and 42 °C, respectively. The entropy change and enthalpy change were positive, which indicated that the interaction of baicalin and BSA was driven mainly by hydrophobic forces. The process of binding was a spontaneous process in which Gibbs free energy change was negative.

Acknowledgments

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References

[1] S. Roufik, S.F. Gauthier, X. Leng, S.L. Turgeon, Biomacromolecules 7 (2006) 419–426.
[2] W. Ke, D. Zhou, J. Wu, K. Ji, Appl. Spectrosc. 59 (2005) 418–423.

[3] T.H. Zhang, J. Luo, J.M. Zhou, Biochimie 84 (2002) 335–339.
[4] H. Malonga, J.F. Neault, H.A. Tajmir-Riahi, DNA Cell Biol. 25 (2006) 393–398.
[5] C. Mancuso, A. Bonsignore, C. Capone, E. Di Stasio, G. Pani, Antioxid. Redox. Signal. 8 (2006) 487–494.
[6] L. Shang, X. Jiang, S. Dong, J. Photochem. Photobiol. A 184 (2006) 93–97.
[7] B. Bose, A. Dube, J. Photochem. Photobiol. B 85 (2006) 49–55.
[8] N. Zhou, Y.Z. Liang, P. Wang, J. Photochem. Photobiol. A 185 (2007) 271–276.
[9] Y.J. Hu, Y. Liu, R.M. Zhao, J.X. Dong, S.S. Qu, J. Photochem. Photobiol. A 179 (2006) 324–329.
[10] Y.P. Wang, Y.L. Wei, C. Dong, J. Photochem. Photobiol. A. 177 (2006) 6–11.
[11] W.T. Seo, Y.H. Park, T.B. Choe, Plant Cell Rep. 12 (1993) 414–417.
[12] Y.Y. Zhang, Y.Z. Guo, H. Ageta, Y. Harigaya, M. Onda, K. Hashimoto, Y. Ikeya, M. Okada, M. Maruno, Planta Med. 63 (1997) 536–539.
[13] T. Akao, M. Hanada, Y. Sakashita, K. Sato, M. Morita, T. Imanaka, J. Pharm. Pharmacol. 59 (2007) 87–93.
[14] Z.J. Zhang, P. Li, Z. Wang, P.T. Li, W.S. Zhang, Z.H. Sun, X.J. Zhang, Y.Y. Wang, Brain Res. 1123 (2006) 188–195.
[15] C.C. Tsai, M.T. Lin, J.J. Wang, J.F. Liao, W.T. Huang, Neuropharmacology 51 (2006) 709–917.
[16] C.P. Chang, W.T. Huang, B.C. Cheng, C.C. Hsu, M.T. Lin, Neuropharmacology 52 (2007) 1024–1033.
[17] G. Zhu, C. Li, Z. Cao, J. Ethnopharmacol. 109 (2007) 325–330.
[18] R.F. Pasternack, C. Bustamante, P.J. Colling, A. Giannetto, E.J. Gibbs, J. Am. Chem. Soc. 115 (1993) 5393–5399.
[19] R.F. Pasternack, C. Bustamante, P.J. Collings, Science 269 (1995) 935–939.
[20] Z.D. Liu, C.Z. Huang, Y.F. Li, Y.F. Long, Anal. Chim. Acta 577 (2006) 244–249.
[21] J.B. Xiao, C.S. Yang, F.L. Ren, X.Y. Jiang, M. Xu, Meas. Sci. Technol. 18 (2007) 859–866.
[22] J.B. Xiao, J.W. Chen, F.L. Ren, C.S. Yang, M. Xu, Anal. Chim. Acta 589 (2007) 186–191.
[23] J.W. Chen, C.S. Yang, F.L. Ren, J.B. Xiao, M. Xu, Meas. Sci. Technol. 18 (2007) 2043–2047.
[24] Z.G. Chen, T.Y. Zhang, F.L. Ren, W.F. Ding, Microchim. Acta 153 (2006) 65–71.
[25] Z.G. Chen, J.B. Liu, Y.L. Han, L. Zhu, Anal. Chim. Acta 570 (2006) 109–115.
[26] A. Papadopoulou, R.J. Green, R.A. Frazier, J. Agric. Food Chem. 53 (2005) 158–163.
[27] J.R. Lakowicz, Principles of fluorescence spectroscopy, Plenum Press, New York, London, 1983.
[28] J.R. Lakowicz, G. Weber, Biochemistry 12 (1973) 4161–4170.
[29] J.R. Lakowicz, G. Weber, Biochemistry 12 (1973) 4171–4179.
[30] C.Q. Jiang, M.X. Gao, J.X. He, Anal. Chim. Acta 452 (2002) 185–189.
[31] C. Wang, Q.H. Wu, Z. Wang, J. Zhao, Anal. Sci. 22 (2006) 435–438.
[32] W.D. Horrocks, W.E. Collier, J. Am. Chem. Soc. 103 (1981) 2856–2862.
[33] T. Förster, in: O. Sinanoglu (Ed.), Modern Quantum Chemistry, vol. 3, Academic Press, New York, 1965, pp. 93–137.
[34] N. Shaklai, J. Yguerabide, H.M. Ranney, Biochemistry 16 (1977) 5585–5592.
[35] P.D. Ross, S. Subramanian, Biochemistry 20 (1981) 3096–3102.