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ORIGINAL PAPER

Spectroscopic Studies on the Toxic Interaction of Sodium Oleate with Bovine Serum Albumin

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Abstract The effects of sodium oleate on Bovine Serum Albumin (BSA) were investigated by fluorescence, synchronous fluorescence, ultraviolet–visible and circular dichroism spectroscopy. According to the experiment results, we found that the fluorescence intensity of BSA was quenched by sodium oleate following a static mode, a sodium oleate-BSA complex was formed and the binding site was calculated approximately equal to 1. The experimental results showed that the hydrophilic group (—COONa) can bind to the BSA and lead to the looser of the protein conformation, the microenvironment and the secondary structure elements were changed in the presence of sodium oleate. This work reflected the toxic interaction mechanism of BSA and sodium oleate from the perspective of spectroscopy.

Keywords Sodium oleate · Bovine serum albumin · Spectroscopy · Interaction

Introduction

Proteins are the basic substance of body used to maintain the normal body metabolism. Protein denaturation [1] will damage body function and structure, which can cause many diseases. Many pollutants in environment will have a toxic effect on protein by causing its degeneration. Thus, studying the process of protein-depth in vitro has an important role in the prevention and treatment of diseases [2]. Serum albumin, a representative protein widely used in molecular biological experiments, is the most abundant carrier protein in the blood of all mammals [3]. It has a good biocompatibility, no cytotoxicity and can interact with endogenous and exogenous substances. Bovine serum albumin is similar to human serum albumin (HSA) [4], stable, abundant source, thus can be used to instead of HSA in protein and molecular binding assay.

Surfactant can highly decrease the surface tension of the material, which has been widely used in textile, food, medicine, pesticide, cosmetic and other industries. With its wide usage, it brings a negative impact to environment. When surfactant comes into the water, it will change the water quality, water odor, foam, reduce the dissolved oxygen and impact water bodies reoxygenation. When comes into the soil, surfactant will change the microenvironment of the soil, having a negative effect on the growth of the plant. In the research of water treatment, it is showed that surfactant can inhibit the growth of microorganisms and reduce water treatment efficiency [5, 6]. Some studies also found that surfactant in the water would bring toxic effects on fish, algae, even lead to lethal effects at high concentration [7]. Through food chain, surfactant comes into the human body and interacts with functional molecules—proteins, nucleic acids, which has a threaten to life health [8]. Therefore, establishing a way to evaluate the toxicity of surfactants on body is essential.

Sodium oleate ($C_{18}H_{33}NaO_2$), a white powder with a slightly yellow, is an anionic surfactant. When dissolved in water, sodium oleate will form many small balls dispersed in water, the outside of the ball is hydrophilic carboxylic acid sodium salt $-COO^- Na^+$, the long alkyl part is hydrophobic and locates inside the ball. The structure of sodium oleate is showed as follows (Scheme 1):

The interaction mechanism of sodium oleate with the protein is rarely reported. So in this paper, we studied the toxic interaction of sodium oleate with bovine serum

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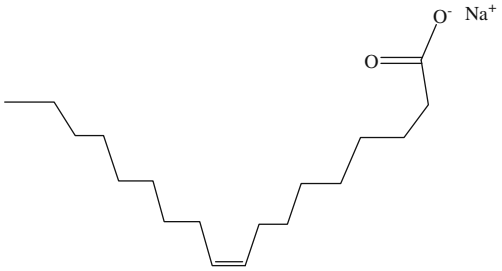

Scheme 1 The structure of sodium oleate

albumin (BSA) through fluorescence spectroscopy, synchronous fluorescence spectroscopy, UV absorption, CD spectra, and provided a reference to the interaction of carboxylic acids salt anionic surfactants on protein.

Experimental

Reagents

Bovine serum albumin (BSA) was bought from Sinopharm Chemical Reagent Beijing Co., Ltd., and was dissolved in ultrapure water to form a 1.0×10^{-5} mol L^{-1} solution, then preserved at 4 °C for later use.

Sodium oleate was purchased from Tianjin Tianda Chemical Reagent Co., Ltd., and the concentration of the stock solution was 1.0×10^{-3} mol L^{-1}.

Physiological buffer (Tris-saline buffer) was 0.1 mol L^{-1}, pH 7.4. All solution were prepared with ultra-pure water (18.25 MΩ), and diluted as required.

Apparatus

Fluorescence spectra were recorded on an F-4600 fluorescence spectrophotometer (Hitachi, Japan) equipped with a xenon lamp and 1.0 cm quartz cells. The absorption spectra were collected on a double beam UV-2450 spectrophotometer (Shimadzu, Japan) in 1.0 cm quartz cells. Circular dichroism (CD) measurements were made on a J-810 Spectropolarimeter (Jasco, Tokyo, Japan) at room temperature under constant nitrogen flush. A pHs-3C acidometer (Shanghai Pengshun Scientific Instrument Co., Ltd.), and KQ-100E ultrasonic cleaner (Jiangsu Kunshan Ultrasonic Instrument Co., Ltd.) were used for sample preparation.

Measurements

For fluorescence spectra, 1.0 ml BSA solution, 1.0 ml Tris–HCl buffer solution and a known volume of sodium oleate solution were placed in a 10 ml standard volumetric flask, the mixture diluted to the volume with water. After preparation, the reaction systems were incubated for 20 min, then the spectra were measured on the HITACHI F-4600 fluorescence spectrophotometer. The excitation wavelength was set at 280 nm and scan speed was 1,200 nm/min. The excitation and emission slit widths were set at 5 nm. PMT (Photo Multiplier Tube) voltage was 700 V.

Synchronous fluorescence spectra of solutions prepared as above were measured on the HITACHI F-4600 fluorescence spectrophotometer. The excitation wavelength (λ_{ex}) was set at 280 nm. The excitation and emission slit widths were set at 5.0 nm. The D-value ($\Delta\lambda$) between the excitation and emission wavelengths was set at 15 or 60 nm. PMT voltage was 700 V.

All absorption spectra used the same concentration of sodium oleate as the reference solution, and measured on the UV-2450 spectrophotometer. The slit width was set at 2.0 nm. The wavelength range was 500– 200 nm.

The Circular dichroism (CD) spectra was measured from 190–240 nm at a scan speed of 200 nm/min. Each result was the average of the two scans.

Results and Discussion

The Influence of Sodium Oleate Concentration on the Fluorescence Intensity of BSA

There are three intrinsic fluorophores tryptophan, tyrosine and phenylalanine in BSA. The intrinsic fluorescence of BSA is almost entirely due to tryptophan [4], for the quantum yield of phenylalanine is very low and the fluorescence of tyrosine is almost totally quenched if it is ionized, or near an amino group, a carboxyl group, or a tryptophan [9]. There are two tryptophans within a BSA molecule, which are located at amino acid positions 134 and 212. Trp-212 is located in a hydrophobic environment, and contributes more to the fluorescence intensity than Trp-134, which is located on the surface of BSA [10, 11]. The change of the emission spectra can reflect the information about the structure and dynamics of BSA. Fluorescence emission spectra of BSA were recorded (Fig. 1). It can be seen from Fig. 1 that the fluorescence intensity decreased with the increasing concentration of sodium oleate. The maximum emission wavelength had a blue shift from 335 nm to 328 nm. These results indicated that sodium oleate can bind to BSA, the hydrophilic group (—COONa) bound to the BSA and leaded to the exposure of Trp-212, the microenvironment of Trp-212 was changed.

The Fluorescence Quenching Mechanism

The quenching mechanisms are usually classified into dynamic quenching and static quenching [12]. When the dynamic quenching occurred, the quencher and fluorophore

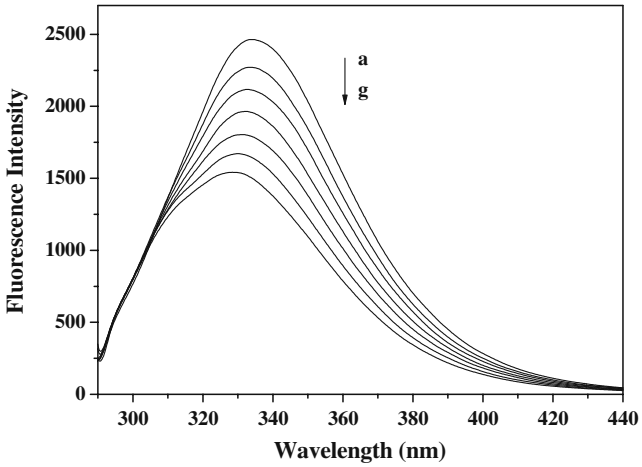

Fig. 1 The influence of sodium oleate on the fluorescence spectra of BSA. Conditions: BSA 1.0×10^{-6} molL^{-1}; Sodium oleate **a-g** 0, 1×10^{-6}, 2×10^{-6}, 3×10^{-6}, 4×10^{-6}, 6×10^{-6} and 8×10^{-6} molL^{-1}; Tris–HCl 0.1 molL^{-1} (pH=7.4)

encounters during the excited state, the quencher atom or molecular can facilitate non-radioactive transitions to the ground state. When static quenching occurred, it is usually forming a complex between quencher and fluorophore, the ground state is non-fluorescent. So in this experiment, we assumed that the interaction of sodium oleate with BSA was a dynamic quenching process, and used the Sterne-Volmer equation to analyze the quenching data [13, 14]. The Sterne-Volmer equation is:

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

Where F_0 and F are the steady-state fluorescence intensities in the absence and presence of quencher, in this experiment is sodium oleate. K_{SV} is the Stern-Volmer quenching constant, and [Q] is the concentration of quencher. K_q is the quenching rate constant of the biological macromolecule. τ_0 is the average lifetime of the molecule without any quencher ($\tau_0=10^{-8}$ s) [15].

The fluorescence intensity data were analyzed by plotting F_0/F against [Q], K_{SV} and K_q were calculated as 7.57×10^4 L mol^{-1} and 7.57×10^{12} L mol^{-1} s^{-1} (Fig. 2). Because the maximum quenching constant of various quenchers with a biopolymer is usually 2.0×10^{10} L mol^{-1} s^{-1} [16]. The value of K_q was much larger than 2.0×10^{10} L mol^{-1} s^{-1}. So the probable quenching mechanism was a static quenching, in this process a sodium oleate-BSA complex was formed.

Binding Constant and Binding Capacity

For static quenching, the following equation can be used to calculate the binding constant and binding sites [17, 18].

$$\log(F_0 - F)/F = \log K_A + n\log[Q] \quad (2)$$

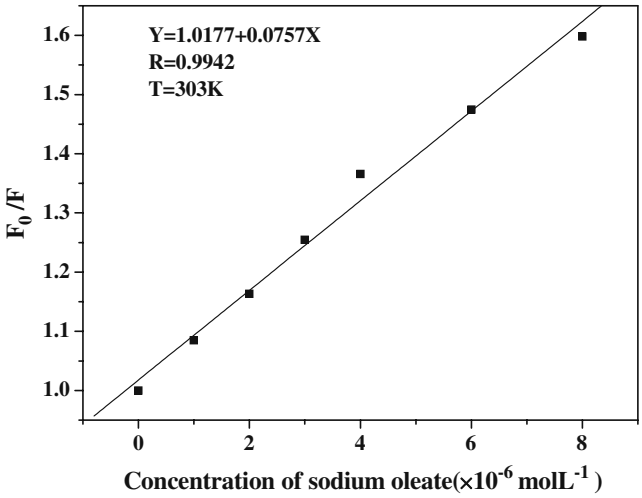

Fig. 2 Stern-Volmer plots for the quenching of BSA by sodium oleate

Where F_0, F and [Q] are the same as in Eq. 1, K_A is the binding constant and n is the number of binding sites. The values of K_A and n were calculated as 4.742×10^4 L mol^{-1} and 0.9561, respectively (Fig. 3). The value of n is approximately equal to 1, indicating that there is one type of binding site for sodium oleate in BSA, and the value of K_A is 4.742×10^4 L mol^{-1}, indicating that there is a strong interaction between sodium oleate and BSA.

Conformational Investigation

To further investigate the conformational changes of the BSA, synchronous fluorescence, UV–vis absorption and CD were used in this work.

Synchronous Fluorescence Spectroscopy

Synchronous fluorescence spectroscopy was usually used to study the conformation change of protein [19].

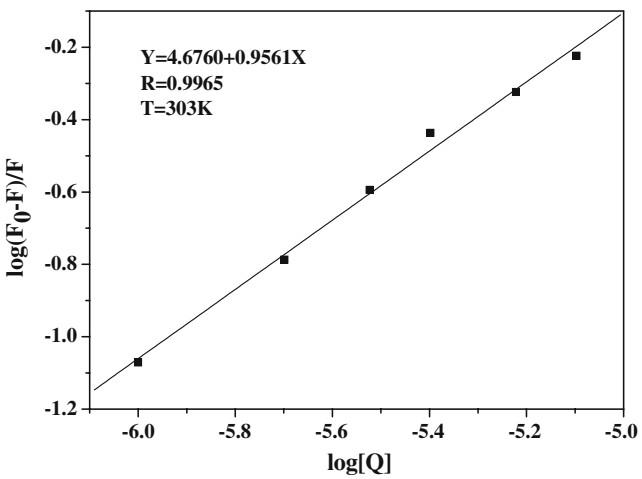

Fig. 3 Double-reciprocal curves of the fluorescence intensity

The excitation and emission monochromators were scanned at the same time while maintaining a constant wavelength interval between them. The shift in the emission maximum (λ_{em}) reflects the changes of polarity around the chromophore molecule [20]. When $\Delta\lambda$ are set at 15 nm and 60 nm, the tyrosine and tryptophan character spectrum were obtained, respectively [21]. The effects of sodium oleate on the BSA synchronous fluorescence spectra are shown in Fig. 4, from which it is showed that the synchronous fluorescence intensity decreased as the concentration of sodium oleate increased. In Fig. 4(a), the maximum emission wavelength of tyrosine kept its position over the investigated concentration range, which indicated that there was little change of the microenvironment of the tyrosine residues, while in Fig. 4(b), the maximum emission wavelength of tryptophan had a slight blue shift, which suggested that the conformation of BSA had changed and the tryptophan moved to a less polar environment [22].

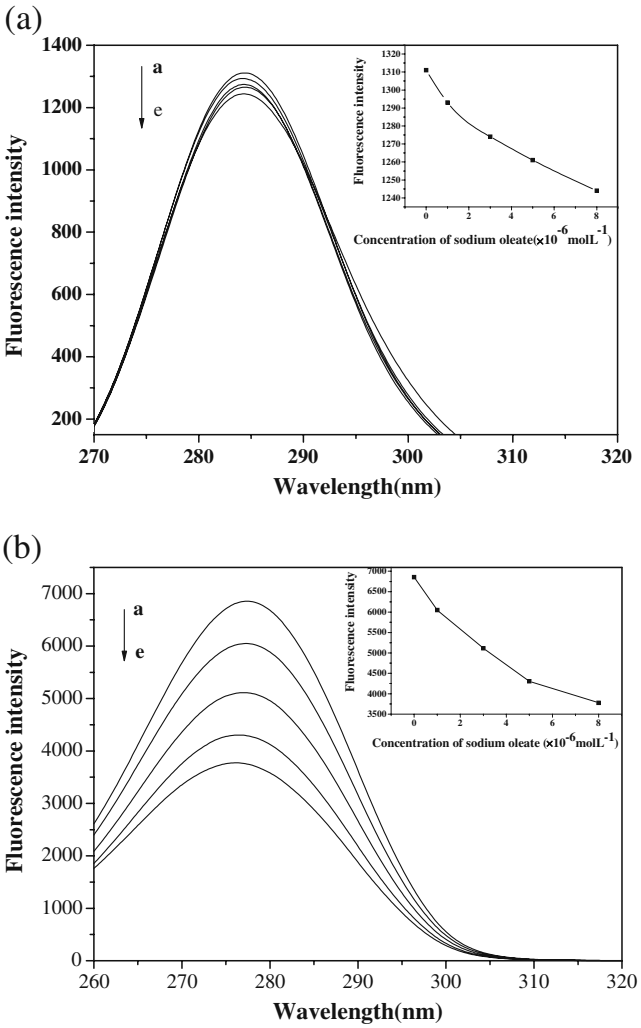

Fig. 4 Synchronous fluorescence spectra of BSA. Conditions: **a** $\Delta\lambda$= 15 nm and **b** $\Delta\lambda$=60 nm. BSA 1×10^{-6} molL^{-1}; Sodium oleate (**a-e**): 0, 1×10^{-6}, 3×10^{-6}, 5×10^{-6} and 8×10^{-6} molL^{-1}; Tris–HCl 0.1 molL^{-1} (pH=7.4)

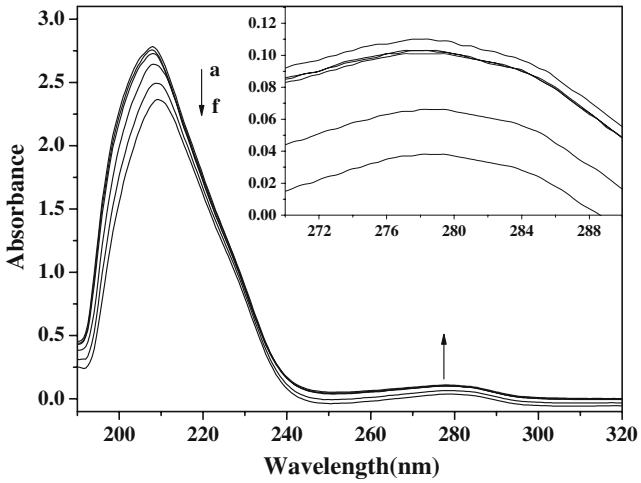

Fig. 5 Absorption spectra of BSA and sodium oleate. Conditions: BSA 1×10^{-6} molL^{-1}; Sodium oleate (**a-f**) 0, 4×10^{-6}, 1×10^{-5}, 3×10^{-5}, 5×10^{-5} and 7×10^{-5} molL^{-1}; Tris–HCl 0.1 molL^{-1} (pH=7.4). Sodium oleate of the same concentration was used as the reference solution **a-f**

UV–vis Absorption Spectroscopy

In this work, UV–vis absorption was used to explore protein structural changes and to investigate protein-ligand complex formation. BSA has two absorption peaks, the absorption peak at about 208 nm reflects the conformation of the peptide bonds, while the peak of 278 nm reflects the aromatic ring amino acids [23]. We recorded the UV–vis absorption spectra of BSA in the absence and presence of sodium oleate, in this experiment we used the corresponding concentration of sodium oleate as the reference solution. It is apparent in Fig. 5 that with the concentration of sodium oleate increased, the absorbance of BSA decreased and the maximum peak position of the sodium oleate-BSA had a light red shifted at about 208 nm. The red shift indicated sodium oleate changed the peptide strands of the BSA, the skeleton of BSA became looser and the hydrophobicity of BSA was decreased [24]. The absorption peak at about 278 nm can provide us with information about the three buried aromatic amino acids: tryptophan, tyrosine, and phenylalanine. With the concentration of sodium oleate increased, the BSA molecules gradually denatured and the main chain uncurled. Trp-212, which was originally buried in a hydrophobic pocket, was exposed to an aqueous environment. So we indicated that the microenvironment of the three aromatic acid residues was altered and the structure of BSA was destroyed.

Circular Dichroism Spectroscopy

CD spectroscopy is a sensitive technique to study the secondary conformation of protein. The conformational information of BSA in the absence and presence of sodium oleate is shown in Fig. 6. The two negative bands at 208 and 222 nm indicates the α-helical character of the protein [25]. The α-helical content were calculated from Eq. 3 and Eq. 4 [26, 27].

$$MRE = \frac{ObservedCD(m\,\mathrm{deg})}{C_p nl \times 10} \tag{3}$$

where C_p is the molar concentration of the protein, n is the number of amino acid residues and l is the path length.

$$\alpha - Helix(\%) = \frac{-\mathrm{MRE}_{208} - 4000}{33,000 - 4000} \times 100 \tag{4}$$

where MRE_{208} is the observed MRE value at 208 nm, 4,000 is the MRE of the β-form and random coil conformation cross at 208 nm, and 33,000 is the MRE value of a pure α-helix at 208 nm.

From the above equations, we calculated the α-helix content of BSA in absence and presence of sodium oleate. The content of α-helix decreased from 55.24% to 52.96% when sodium oleate was added. The decreased of α-helix content indicates that sodium oleate combines with the amino acid residues of the main polypeptide chain of the protein and destroys their hydrogen bond networks [28]. So we can conclude that the protein skeleton of BSA became looser, the amino acid residues were exposed, and the hydrophobicity of BSA was decreased.

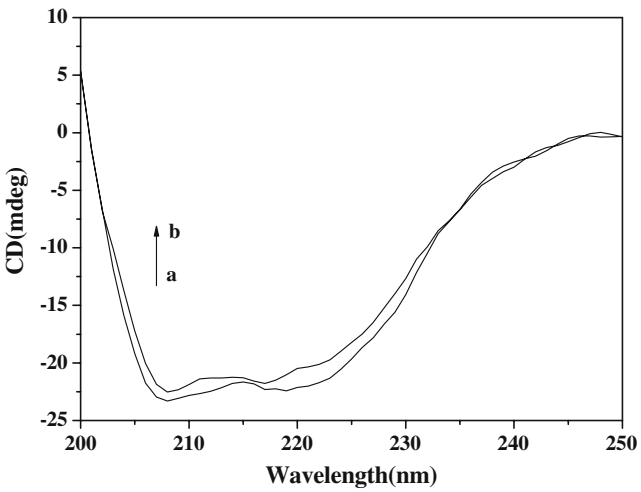

Fig. 6 CD spectra of the sodium oleate—BSA system at room temperature. Conditions: BSA 1×10^{-7} molL^{-1}; Sodium oleate **a-b** 0, 2×10^{-6} molL^{-1}; Buffer: PBS 0.1 molL^{-1}

Conclusions

The interaction mechanisms of sodium oleate with BSA were investigated by the fluorescence spectra, synchronous fluorescence spectra, UV–vis absorption spectra and CD spectra, the results were all agreed with each other. It is showed that the intrinsic fluorescence of BSA was quenched by sodium oleate at a static mode. The absorption spectra and CD spectra showed that sodium oleate bound to the BSA and induced the conformation change of the BSA, some of the functional groups were exposed. Further, the function of the BSA may be changed, some diseases would be caused by these changes.

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References

1. Schellman JA (2002) Fifty years of solvent denaturation. Biophys Chem 96:91–101
2. Liu R, Zong W, Jin K, Lu X, Zhu J, Zhang L, Gao C (2008) The toxicosis and detoxifcation of anionic/cationic surfactants targeted to bovine serum albumin. Spectrochim Acta A Mol Biomol Spectrosc 70:198–200
3. Ercelen S, Klymchenko AS, Mely Y, Demchenko AP (2005) The binding of novel two-color fluorescence probe FA to serum albumins of different species. Int J Biol Macromol 35:231–242
4. Zhang YZ, Zhou B, Liu YX, Zhou CX, Ding XL, Liu Y (2008) Fluorescence study on the interaction of bovine serum albumin with p-aminoazobenzene. J Fluoresc 18:109–118
5. Schoeberl P (1991) Coupling the OECD confirmatory test with continuous ecotoxicity tests. Tenside Surf Det 28:6–14
6. Lina SH, Lina CM, Leu HG (1999) Operating characteristics and kinetic studies of surfactant wastewater treatment by Fenton oxidation. Water Res 33:1735–1741
7. Wang X, Li WQ, Zheng AR et al (1996) The influence of detergent (LAS) on the growth of algae in the sea. Acta Oceanolog Sin 18(3):128–132
8. Fox K, Holt M, Daniel M et al (2000) Removal of linear alkylbenzene sulfonate from a small Yorkshire stream: contribution to GREAT-ER project. Sci Total Environ 251:171–185
9. Sulkowska (2002) Interaction of drugs with bovine serum and human serum albumin. J Mol Struct 614:227–232
10. Eftink MR, Ghiron CA (1977) Exposure of tryptophanyl residues and protein dynamics. Biochemistry 16:5546–5551
11. Peterman BF, Laidler KJ (1980) Study of reactivity of tryptophan residues in serum albumins and lysozyme by N-bromosuccinamide fluorescence quenching. Arch Biochem Biophys 199:158–164
12. Hu YJ, Liu Y, Pi ZB, Qu SS (2005) Interaction of cromolyn sodium with human serum albumin: a fluorescence quenching study. Bioorg Med Chem 13:6609–6614
13. Gelamo EL, Silva CH, Imasato H, Tabak M (2002) Interaction of bovine (BSA) and human (HSA) serum albumins with ionic surfactants: spectroscopy and modelling. Biochim Biophys Acta 1594:84–99

14. Kang J, Liu Y, Xie MX, Li S, Jiang M, Wang YD (2004) Interactions of human serum albumin with chlorogenic acid and ferulic acid. Biochim Biophys Acta 1674:205–214
15. Lakowicz JR, Weber G (1973) Quenching of fluorescence by oxygen. A probe for structural fluctuations in macromolecules. Biochemistry 12:4161–4170
16. Sun SF, Zhou B, Hou HN, Liu Y, Xiang GY (2006) Studies on the interaction between Oxaprozin-E and bovine serum albumin by spectroscopic methods. Int J Biol Macromol 39:197–200
17. Barik A, Priyadarsini KI, Mohan H (2003) Photophysical studies on binding of curcumin to bovine serum albumins. Photochem Photobiol 77:597–603
18. Tian J, Liu J, Zhang J, Hu Z, Chen X (2003) Fluorescence studies on the interactions of barbaloin with bovine serum albumin. Chem Pharm Bull (Tokyo) 51:579–582
19. Lu JQ, Jin F, Sun TQ, Zhou XW (2007) Multi-spectroscopic study on interaction of bovine serum albumin with lomefloxacin-copper (II) complex. Int J Biol Macromol 40:299–304
20. Wang YQ, Zhang HM, Zhang GC, Liu SX, Zhou QH, Fei ZH, Liu ZT (2007) Studies of the interaction between paraquat and bovine hemoglobin. Int J Biol Macromol 41:243–250
21. Wang YQ, Zhang HM, Wang RH (2008) Investigation of the interaction between colloidal TiO(2) and bovine hemoglobin using spectral methods. Colloids Surf B Biointerfaces 65:190–196
22. Pan XP, Liu RT, Qin PF (2010) Spectroscopic studies on the interaction of acid yellow with bovine serum albumin. J Lumin 130:611–617
23. Zhang YZ, Chen XX, Dai J, Zhang XP, Liu YX, Liu Y (2008) Spectroscopic studies on the interaction of lanthanum(III) 2-oxo-propionic acid salicyloyl hydrazone complex with bovine serum albumin. Luminescence 23:150–156
24. Hu YJ, Liu Y, Wang JB, Xiao XH, Qu SS (2004) Study of the interaction between monoammonium glycyrrhizinate and bovine serum albumin. J Pharm Biomed Anal 36:915–919
25. Venkateshrao S, Manoharan PT (2004) Conformational changes monitored by fluorescence study on reconstituted hemoglobins. Spectrochim Acta A Mol Biomol Spectrosc 60:2523–2526
26. Li Y, He W, Liu J, Sheng F, Hu Z, Chen X (2005) Binding of the bioactive component jatrorrhizine to human serum albumin. Biochim Biophys Acta 1722:15–21
27. Merchant JR, Chothia DS (1970) Antituberculous Schiff bases. J Med Chem 13:335–336
28. Cheng XX, Lui Y, Zhou B, Xiao XH, Liu Y (2009) Probing the binding sites and the effect of berbamine on the structure of bovine serum albumin. Spectrochim Acta A Mol Biomol Spectrosc 72:922–928