ISSN : 0974 - 7524 Volume 10 Issue 5

Physical CHEMISTRY
An Indian Journal

Full Paper

PCAIJ, 10(5), 2015 [163-172]

# Studying the interaction of bovine serum albumin with cefpirome sulfate by synchronous fluorescence spectroscopy

**Qiu-Ju Zhang, Bao-Sheng Liu*, Rong Han, Gai-Xia Li**

**Key Laboratory of Analytical Science and Technology of Hebei Province, College of Chemistry & Environmental Science, Hebei University, Baoding 071002, Hebei Province, (P.R.CHINA)**

**E-mail: lbs@hbu.edu.cn**

## ABSTRACT

The interaction of cefpirome sulfate with bovine serum albumin in aqueous solution was studied by synchronous fluorescence and verified by fluorescence quenching spectroscopy. The data of synchronous fluorescence spectroscopy was used to infer the mechanism of the system and calculate the relevant parameters. The experimental results showed that the static mechanism played a role in the system; there was only one site for cefpirome sulfate on bovine serum albumin and the binding site was located in sub-domain IIA of BSA. Thermodynamic parameters were calculated, suggesting that hydrogen bond and hydrophobic interactions played a major role in stabilizing the complex. Based on the theory of Forester's non-radiation energy transfer, binding distance is < 7 nm. In addition, synchronous fluorescence spectroscopy also provided information about the change of the molecular environment.



## KEYWORDS

Bovine serum albumin;
Cefpirome sulfate;
Synchronous fluorescence spectroscopy;
Fluorescence quenching spectroscopy.

## INTRODUCTION

Serum albumins have an important role in the transporter of a variety of compounds, such as drugs, fatty acids, and amine-terminated dendrimers[1]. And it is common to understand the structure change of the protein by studying the interaction between small molecular and protein. Bovine serum albumin (BSA) is one of the most extensively studied proteins and has many superior properties, including structural homology with human serum albumin, medical importance, abundance, low-cost, ease of purification and stability[2].

Cefpirome is a new C-3' quaternary ammonium cephalosporin which bears a 2,3-cyclopentano pyridinium at the C-3 position of the cephem nucleus and has been classified as a fourth generation cephalosporin[3]. Cefpirome sulfate (CFS) (Figure 1) is sulfate salt of cephalosporin for parenteral administration, originally created by Hoechst AG Frankfurt am Main, Germany and Roussel Uclaf Paris, France[4]. It has a potential clinical advantage against gram-positive bacteria[5].

The fluorescence measurement has been used as a tool to study the interaction between small molecule and protein in an attempt to characterize the

Full Paper

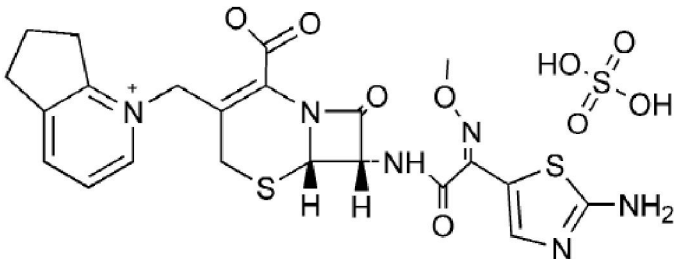

**Figure 1 : Chemical structure of CFS**

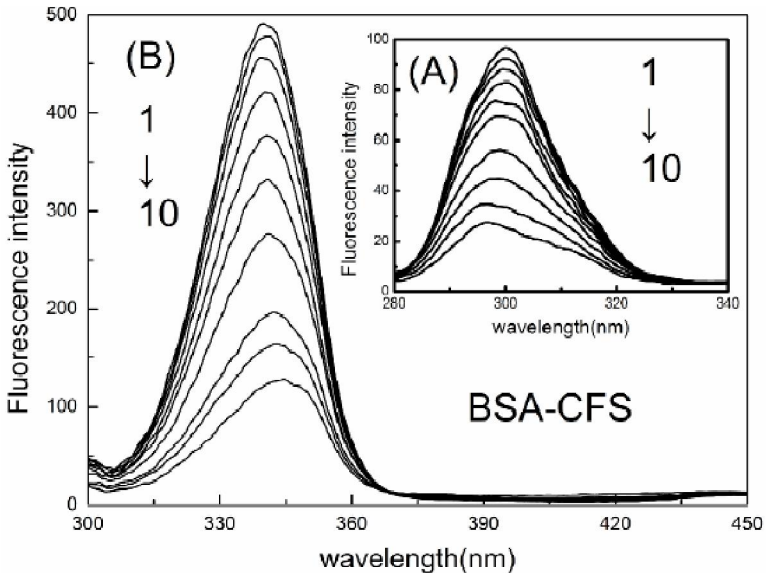


**Figure 2 : Synchronous fluorescence spectrum of BSA-CFS system (*T*=298 K) (A: Δλ=15 nm, B: Δλ=60 nm) $C_{BSA}$= 2.0 ×10$^{-7}$ mol·L$^{-1}$, 1~10 $C_{CFS}$= (0, 0.4, 1.2, 4.0, 8.0, 10, 20, 30, 40, 50) ×10$^{-6}$ mol·L$^{-1}$**

type of chemical association[6]. Synchronous fluorescence spectroscopy was introduced by Lloyd in 1971[7]. In the synchronous fluorescence technique, both excitation and emission monochromators are scanned in the same instant with a constant interval between excitation and emission wavelength maintained[8]. It is simple, less costly and rapid, and it avoids taking special sample preparation steps[9]. And it has been successfully used for the analysis of various multifluorophoric mixtures such as drink[10], olive oil[11], pesticide[12], etc. Synchronous fluorescence spectroscopy is also applied in exploring the change of the molecular environment in the vicinity of the chromosphere molecules in low concentration under physiological condition[13]. To the best of our knowledge, there are a few papers about using the data of synchronous fluorescence spectroscopy to study the mechanism of the interaction between small molecules and protein.

We described in this article the interaction mechanism of CFS and BSA using synchronous fluorescence, and verifying by fluorescence quenching spectroscopy. The results showed that the two methods had consistent conclusion, indicating synchronous fluorescence spectrometry could be used to study the interaction between drugs and protein.

## EXPERIMENTAL

### Apparatus

Fluorescence spectra were recorded on a Shimadzu RF-5301 fluorescence spectrophotometer (Japan). Synchronous fluorescence spectra were obtained by setting the excitation and emission wavelength intervals (Δλ) at 15 and 60 nm. The absorption spectra were obtained from a Shimadzu UV-265 spectrophotometer. pH measurements were made with a Leici pHS-3C digital pH meter (Shanghai, China). All temperatures were controlled by a SYC-

$15_B$ superheated water bath (Nanjing, China).

## Reagent

Cefpirome sulfate (CFS, CAS: 98753-19-6, $1.0\times10^{-3}$ mol·L$^{-1}$) was prepared as a stock solution. BSA was purchased from Sigma and solutions of BSA ($2.0\times10^{-5}$ mol·L$^{-1}$) were prepared as a stock solution. Tris/HCl buffer (0.05 mol·L$^{-1}$ Tris, 0.15 mol·L$^{-1}$ NaCl) containing 0.15 mol·L$^{-1}$ NaCl was selected to keep the pH constant and to maintain the ionic strength of the solution. All other reagents were of analytical grade and all the water used in the experiments was double-distilled. All aqueous solutions were stored at 277 K.

## Procedures

1.0 mL Tris/HCl (pH 7.40), 1.0 mL BSA solution ($2\times10^{-6}$ mol·L$^{-1}$) and different volumes of CFS were successively added to a 10 mL colorimetric tube and the samples were diluted to scaled volume with water. Fluorescence quenching spectra were measured in the range of 285–450 nm at an excitation wavelength of 280 and 295 nm. Synchronous fluorescence spectra of BSA were recorded from 280 to 340 nm ($\lambda ex - \lambda em = \Delta\lambda = 15$ nm) and from 300 to 450 nm ($\lambda ex - \lambda em = \Delta\lambda = 60$ nm) at three temperatures.

1.0 mL Tris-HCl (pH 7.40), 1.0 mL BSA solution ($2\times10^{-5}$ mol·L$^{-1}$) solution and different volumes of CFS solution were added into 10 mL colorimetric tube successively. UV–vis measurements were made against blank solutions treated in the same way without BSA. UV–Vis absorption spectra of BSA solution were measured in the range of 190–400 nm at 298 K.

# RESULTS AND DISCUSSION

## Conformation investigation by synchronous fluorescence

Synchronous fluorescence spectroscopy can provide information about the change of the molecular microenvironment. A shift of the maximum emission wavelength is related to the alteration of the polarity in the microenvironment around the chromophore and $\Delta\lambda$, representing the value of the difference between the excitation and emission wavelengths, is an important operating parameter[14]. When the wavelength interval between excitation and emission is 15 or 60 nm, the synchronous fluorescence spectra give information about the molecular environment in the vicinity of tyrosine or tryptophan residues, respectively[15]. The effect of CFS on the synchronous fluorescence spectra of BSA at $\Delta\lambda$=15 nm and $\Delta\lambda$=60 nm are presented in Figure 2. It is apparent from the Figure 2 that the emission wavelength of the tyrosine residues is blue-shifted with increasing concentration of CFS. This blue shift expressed that the polarity around tyrosine residues weakened and the hydrophobicity strengthened[16]. This may be due to the changes of residue micro-environment with the insertion of CFS. At the same time, the $\lambda_{max}$ has a red shift when $\Delta\lambda$=60 nm, which indicated that the conformation of BSA was changed, the polarity around the tryptophan residues was increased and the hydrophobicity was decreased[17]. In a word, the conformation of BSA was changed by adding CFS.

## Analysis of synchronous fluorescence spectra

Two quenching processes are known: static and dynamic quenching. Dynamic quenching resulted from the diffusive encounter between quencher and fluorophore during the lifetime of the excited state; static quenching resulted from the formation of a non-fluorescent ground-state complex (fluorophore–quencher)[18]. Fluorescence quenching is described by the Stern–Volmer equation (Eq. 1)[19]:

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

Where, $F_0$ and $F$ are the fluorescence intensities before and after the addition of the quencher. $K_q$, $K_{sv}$, $\tau_0$ and [$Q$] are the bimolecular quenching rate constant, the Stern–Volmer quenching constant, the average lifetime of the biomolecule without quencher ($10^{-8}$ s), the concentration of the quencher, respectively. Both quenching constant and quenching rate constant are shown in TABLE 1. The results show the value of $K_q$ is the rate constants of BSA quench by CFS, which is greater than the limiting diffusion rate constants of the biomolecule ($2\times10^{10}$ L·mol$^{-1}$·s$^{-1}$)[20] and the Sterne–Volmer quenching constants are inversely correlated with the temperatures which indicates that the probable quenching mechanism is the for-

Full Paper

**TABLE 1 : Quenching constants of BSA–CFS system at different temperatures**

| Method | | T/K | $K_q/(L\cdot mol^{-1}\cdot s^{-1})$ | $K_{sv}/(L\cdot mol^{-1})$ | $r_1$ |
|---|---|---|---|---|---|
| Synchronous Fluorescence Spectroscopy | Δλ=15 nm | 298 | $5.19\times10^{12}$ | $5.19\times10^{4}$ | 0.994 2 |
| | | 310 | $4.34\times10^{12}$ | $4.39\times10^{4}$ | 0.993 1 |
| | | 318 | $4.11\times10^{12}$ | $4.11\times10^{4}$ | 0.994 7 |
| | Δλ=60 nm | 298 | $5.78\times10^{12}$ | $5.78\times10^{4}$ | 0.993 1 |
| | | 310 | $5.18\times10^{12}$ | $5.18\times10^{4}$ | 0.996 4 |
| | | 318 | $4.92\times10^{12}$ | $4.92\times10^{4}$ | 0.997 5 |
| Fluorescence Quenching Spectroscopy | $\lambda_{ex}$=280 nm | 298 | $6.51\times10^{12}$ | $6.51\times10^{4}$ | 0.994 7 |
| | | 310 | $6.09\times10^{12}$ | $6.09\times10^{4}$ | 0.993 7 |
| | | 318 | $5.81\times10^{12}$ | $5.81\times10^{4}$ | 0.994 7 |
| | $\lambda_{ex}$=295 nm | 298 | $3.73\times10^{12}$ | $3.73\times10^{4}$ | 0.995 7 |
| | | 310 | $2.88\times10^{12}$ | $2.88\times10^{4}$ | 0.996 6 |
| | | 318 | $2.72\times10^{12}$ | $2.72\times10^{4}$ | 0.998 5 |

**$K_q$ is the quenching rate constant; $K_{sv}$ *is* the Stern–Volmer quenching constant; $r_1$ is the linear relative coefficient of $F_0/F$~[Q].**

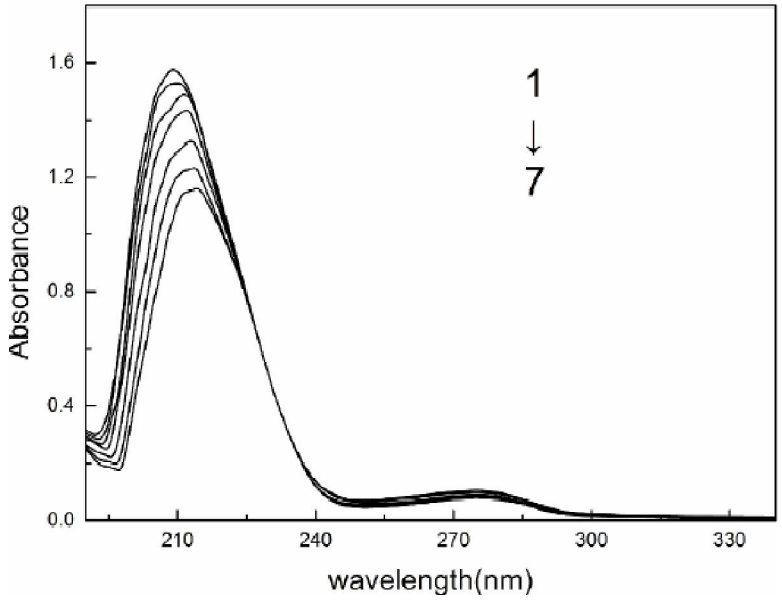


**Figure 3 UV absorption spectrum of BSA-CFS system; ($T$ = 298 K) $C_{BSA}$ = 2.0 ×$10^{-6}$ mol·$L^{-1}$; 1~7 $C_{CFS}$ = (0, 4.0, 10, 20, 30, 40, 50) ×$10^{-6}$ mol·$L^{-1}$**

mation of BSA-CFS complex (static quenching) rather than by dynamic collision (dynamic quenching)[21]. Comparing the data, it shows the mechanism obtained by synchronous fluorescence spectroscopy are similar with fluorescence quenching spectroscopy, and the linear relative coefficients are all above 0.99, suggesting that synchronous fluorescence spectroscopy can be applies to study the interaction between drugs and protein. In addition, during the experiment, it was found that at relatively high temperatures, synchronous fluorescence compared to fluorescence quenching method is more stable.

Figure 3 is the UV/vis absorption spectra, it can be seen that with gradual addition of CFS to BSA solution, the intensity of the peak at 208 nm decreases with red shift. The results indicate that the interaction between BSA and CFS resulted in the formation of a complex[22]. This also implies that the mechanism of the interaction between CFS and BSA is static.

## Binding constant and binding sites

For static quenching, the relation between fluo-

**TABLE 2 : Binding constants *Ka* and binding sites *n* at different temperatures**

| *Method* | | *T/K* | $K_a/(L \cdot mol^{-1})$ | *n* | $r_2$ |
|---|---|---|---|---|---|
| Synchronous Fluorescence Spectroscopy | **Δλ=15 nm** | **298** | **$4.35\times10^4$** | **0.97** | **0.992 3** |
| | | 310 | $3.88\times10^4$ | 1.04 | 0.995 3 |
| | | 318 | $3.45\times10^4$ | 1.00 | 0.994 2 |
| | Δλ=60 nm | 298 | $4.97\times10^4$ | 1.06 | 0.997 1 |
| | | 310 | $4.85\times10^4$ | 1.02 | 0.999 0 |
| | | 318 | $4.12\times10^4$ | 1.10 | 0.993 4 |
| Fluorescence Quenching Spectroscopy | $\lambda_{ex}$=280 nm | 298 | $5.71\times10^4$ | 1.01 | 0.995 7 |
| | | 310 | $5.65\times10^4$ | 1.00 | 0.992 9 |
| | | 318 | $4.72\times10^4$ | 1.07 | 0.994 0 |
| | $\lambda_{ex}$=295 nm | 298 | $3.35\times10^4$ | 1.02 | 0.998 3 |
| | | 310 | $3.28\times10^4$ | 1.03 | 0.997 4 |
| | | 318 | $2.48\times10^4$ | 1.02 | 0.996 0 |

***Ka* is the binding constant; *n* is the number of binding site. $r_2$ is the linear relative coefficient of $\log(F_0\text{-}F)/F \sim \log\{[Q_t]\text{-}n[P_t](F_0\text{-}F)/F_0\}$.**

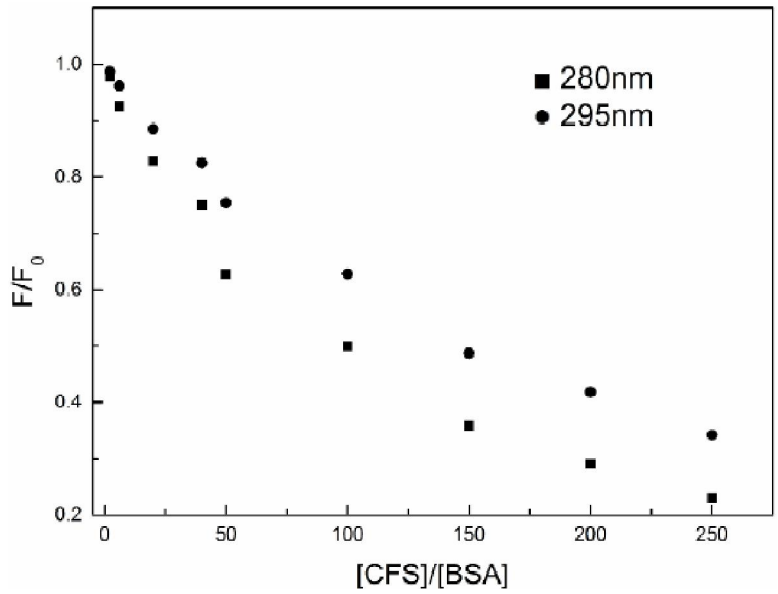


**Figure 4 : Quenching curves of BSA-CFS at $\lambda_{ex}$ = 280 nm and $\lambda_{em}$ = 295 nm ($T$ =298 K); $C_{BSA}$=2.0 ×10$^{-7}$ mol·L$^{-1}$, 1~10 $C_{CFS}$= (0, 0.4, 1.2, 4.0, 8.0, 10, 20, 30, 40, 50) ×10$^{-6}$ mol·L$^{-1}$**

rescence intensity and the amount of quencher can be defined using the 'modified' Stern–Volmer equation (Eq. 2)[23]:

$$\lg[(F_0 - F)/F] = n\lg K_a + n\lg\{[Q_t] - n[P_t][(F_0 - F)/F_0]\} \quad (2)$$

Where, $F_0$ and $F$ are the fluorescence intensity of protein when the quencher $Q$ is present and absent. $[Q_t]$ is the total quencher concentration and $[P_t]$ is the total protein concentration. From the plot of lg $[(F_0\text{-}F)/F]$ versus lg $([Q_t]\text{-}n[P_t](F_0\text{-}F)/F_0)$, binding constants $K_a$ and the number of binding sites $n$ are calculated from the intercept and slope. The values of $K_a$ and $n$ from synchronous fluorescence spectroscopy are calculated at different temperatures and listed in TABLE 2. From TABLE 2, the values of $n$ are almost equal to 1, which shows that the binding mode of CFS to BSA are 1:1[24], Furthermore, the binding constants decreased with rising temperatures, which confirms that their fluorescence quenching was static[25]. The value of $K_a$ when Δλ=60 nm is greater than the value of $K_a$ when Δλ=15 nm, implying that CFS is probably closer to tryptophan residues compared to tyrosine residues. Comparing the

**TABLE 3 : Thermodynamic parameters of BSA and CFS at different temperatures**

| Method | | T /K | Ka /($M^{-1}$) | ΔH /($KJ{\cdot}mol^{-1}$) | ΔS/($J{\cdot}mol^{-1}{\cdot}K^{-1}$) | ΔG /($KJ{\cdot}mol^{-1}$) |
|---|---|---|---|---|---|---|
| Synchronous Fluorescence Spectroscopy | Δλ= 15 nm | 298 | $4.35\times10^4$ | -8.96 | 58.72 | -26.46 |
| | | 310 | $3.88\times10^4$ | | 58.94 | -27.23 |
| | | 318 | $3.45\times10^4$ | | 58.69 | -27.62 |
| | Δλ= 60 nm | 298 | $4.97\times10^4$ | -7.39 | 65.11 | -26.79 |
| | | 310 | $4.85\times10^4$ | | 65.87 | -27.81 |
| | | 318 | $4.12\times10^4$ | | 65.11 | -28.09 |
| Fluorescence Quenching Spectroscopy | λex= 280 nm | 298 | $5.71\times10^4$ | -7.50 | 65.89 | -27.14 |
| | | 310 | $5.65\times10^4$ | | 66.78 | -28.20 |
| | | 318 | $4.72\times10^4$ | | 65.89 | -28.45 |
| | λex= 295 nm | 298 | $3.35\times10^4$ | -10.87 | 50.16 | -25.81 |
| | | 310 | $3.28\times10^4$ | | 51.39 | -26.80 |
| | | 318 | $2.48\times10^4$ | | 49.95 | -26.75 |

**$K_a$ is the binding constant; Δ*H* is the enthalpy change; Δ*S* is the entropy change; Δ*G* is the free energy**

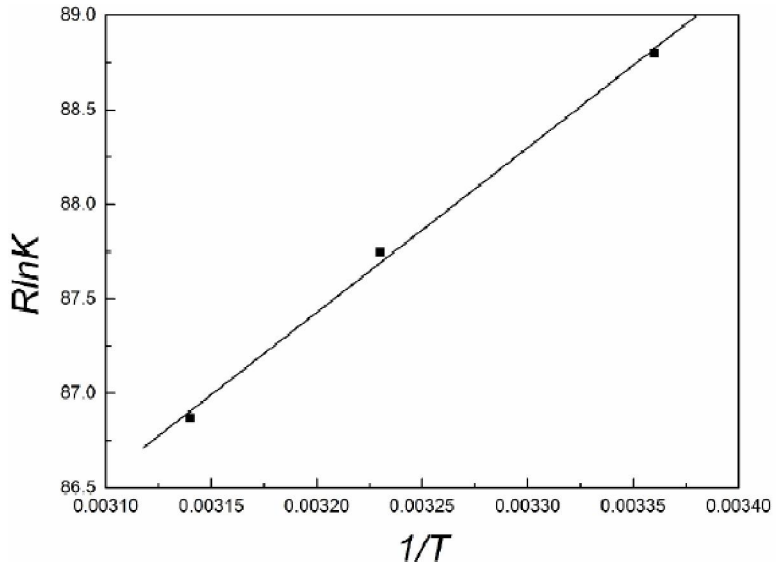


**Figure 5 : Van't Hoff plot of the interaction of BSA- CFS (Δλ= 60 nm)**

results of two methods, it is can be seen that the value of $K_a$ and *n* are similar, indicating synchronous fluorescence spectroscopy can be used to calculate the $K_a$ and *n*.

## The primary binding site studies

BSA is composed of three linearly arranged, structurally homologous domains (I–III), and each domain in turn is the product of two sub-domains (A and B)[26]. In the sub-domains of BSA, IIA subunit (tryptophan and tyrosine) and IIIA (tyrosine) are also considered as the main binding sites for small drug molecule. It is generally accepted that the intrinsic fluorescence of BSA, when excited at 280 nm, arises mainly from tryptophan and tyrosine residues; when excited at 295 nm, arises mainly from tryptophan residue[27]. Based on the Stern–Volmer equation, comparing the fluorescence quenching of BSA excited at 280 nm and 295 nm allows us to estimate the participation of tryptophan and tyrosine groups in the system. In Figure 4, in the presence of CFS, the quenching curves of BSA that are excited at 280 and 295 nm do not overlap, and at the same temperature, $K_a$ values at excitation wavelengths of 280 nm are greater than 295 nm from TABLE 2, suggesting that tryptophan and tyrosine residues play an important

role. In this way, it could confirm that the primary binding site for CFS is located in sub-domain IIA of BSA.

**Thermodynamic parameters and binding forces**

The forces acting between a small molecule and macromolecule include hydrogen bonding, the van der Waals force, the electrostatic force, the hydrophobic force, etc[28]. The thermodynamic parameters, such as free energy (ΔG), enthalpy (ΔH) and entropy (ΔS) of binding reaction, are the main evidence for confirming acting forces. If ΔH (enthalpy change) is little changed within the studied temperature range, then the reaction ÄH can be regarded as constant[29]. The thermodynamic parameters can be calculated on the basis of van't Hoff equation[30]:

$$R\ln K = \Delta S - \Delta H / T \quad (3)$$

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

Where, $K$ is the binding constant, and $R$ is the universal gas constant. For the system of BSA-CFS, according to Eq. (3), (4), the values of ΔH were obtained from the linear van't Hoff plot (Figure 5) the other thermodynamic parameters for the interaction of BSA-CFS calculated at three temperatures are listed in TABLE 3. From TABLE 3, it can be seen that the negative sign for ΔG indicates the spontaneity of the binding of CFS with BSA. For drug–protein interaction, the positive ΔS value is frequently taken as evidence for the hydrophobic interaction[31] because the water molecules that are arranged in an orderly fashion around the ligand and protein acquire a more random configuration. In this way, the destruction of the water structure was induced by hydrophobic interactions. The negative ΔH value observed in the present study cannot be attributed to electrostatic interactions since for electrostatic interactions ΔH is very small, almost zero[32, 33]. Accordingly, it is not possible to account for the thermodynamic parameters of BSA-CFS coordination compound on the basis of a single intermolecular force model[34]. The negative ΔH and positive ΔS values, therefore, showed that both hydrogen bond and hydrophobic interactions play a role in the binding of CFS to BSA[35]. BSA molecule has two tryptophan residues (Trp-134 and Trp-213), which possess intrinsic fluorescence: Trp-134 in the first sub-domain IB of the albumin molecule and Trp-213 in sub-domain IIA. Trp-213 is located within a hydrophobic binding pocket of the protein and Trp-134 is mainly exposed to the hydrophilic environment. According to the values of the thermodynamic parameters, it can be deduced that the compounds tended to bind in the vicinity of Trp-213[36]. This again demonstrates that the primary binding site for CFS is located in sub-domain IIA of BSA.

Comparing TABLES 3, it shows that ΔH0ΔS0ΔG obtained by two methods are similar, this indicates synchronous fluorescence and fluorescence quenching method are consistent with the conclusions about binding forces.

**Energy transfer between CFS and BSA**

Förster's theory of dipole–dipole energy transfer is used to determine the distances between the protein residue (donor) and the bound drug (acceptor) in BSA[37]. Generally speaking, fluorescence resonance energy transfer occurs when the emission spectrum of a fluorophore overlaps the absorption spectrum of another molecule[38]. The absorption spectrum of BSA was recorded in $4.0\times10^{-7}$ mol·L$^{-1}$ liposome solution. The emission spectrum of CFS was also recorded under the same condition. The energy transfer effect is related not only to the distance between the acceptor and donor, but also to the critical energy transfer distance $R_0$:

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

Where $F$ and $F_0$ are the fluorescence intensities of BSA in the presence and absence of CFS, $E$ is the energy transfer efficiency, $r$ is the distance between acceptor and donor, and $R_0$ is the critical distance when the transfer efficiency is 50%. The quantity $R_0^{\,6}$ is calculated using the equation:

$$R_0^{\,6} = 8.78\times10^{-25} K^2 \phi N^{-4} J \quad (6)$$

Where $K^2$ is the spatial orientation factor of the dipole, $N$ is the refractive index of the medium, $\Phi$ is the fluorescence quantum yield of the donor, and $J$ is the overlap integral between the fluorescence emission spectrum of the donor and the absorption spectrum of the acceptor. In this case, $K^2 = 2/3$, $N = 1.336$ and $\Phi = 0.118$[39], $J$ is given by:

Full Paper

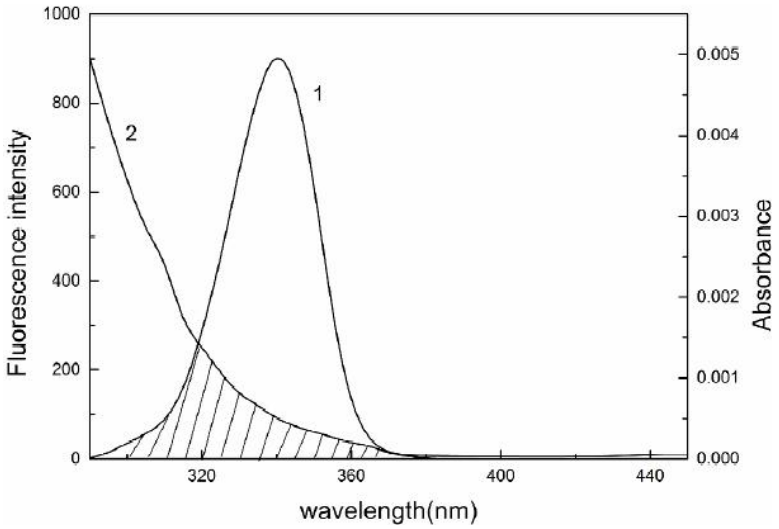


**Figure 6 : Overlap of synchronous fluorescence spectrum of BSA (1) with the absorption spectrum of CFS (2) at 298 K ($C_{BSA}$= $C_{CFS}$ =4.0 ×$10^{-7}$ mol·$L^{-1}$) (Δλ= 60 nm)**

**TABLE 4 : Energy transfer parameters in the system between BSA and CFS at different temperatures**

| Method | | T/K | E/ (%) | J/($cm^3$·$M^{-1}$) | $R_0$/(nm) | r/(nm) |
|---|---|---|---|---|---|---|
| Synchronous Fluorescence Spectroscopy | Δλ=15 nm | 298 | 1.93 | $5.98\times10^{-15}$ | 2.25 | 4.33 |
| | | 310 | 3.84 | $6.04\times10^{-15}$ | 2.25 | 3.86 |
| | | 318 | 2.48 | $5.93\times10^{-15}$ | 2.25 | 4.14 |
| | Δλ=60 nm | 298 | 2.16 | $2.11\times10^{-15}$ | 1.89 | 3.57 |
| | | 310 | 1.79 | $2.24\times10^{-15}$ | 1.91 | 3.72 |
| | | 318 | 3.36 | $2.41\times10^{-15}$ | 1.93 | 3.38 |
| Fluorescence Quenching Spectroscopy | λex=280 nm | 298 | 2.13 | $3.71\times10^{-15}$ | 2.08 | 3.93 |
| | | 310 | 4.51 | $3.74\times10^{-15}$ | 2.08 | 3.46 |
| | | 318 | 2.71 | $3.79\times10^{-15}$ | 2.09 | 3.79 |
| | λex=295 nm | 298 | 6.81 | $2.22\times10^{-15}$ | 1.91 | 2.95 |
| | | 310 | 1.23 | $2.99\times10^{-15}$ | 2.00 | 4.16 |
| | | 318 | 3.46 | $2.91\times10^{-15}$ | 2.00 | 3.48 |

$$J = \sum F(\lambda)\varepsilon(\lambda)\lambda^4\Delta\lambda / \sum F(\lambda)\Delta\lambda \quad (7)$$

Where $F(\lambda)$ is the fluorescence intensity of the fluorescence donor at wavelength $\lambda$, and $\varepsilon(\lambda)$ is the molar absorption coefficient of the acceptor at this wavelength. Figure 6 showed the spectral overlap between absorption spectrum of CFS and fluorescence spectrum of BSA in the wavelength range of 300–450 nm.

According to the above equations and experimental data, the calculated results of $J$, $R_0$, $E$ and $r$ are listed TABLE 4. From TABLE 4, it can be seen that the distance between donor molecule and acceptor molecule is less than 7 nm, which indicates the non-radiative energy transfer from CFS to BSA[40]. The distance of the system i.e. $r$ compared to that of $R_0$ value in the present study also reveals the presence of static type quenching mechanism to a larger extent[41]. With the temperatures increasing, the distance between BSA and CFS has a little change, indicating temperature has little effect on the non-radiative energy transfer. Comparing the results in TABLE 4, it shows the distance ($r$) of synchronous fluorescence spectroscopy are similar with the resultes obtained by fluorescence quenching spectroscopy, and energy transfer theory obtained by two methods are consistent. This implies synchronous fluorescence spectroscopy can

be used to study the energy transfer theory of the interaction.

## CONCLUSIONS

In this paper, the interaction between BSA and CFS was investigated by synchronous fluorescence spectroscopy at pH7.40. Studying the mechanism of the small molecule and protein, it is universal to calculate $K_{sv}$, $K_q$, $K_a$, $n$, thermodynamic parameters ($\Delta H$、$\Delta S$、$\Delta G$), $r$ (the distance between acceptor and donor). The experimental data obtained by synchronous fluorescence method are used to calculate the above experimental parameters, and compares with the results obtained by fluorescence quenching method which is generally accepted. It shows that the quenching mechanism, binding forces, energy transfer theory of two methods are similar. This indicates synchronous fluorescence spectrometry could be used to study the interaction between drugs and proteins. In addition, synchronous spectroscopy showed that the conformation of BSA was changed in the presence of CFS.

## ACKNOWLEDGEMENTS

The authors gratefully acknowledge the financial support of National Science Foundation of China (Grant no. 21375032).

## REFERENCES

**[1]** H.X.Yang, Q.C.Liu, L.Zhao, Y.Yuan, D.D.Fan, J.J.Deng, R.J.Zhang; J Solution Chem., **43**, 774 **(2014)**.
**[2]** C.M.Dai, C.W.Ji, H.X.Lan, Y.Z.Song, W.Yang, D.Zheng; Toxicol Phar., **37,** 870 **(2014)**.
**[3]** A.Chaudhury; Indian J Med Microbi., **21**, 52 **(2003)**.
**[4]** P.Zalewski, R.Skibinski, J.Cielecka-Piontek; J Pharmaceut Biomed., **92,** 22 **(2014).**
**[5]** M.Bergeret, N.Boutros, J.Raymond; Int J Antimicrob Ag., **23,** 247 **(2004)**.
**[6]** A.Kathiravan, M.Chandramohan, R.Renganathan, S.Sekar; J.Mol.Struct., 919, 210 **(2009)**.
**[7]** J.B.F.Lloyd; Nature Phys Sci., **231**, 64 **(1971)**.
**[8]** K.Kumar, P.Bairi, K.Ghosh, K.K.Mishra, A.K.Mishra; Curr Sci India., **107**, 470 **(2014)**.
**[9]** A.Dankowska, M.Małecka, W.Kowalewski; Grasas Aceites., **64**, 425 **(2013)**.
**[10]** L.Ziak, P.Majek, K.Hrobonova, F.Cacho, J.Sadecka; Food Chem., **159, 282 (2014).**
**[11]** F.Mabood, R.Boqué, R.Folcarelli, O.Busto, A.Al-Harrasi, J.Hussain; Spectrochim Acta A, **143**, 298 **(2015)**.
**[12]** J.A.M.Pulgarin, L.F.G.Bermejo, S.B.Rodriguez; RSC Adv., **4**, 5671 **(2014)**.
**[13]** Y.Z.Zhang, B.Zhou, Y.X.Liu, C.X.Zhou, X.L.Ding, Y.Liu; J Fluoresc., **18**, 109 **(2008)**.
**[14]** M.Y.Hu, X.Wang, H.Wang, Y.Chai, Y.He, G.W.Song; Luminescence, **27**, 204 **(2012)**.
**[15]** B.Kaboudin, K.Moradi, M.R.Faghihi, F.Mohammadi; J Lumin., **139,** 104 **(2013).**
**[16]** Y.Shu, W.W.Xue, X.Y.Xu, Z.M.Jia, X.J.Yao, S.W.Liu, L.H.Liu; Food Chem., **173,** 31 **(2015).**
**[17]** Y.Q.Wang, H.M.Zhang, G.C.Zhang, W.H.Tao, S.H.Tang; J Lumin., **126,** 211 **(2007).**
**[18]** S.Y.Bi, B.Pang, T.J.Wang, T.T.Zhao, W.Yu; Spectrochim Acta A, **120**, 456 **(2014)**.
**[19]** S.Y.Bi, L.L.Yan, Y.T.Sun, H.Q.Zhang; Spectrochim Acta A, **78**, 410 **(2011)**.
**[20]** M.M.Zhao, R.R.Tang, S.Xu; Spectrochim Acta A, **135**, 953 **(2015)**.
**[21]** Y.J.Hu, H.L.Yue, X.L.Li, S.S.Zhang, E.Tang, L.P.Zhang; J Photoch Photobio B, **112,** 16 **(2012).**
**[22]** X.N.Yan, B.S.Liu, B.H.Chong, S.N.Cao; J Lumin., **142,** 155 **(2013).**
**[23]** S.N.Cao, B.S.Liu, Z.Y.Li, B.H.Chong; J Lumin., **145,** 94 **(2014).**
**[24]** D.D.Chen, Q.Wu, J.Wang, Q.Wang, H.Qiao; Spectrochim Acta A, **135**, 511 **(2015)**.
**[25]** X.Luo, C.R.Du, J.R.Wei, J.Deng, Y.J.Lin, C.W.Lin; Luminescence, **28**, 202 **(2013)**.
**[26]** S.Z.Hao, S.D.Liu, X.H.Wang, X.J.Cui, L.P.Guo; J Lumin., **129**, 1320 **(2009)**.
**[27]** S.C.Liu, J.Tang, X.H.Zhang, Y.Y.Gao, F.Ma, Q.Y.Yang; Spectroscopy-US, **27**, 19 **(2012)**.
**[28]** M.D.Meti, S.D.Gunagi, S.T.Nandibewoor, S.A.Chimatadar; Monatsh Chem., **144**, 1253 **(2013)**.
**[29]** H.L.Xu, N.N.Yao, G.Y.Li, Z.Q.Li; Spectrosc Lett., **47**, 119 **(2014)**.
**[30]** X.N.Zhao, Y.Liu, L.Y.Niu, C.P.Zhao; Spectrochim Acta A, **94**, 357 **(2012)**.
**[31]** Y.Q.Wang, H.M.Zhang, G.C.Zhang, Q.H.Zhou, Z.H.Fei, Z.T.Liu, Z.X.Li; J Mol Struct., **886,** 77 **(2008).**
**[32]** P.D.Ross, S.Subramanian; Biochemistry-US, **20**, 3096 **(1981)**.
**[33]** P.B.Kandagal, S.Ashoka, J.Seetharamappa,

S.M.T.Shaikh, Y.Jadegoud, O.B.Ijare; J Pharmaceut Biomed, **41**, 393 **(2006)**.

**[34]** J.N.Tian, J.Q.Liu, W.Y.He, Z.D.Hu, X.J.Yao, X.G.Chen; Biomacromolecules, **5**, 1956 **(2004)**.

**[35]** P.B.Kandagal, S.Ashoka, J.Seetharamappa, V.Vani, S.M.T.Shaikh; J Photoch Photobio A, **179,** 161 **(2006).**

**[36]** B.Kaboudin, K.Moradi, M.R.Faghihi, F.Mohammadi; J Lumin., **139**, 104 **(2013)**.

**[37]** S.Y.Cui, X.L.Hu, J.Q.Liu; J Solution Chem., **40**, 764 **(2011)**.

**[38]** X.L.Shi, X.W.Li, M.Y.Gui, H.Y.Zhou, R.J.Yang, H.Q.Zhang, Y.R.Jin; J Lumin., **130**, 637 **(2010)**.

**[39]** H.Lin, J.F.Lan, M.Guan, F.L.Sheng, H.X.Zhang; Spectrochim Acta A, **73**, 936 **(2009)**.

**[40]** X.Y.Yu, Z.X.Liao, Q.Yao, H.T.Liu, X.F.Li, P.G.Yi; Spectrochim Acta A, **118**, 331 **(2014)**.

**[41]** P.Mandal, M.Bardhan, T.Ganguly; J Photoch Photobio B**, 99**, 78 **(2010)**.