Comparative studies on the interaction of tartrazine with bovine serum albumin by fluorescence quenching spectroscopy and synchronous fluorescence spectroscopy

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ABSTRACT

Under simulated physiological conditions, the reaction mechanism between tartrazine (TTZ) and bovine serum albumin (BSA) at different temperatures (293 K, 303 K, 310 K) was investigated by fluorescence quenching method and synchronous fluorescence method, respectively. The results indicated that the fluorescence intensity and synchronous fluorescence intensity of BSA decreased regularly with the addition of TTZ, in addition, the quenching mechanism, binding constants ($K_a$), the number of binding site ($n$), type of interaction force and energy transfer parameters of TTZ with BSA obtained from two methods by the same equation were consistent, which indicated synchronous fluorescence spectrometry was a new method of studying the binding mechanism between drug and protein, and it was a useful supplement to the classical method.

INTRODUCTION

Serum albumin, the most abundant protein constituent in blood plasma, can be combined with a lot of endogenous and exogenous compounds and plays a fundamental role in the disposition and transportation of various molecules. Therefore, investigating the binding mechanism of endogenous or exogenous compounds and serum albumins has very significant value in life sciences, chemistry, pharmacy and clinical medicine. The classical fluorescence spectroscopy studies the reaction mechanism of small molecule drugs and proteins, mainly by studying the change of fluorescence intensity of protein in the maximum emission wavelength before and after adding the drugs, and derives the binding constants, binding sites and the donor-to-acceptor distance with other information between proteins and drugs[1-3]. Synchronous fluorescence spectrometry technology was first proposed by Lloyd[4], and the biggest difference between this and fluorescence measurement method is that the excitation and emission monochromators were scanned simultaneously. Comparing with classical fluorescence spectroscopy, synchronous fluorescence method has some advantages such as good selectivity, high sensitivity, less interference etc[5-6], and it can be used for the simultaneous determination of multi-com-
ponent mixture. However, studies on the binding con-
stant and binding sites between the protein and TTZ
with this method have not been reported.

Food colorants are a very important class of food
additives and may be natural or synthetic. Synthetic food
colorants are widely used due to their low price, high
effectiveness and excellent stability.[7] Tartrazine (TTZ)
(the structure shown in Figure 1) is common synthetic
food colorants, which is used in a wide variety of food
such as beverages, dairy products, cereals, snack foods
and ice creams. In this study, we study the binding
mechanism of drugs and proteins by utilizing classical
fluorescence quenching method and synchronous fluo-
rescence method with the research object being TTZ.
The data were obtained and analyzed. It shows that the
research of combination mechanism of proteins and
drugs has become more abundant with the new method
and it is a useful supplement to the application of fluo-
rescence spectroscopy in the field.

Figure 1 : Chemical structure of tartrazine

EXPERIMENTAL

Apparatus

All fluorescence spectra were recorded with a
Shimadzu RF-5301PC spectro- fluorophotometer. Ab-
sorption was measured with an UV-Vis recording spec-
trophotometer (UV-265 Shimadzu, Japan). All pH
measurements were made with a pH5-3C precision
acidity meter (Leici, Shanghai). All temperatures were
controlled by a CS501 super-heated water bath
(Nantong Science Instrument Factory).

Materials

Bovine serum albumin was purchased from Sigma
Company and of the purity grade inferior 99%.
Tartrazine (TTZ) was purchased from Aldrich (Wis-
consin, USA). Stock solutions of BSA (1.0 × 10^{-5} mol·L^{-1}),
and TTZ (1.0 × 10^{-4} mol·L^{-1}) were prepared. All the
stock solutions were further diluted as working solu-
tions prior to use. Tris-HCl buffer solution (0.05 mol·L^{-1}
of Tris, 0.15 mol·L^{-1} of NaCl) was used to keep the
pH of the solution at 7.40, and NaCl solution was used
to maintain the ionic strength of the solution. All other
reagents were analytical grade and all aqueous solu-
tions were prepared with newly double-distilled water
and stored at 277 K.

The fluorescence intensities were corrected for the
absorption of excitation light and re-absorption of emi-
ted light to decrease the inner filter effect using the fol-
lowing relationship[8]:
F_{cor} = F_{obs} \times e^{A_{ex} \times A_{em}^{1/2}} \quad (1)
Where, $F_{cor}$ and $F_{obs}$ are the corrected and observed
fluorescence intensities respectively. $A_{ex}$ and $A_{em}$ are the
absorbance values of TTZ at excitation and emission
wavelengths, respectively. The fluorescence intensity
used in this paper was corrected.

Procedures

Fluorescence measurements

In a typical fluorescence measurement, 1.0 mL of
Tris-HCl buffer, pH 7.4, 1.0 mL of 4.0 × 10^{-6} mol·L^{-1}
BSA solution and different concentrations of TTZ were
added into 10 mL colorimetric tube successively. The

Figure 2 : Fluorescence spectra of TTZ-BSA system ($\lambda_{ex}$ =280
nm, $T$ = 293 K); $C_{BSA} = 4.0 \times 10^{-7}$ mol·L^{-1}; $1$-$10$ $C_{TTZ} = (0, 0.4,
0.7, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0) \times 10^{-6}$ mol·L^{-1}
samples were diluted to scaled volume with water, mixed thoroughly by shaking, and kept static for 30 minutes at different temperatures (293, 303 and 310 K). Excitation wavelength for BSA was 280 nm (295 nm), with a 10 mm pathlength cell. The excitation and emission slits were set at 5 nm each. The solution was subsequently scanned on the fluorophotometer and recorded the fluorescent intensity at 340 nm.

**Synchronous fluorescence measurements**

The solution preparation was the same as in Section 2.3.1, we recorded the fluorescence spectra of BSA-TTZ system when the value between the excitation and emission wavelengths, \( \Delta \lambda \) was 15 and 60 nm.

**RESULT AND DISCUSSION**

**Fluorescence quenching spectra of BSA-TTZ system**

Proteins were considered to have intrinsic fluorescence due to the presence of amino acids, mainly tryptophan (Trp), tyrosine (Tyr). When the excitation wavelength was at 280 nm (or 295 nm), BSA has a strong fluorescence emission peak at 340 nm. The fluorescence spectra of BSA-TTZ system was shown in Figure 2. As shown in Figure 2, the fluorescence intensity of BSA decreased regularly with the addition of TTZ when the excitation wavelength is 280 nm (it was similar to 295 nm). This result showed that TTZ could quench the intrinsic fluorescence of BSA and there was an interaction between TTZ and BSA, it could also reveal that a new complex is being formed\(^9\).

In order to confirm the quenching mechanism, the fluorescence quenching data are analyzed by the Stern-Volmer equation\(^10\):

\[
\frac{F}{F_0} = 1 + K_{sv} \tau_f [L] = 1 + K_{sv} [L]
\]  
(2)

Where, \( F_0 \) and \( F \) represent the fluorescence intensities in the absence and in the presence of quencher, respectively. \( \tau_f \) is the average lifetime of fluorescence without quencher, which is about \( 10^{-8} \) s. \( K_{sv} \) is the Stern-Volmer quenching constant. \( K_q \) is the bimolecular quenching constant, and \( [L] \) is the concentration of the quencher. According to Eq. (2), based on the linear fit plot of \( F/F_0 \) versus \( [L] \), the \( K_{sv} \) values can be obtained. The calculated results were shown in TABLE 1. Different mechanisms of quenching are usually classified as the dynamic quenching and the static quenching. Dynamic and static quenching can be distinguished by their different dependence on temperature. The quenching rate constants decrease with the rising temperature for the static quenching, but the reverse effect is observed for the dynamic quenching\(^11\). In TABLE 1, the values of \( K_{sv} \) decreased with the rising temperature in all systems, which indicated that the probable quenching mechanism of the interaction between BSA and TTZ was initiated by complex formation rather than by dynamic collision\(^12\). In addition, all the values of \( K_q \) were much greater than the maximum scatter collision quenching constant of various quenchers (\( 2 \times 10^{10} \) L·mol\(^{-1}\)·s\(^{-1}\)), this also suggested that the quenching was a static process\(^13\).

For static quenching process, the relationship between the fluorescence intensity and the concentration of quencher can be usually described by Eq. (3)\(^14\) to obtain the binding constant (\( K_a \)) and the number of binging sites (\( n \)) in most paper:

\[
\log \left[ \frac{(F_0 - F)}{F_0} \right] = n \log K_a + n \log \left[ [D] - [B][F_0 - F]/F_0 \right]
\]  
(3)

Where, \( [D] \) and \( [B] \) are the total concentrations of TTZ and BSA, respectively. On the assumption that \( n \) in the bracket is equal to 1, the curve of log \( [(F_0 - F)/F] \) versus log \( [[D] - [B][F_0 - F]/F_0] \) is drawn and fitted linearly, then the value of \( n \) can be obtained from the slope of the plot. If the \( n \) value obtained is not equal to 1, then it is substituted into the bracket and the curve of log \( [(F_0 - F)/F] \) versus log \( [[D] - n[B][F_0 - F]/F_0] \) is drawn again. The above process was repeated again and again till \( n \) obtained was only a single value. Based on the \( n \) obtained the binding constant \( K_a \) can be also obtained. In the work, a calculation program was developed. The calculation process can be finished with calculator based on the above simple program and the calculating result can be obtained by inputting \( F, [D] \) and \( [B] \). The calculated result was shown in TABLE 1. As shown in TABLE 1, the fact that the values of \( n \) were all approximately to 1 implied that just one binding site for TTZ existed in BSA. Meanwhile, binding constants \( K_a \) were decreased with the rising temperature, further suggested that the quenching was a static process\(^15\).
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At 280 nm wavelength the Trp and Tyr residues in BSA are excited, whereas the 295 nm wavelength excites only Trp residues\(^{16}\). In BSA sub-hydrophobic domain, IIA (containing both Trp and Tyr) and IIIA (containing only Tyr) is the major binding site of small molecule ligands\(^{17}\). Based on the Stern-Volmer equation, comparing the fluorescence quenching of BSA excited at 280 nm and 295 nm allows to estimate the participation of Trp and Tyr groups in the system\(^{18}\). As seen in Figure 3, in the presence of TTZ, the quenching curves of BSA excited at 280 nm and 295 nm overlap approximately below the molar ratio of TTZ:BSA=10:1, which indicated that only Trp residues played an important role in the interaction of TTZ with BSA. The quenching of BSA fluorescence exited at 280 nm above this molar ratio was slightly higher than that excited at 295 nm which means that Tyr residues began to take part in the interaction. According to this conclusion, it could be inferred that TTZ molecules only take interaction with Trp residues of BSA at low concentration, whereas both Trp and Tyr residues at high concentration, but the interaction of Tyr with TTZ is weak in terms of Trp. From TABLE 1, in the same temperature the \(K_q\) values of BSA at 280 nm was slightly greater than 295 nm, this also suggested that only Trp residues played an important role in the interaction between TTZ and BSA.

Synchronous fluorescence spectra studies

Synchronous fluorescence spectra are used to investigate the protein conformational change, as it has been shown to give narrow and simple spectra\(^{19}\). When the \(\Delta \lambda\) value between the excitation and emission wave-lengths were stabilized at either 15 or 60 nm, the synchronous fluorescence gives characteristic information for tyrosine residues or tryptophan residues, respectively\(^{20}\). It can be seen from Figure 4, when the \(\Delta \lambda\) value was 15 nm, the fluorescence intensities of BSA-TTZ decreased not obviously with no shifts with increasing concentration of TTZ, this indicated that Tyr residues hardly played an important role in the interaction between TTZ and BSA. When the \(\Delta \lambda\) value was 60 nm the synchronous fluorescence intensities of TTZ-BSA decreased regularly with some shifts, which indicated that the interaction of BSA with TTZ changed the microenvironment of tryptophan residues\(^{21}\), which was coincident with the result of Section 3.2. The polarity of hydrophobic environment was enhanced and hydrophobicity was reduced in the BSA cavity due to the changes of tryptophan residues microenvironment with

### TABLE 1: Quenching reactive parameters of BSA and TTZ at different temperatures

<table>
<thead>
<tr>
<th>(\lambda_{ex}(\text{nm}))</th>
<th>(T/(\text{K}))</th>
<th>(K_q/(\text{L} \cdot \text{mol}^{-1} \cdot \text{s}^{-1}))</th>
<th>(K_a/(\text{L} \cdot \text{mol}^{-1}))</th>
<th>(r_1)</th>
<th>(K_a/(\text{L} \cdot \text{mol}^{-1}))</th>
<th>(n)</th>
<th>(r_2)</th>
</tr>
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<tr>
<td>293</td>
<td>280</td>
<td>1.47×10(^{13})</td>
<td>1.47×10(^5)</td>
<td>0.992</td>
<td>1.66×10(^5)</td>
<td>0.89</td>
<td>0.991</td>
</tr>
<tr>
<td></td>
<td>303</td>
<td>1.25×10(^{13})</td>
<td>1.25×10(^5)</td>
<td>0.998</td>
<td>1.33×10(^5)</td>
<td>0.85</td>
<td>0.998</td>
</tr>
<tr>
<td></td>
<td>310</td>
<td>1.20×10(^{13})</td>
<td>1.20×10(^5)</td>
<td>0.998</td>
<td>1.26×10(^5)</td>
<td>0.93</td>
<td>0.998</td>
</tr>
<tr>
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<td>280</td>
<td>1.46×10(^{13})</td>
<td>1.46×10(^5)</td>
<td>0.990</td>
<td>1.20×10(^5)</td>
<td>0.78</td>
<td>0.992</td>
</tr>
<tr>
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<td>303</td>
<td>1.07×10(^{13})</td>
<td>1.07×10(^5)</td>
<td>0.994</td>
<td>1.16×10(^5)</td>
<td>0.86</td>
<td>0.996</td>
</tr>
<tr>
<td></td>
<td>310</td>
<td>1.03×10(^{13})</td>
<td>1.03×10(^5)</td>
<td>0.997</td>
<td>1.08×10(^5)</td>
<td>0.86</td>
<td>0.997</td>
</tr>
</tbody>
</table>

\(K_q\) is the quenching rate constant; \(K_a\) is the binding constant; \(n\) is the number of binding site. \(r_1\) is the linear relative coefficient of \(F_0/F \sim [L]\); \(r_2\) is the linear relative coefficient of \(\log([F_0-F]/F) - \log([D] \cdot [B] \cdot [F_0-F]/F_0)\).
Figure 4: Fluorescence spectrum of TTZ-BSA system ($T = 293\ \text{K}$) (A) $\Delta \lambda = 15\ \text{nm}$; (B) $\Delta \lambda = 60\ \text{nm}$. $C_{\text{BSA}} = 4.0 \times 10^{-7}\ \text{mol} \cdot \text{L}^{-1}$; $1 \sim 10$ $C_{\text{TTZ}} = (0, 0.4, 0.7, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0) \times 10^{-6}\ \text{mol} \cdot \text{L}^{-1}$.

TABLE 2: Quenching reactive parameters of TTZ and BSA at different temperatures ($\Delta \lambda = 60\ \text{nm}$)

<table>
<thead>
<tr>
<th>$\Delta \lambda$ (nm)</th>
<th>$T$/K</th>
<th>$K_q$/(L·mol$^{-1}$·s$^{-1}$)</th>
<th>$K_a$/(L·mol$^{-1}$)</th>
<th>$r_3$</th>
<th>$K_s$/(L·mol$^{-1}$)</th>
<th>$n$</th>
<th>$r_4$</th>
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<td>293</td>
<td>$4.59 \times 10^{12}$</td>
<td>$4.59 \times 10^{4}$</td>
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<td>$4.24 \times 10^{4}$</td>
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<td>0.993</td>
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<td>$4.29 \times 10^{12}$</td>
<td>$4.29 \times 10^{4}$</td>
<td>0.998</td>
<td>$3.33 \times 10^{4}$</td>
<td>0.91</td>
<td>0.991</td>
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<tr>
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<td>310</td>
<td>$4.02 \times 10^{12}$</td>
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<td>$3.29 \times 10^{4}$</td>
<td>0.80</td>
<td>0.992</td>
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<td>$1.84 \times 10^{5}$</td>
<td>0.992</td>
<td>$1.39 \times 10^{5}$</td>
<td>0.74</td>
<td>0.997</td>
</tr>
<tr>
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<td>$1.31 \times 10^{13}$</td>
<td>$1.31 \times 10^{5}$</td>
<td>0.997</td>
<td>$1.34 \times 10^{5}$</td>
<td>0.85</td>
<td>0.995</td>
</tr>
<tr>
<td></td>
<td>310</td>
<td>$1.25 \times 10^{13}$</td>
<td>$1.25 \times 10^{5}$</td>
<td>0.997</td>
<td>$1.33 \times 10^{5}$</td>
<td>0.86</td>
<td>0.997</td>
</tr>
</tbody>
</table>

$K_q$ is the quenching rate constant; $K_a$ is the binding constant; $n$ is the number of binding site. $r_3$ is the linear relative coefficient of $F_0/F - [L]$; $r_4$ is the linear relative coefficient of log[$(F_0 - F)/F_0$] - log([$D$] - $n[B] (F_0 - F)/F_0$).

The insertion of TTZ\cite{22}, leading to the conformation change of BSA. Protein molecules were extended by high concentrations of drugs, which reduced the energy transfer among the amino acid residues and their fluorescence intensity. For the $\Delta \lambda = 15\ \text{nm}$ and $\Delta \lambda = 60\ \text{nm}$, according to Eqs. (2)(3), the corresponding results are shown in TABLE 2. From TABLE 2, it can be seen the values of $K_a$ decreased with the rising temperature in all systems, which indicated that the probable quenching mechanism of the interaction between BSA and TTZ was a static process. The $K_q$ was the order of $10^{12}\ \text{L} \cdot \text{mol}^{-1} \cdot \text{s}^{-1}$. Obviously, the $K_q$ value of protein quenching procedure initiated by TTZ was greater than $2 \times 10^{10}\ \text{L} \cdot \text{mol}^{-1} \cdot \text{s}^{-1}$, this indicated that the quenching was not initiated from dynamic collision but from the formation of a complex. The $n$ value approaches unity, suggesting that one molecule of TTZ combines with one molecule of BSA; the decreasing trend of $K_a$ with the increasing temperature was in accordance with binding constants dependence on the temperature as mentioned above, which indicated that TTZ-BSA would be partly decomposed when the temperature rising, it also indicated it was a static quenching. The quenching mechanism obtained by synchronous fluorescence method was coincident with the one obtained by fluorescence method. Comparing TABLE 1 and TABLE 2, we can know that the quenching parameters obtained by two methods have the same order of magnitude. However, in TABLE 2 the quenching parameters of $\Delta \lambda = 15\ \text{nm}$ are significantly smaller than the ones of $\Delta \lambda = 60\ \text{nm}$, this also suggested that only Trp residues played an important role in the interaction between TTZ and BSA, which was coincident with the result of Section 3.2.

Type of interaction force of BSA-TTZ systems
Generally, the interaction force between the small drug molecule and biological macromolecule include hydrogen bond, Van der Waals force, electrostatic interactions and hydrophobic force, etc. Ross and Subramanian[23] have characterized the sign and magnitude of the thermodynamic parameter, enthalpy change ($\Delta H$), free energy ($\Delta G$) and entropy change ($\Delta S$) of reaction, associated with various individual kinds of interaction. When temperature varies in a small range, the $\Delta H$ could be considered as a constant[24]. Negative $\Delta H$ and positive $\Delta S$ indicate electrostatic interaction plays a major role in the binding reaction. Positive $\Delta H$ and $\Delta S$ are generally considered as the evidence for typical hydrophobic interactions. In addition, Van der Waals force and hydrogen bonding formation in low dielectric media are characterized by negative $\Delta H$ and $\Delta S$. The thermodynamic parameters can be calculated on the basis of the following equation[25]:

$$\frac{T}{K} \ln R = \Delta S - \Delta H / T$$ (4)

$$\Delta G = \Delta H - T \Delta S = -RT \ln K$$ (5)

According to the relevant thermodynamic parameters of small molecule drugs and biological macromolecules, the type of interaction force can be simply judged. According to Eq. (4), based on the linear fit plot of $\ln K$ versus $1/T$, $\Delta H$ values can be obtained and then $\Delta S$ values can be obtained at each temperature from the $K$ values and $\Delta H$ value. According to Eq. (5), the $\Delta G$ values can be obtained at each temperature from the $K$ values. Therefore, the values of $\Delta H$, $S$ and $\Delta G$ were listed in TABLE 3. From TABLE 3, it can be seen that the reaction process of TTZ with BSA was a spontaneous molecular interaction procedure in which entropy increased and Gibbs free energy decreased[26]. The positive value of $\Delta S$ and $\Delta H$ showed that the electrostatic interaction play a major role in the binding process[27]. The conclusions of the synchronous fluorescence method were consistent with fluorescence method, further suggested that synchronous fluorescence spectrometry was a new method of studying the binding mechanism between drug and protein.

**Energy transfer from BSA to TTZ**

According to the Förster nonradioactive resonance energy transfer theory[28] the effective energy transfer from donor to acceptor will occur when two molecules meet the following preconditions: (1) donor is a fluorophore; (2) the overlap is enough between the fluorescence emission spectrum of the donor and UV-vis absorption spectrum of the acceptor; (3) the distance between the donor and the acceptor is within 2-7 nm. The energy transfer effect is not only related to the distance between the donor (tryptophan residue) and acceptor, but also influenced by the critical energy transfer distance $R_0$. It is described by the following equations:[29-30]

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

$$R_0^{-6} = 8.78 \times 10^{-20} \text{K}^2 \Phi N J$$ (7)

Where, $R_0$ is the critical distance when the transfer efficiency is 50%, $r$ is the distance between the acceptor and the donor, and $E$ is the energy transfer efficiency. $K$ is the spatial orientation factor of the dipole, $N$ is refractive index of the medium, $\Phi$ is the fluorescence quantum yield of the donor, and the overlap integral ($J$) between the fluorescence emission spectrum of the donor and the absorption spectrum of the acceptor can be

<table>
<thead>
<tr>
<th>$T$ (K)</th>
<th>$K_a$ (L·mol⁻¹)</th>
<th>$\Delta H$ (KJ·mol⁻¹)</th>
<th>$\Delta S$ (J·mol⁻¹·K⁻¹)</th>
<th>$\Delta G$ (KJ·mol⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>293</td>
<td>1.66×10⁵</td>
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<td>57.00</td>
<td>-29.28</td>
</tr>
<tr>
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<td>1.33×10⁵</td>
<td></td>
<td>56.57</td>
<td>-29.72</td>
</tr>
<tr>
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<td>81.83</td>
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<td></td>
<td>91.58</td>
<td>-30.41</td>
</tr>
</tbody>
</table>

**TABLE 3 : The thermodynamic parameters of TTZ-BSA at different temperatures**
calculated by the equation[29]:

$$J = \sum F(\lambda) \varepsilon(\lambda) \lambda' \Delta \lambda / \sum F(\lambda) \Delta \lambda.$$  \hspace{1cm} (8)

Where, $F(\lambda)$ is the fluorescence intensity of the fluorescent donor at some wavelength, $\varepsilon(\lambda)$ is the molar absorbance of the acceptor at the wavelength $\lambda$. Figure 5 presents the overlap integral of the fluorescence emission spectrum of BSA and the absorption spectrum of TTZ. In the present case, $K^2=2/3$, $N=1.336$ and $\Phi=0.118$[31], according to Eqs. (6)-(8), the corresponding results were shown in TABLE 4. From TABLE 4, it can be seen that the donor-to-acceptor distance $r<7$ nm indicated that the energy transfer from BSA to TTZ occurred with high possibility[32], the distance $r$ increased and the energy efficiency $E$ decreased with increasing temperature (TABLE 4), which resulted in the reduced stability of the binary systems and the values of $K_a$. Moreover, the value of $r$ was greater than $R_0$ in this study which suggested that TTZ could quench the intrinsic fluorescence of BSA by a static quenching mechanism[33]. In addition, from TABLE 4, the data obtained by the two methods were basically consistent, and the conclusions also are the same, which indicated that synchronous fluorescence spectrometry was a new method of studying the binding mechanism between drug and protein, and it was a useful supplement to the classical method.

CONCLUSIONS

In this paper, the binding of TTZ to BSA under simulated physiological conditions was studied by classical fluorescence quenching method and synchronous fluorescence method, which were used the same equation for processing data. From all data, we could see data obtained by both methods were in the same order of magnitude and very close, quenching mechanism and type of interaction force were consistent, which indicated synchronous fluorescence spectrometry was a new method of studying the binding mechanism between drug and protein, and it was a useful supplement to the classical method. In addition, synchronous fluorescence method has some advantages such as good selectivity, high sensitivity, less interference etc, which makes synchronous fluorescence method have more advantages than classical fluorescence quenching method to study the reaction mechanism of drugs with proteins.

ACKNOWLEDGEMENTS

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