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## Study on Interaction of cefoperazone sodium with ofloxacin in the presence of BSA by fluorescence spectroscopy

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### ABSTRACT

Interaction mechanism of cefoperazone sodium (CFP) and ofloxacin (OFX) with bovine serum albumin (BSA) at different temperatures by fluorescence spectrometry method was studied. Results showed that CFP or OFX could quench the fluorescence of BSA. The fluorescence would quench to a larger degree when OFX (or CFP) was added to the system of BSA-CFP (or BSA-OFX). The quenching mechanism of the combination for BSA and drugs was a static procedure. The number of binding sites is 1 in various systems. The values of  $n_H$  were approximately equal to 1. It was proved that the interaction between CFP and OFX simultaneously bound to BSA was weaker and almost no cooperativeness occurred. The site markers competitive experiments indicated that the binding of CFP and OFX to BSA primarily took place in site I. Thermodynamic parameters were also used to identify the force type of drugs with BSA.

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### KEYWORDS

Fluorescence spectrum;  
Cefoperazone sodium;  
Ofloxacin;  
Bovine serum albumin;  
Interaction.

### INTRODUCTION

Serum albumins are the most abundant proteins in the circulatory system of a wide variety of organisms, being the major macromolecule contributing to the osmotic blood pressure. In addition to blood plasma, serum albumins are also found in tissues and bodily secretions throughout the body; the extravascular protein comprises 60% of the total albumin<sup>[1]</sup>. Serum albumin serves as a transport and depot protein for numerous endogenous and exogenous compounds. As the pharmacokinetics and clinical pharmacology are developing rapidly, people have better understandings on the

influences of binding of drugs and protein on pharmacokinetics. Thus, research on the interactions between drugs and BSA is very important not only to clarify the transportation and metabolism process, but also in illustrating the mechanism, pharmacokinetics and toxicity of drugs. In the past decades, researches on the interaction between drugs and BSA using fluorescence method have been intensively reported<sup>[2-3]</sup>. However, most of those researches are of single drug with BSA only. However, people are usually using two or more kinds of drugs in clinical practice, so the existence of the interaction between drugs have inevitable.

Bovine (BSA) and human (HSA) serum albumin

## Regular Paper

tertiary structure is similar in 76%. In this work, bovine serum albumin (BSA) is selected as our protein model because of its medically important, low cost, ready availability, unusual ligand-binding properties<sup>[4]</sup>, and the results of all the studies are consistent with the fact that human and bovine serum albumins are homologous proteins. Crystal structure of BSA is a heart-shaped helical monomer composed of three homologous domains named I, II and III and each domain includes two sub-domains called A and B to form a cylinder<sup>[5]</sup>. The principal regions of ligand-binding sites on albumin are located in hydrophobic cavities in sub-domains IIA and IIIA, which exhibit similar chemical properties<sup>[6]</sup>. These two binding cavities are also referred to sites I, II and III (site I in sub-domain IIA, sites II and III in sub-domain IIIA)<sup>[7]</sup>. In order to identify the binding site on BSA, site marker competitive experiments were carried out, using drug which specially bind to a known site or region on BSA. From X-ray crystallography studies, warfarin<sup>[8]</sup> has been demonstrated to bind to the sub-domain IIA while ibuprofen<sup>[9]</sup> and digoxin<sup>[10]</sup> is considered as IIIA binder site II and site III, respectively. Then information about the PS-BSA binding site can be gained by monitoring the changes in fluorescence of PS bound BSA that brought about by site I (WF), site II (IB) and site III (DG) markers.

Cefoperazone sodium (CFP) is a semi-synthetic 3rd generation cephalosporin effective against a wide range of aerobic and anaerobic gram-positive and gram-negative bacteria<sup>[11]</sup>. Ofloxacin (OFX) belongs to the third generation synthetics of quinolones against bacteria<sup>[12]</sup>. The investigation of CFP and OFX simultaneously binding with BSA was performed by fluorescence spectra, and the cooperativity between drugs was quantitatively investigated by Hill's coefficients, to provide a theoretical basis for research of drugs pharmacology, combination therapy and rational usage of drugs.

## EXPERIMENTAL

### Apparatus

All fluorescence spectra were recorded using Shimadzu RF-540 spectrofluorophotometer and Hitachi F-4500 spectrofluorophotometer. Absorption was measured with an UV-vis recording spectrophotom-

eter (UV-265 Shimadzu Japan). All pH measurements were done with a pHs-3C precision acidity meter (Leici, Shanghai). All temperatures were controlled by CS501 super-heated water bath (Nantong Science Instrument Factory).

### Reagents

BSA was purchased from Sigma Company (no less than 99% pure). Cefoperazone Sodium (CFP) and ofloxacin (OFX) which were obtained from Chinese Institute of Drug and Biological Products. Stock solutions of BSA (100  $\mu\text{mol/L}$ ), CFP (5.0 mmol/L) and OFX (5.0 mmol/L) were prepared. The stock solutions were further diluted as working solutions prior to use. Tris-HCl buffer (50 mmol/L, pH=7.40), which contains 0.2 mol/L NaCl solution, was prepared. All other reagents were of analytical reagent grade and all aqueous solutions were prepared with newly double-distilled water and stored at 277 K.

Under some conditions the fluoresced light is absorbed by quenching groups on neighboring substrates or cleaved product molecules so that only a fraction of the fluoresced light impinges upon the detector system of the fluorometer. This phenomenon is known as the inner filter effect. The fluorescence intensities were corrected for absorption of exciting light and reabsorption of the emitted light to decrease the inner filter effect using the relationship<sup>[13]</sup>

$$F_{\text{cor}} = F_{\text{obs}} \times e^{(A_{\text{ex}} + A_{\text{em}})/2} \quad (1)$$

Where  $F_{\text{cor}}$  and  $F_{\text{obs}}$  are the fluorescence intensities corrected and observed, respectively.  $A_{\text{ex}}$  and  $A_{\text{em}}$  are the absorption of the system at the excitation and the emission wavelength, respectively. The intensity of fluorescence used in this paper was the corrected fluorescence intensity.

### The effect of BSA with single drug (BSA- CPF or OFX)

At 293, 303 and 308 K, 1.0 mL 10  $\mu\text{mol/L}$  BSA aqueous solution was added into a 10.0 mL colorimetric tube, followed by 1.0 mL Tris-HCl buffer solution and a certain amount of 200  $\mu\text{mol/L}$  CFP or OFX aqueous solution. The mixture was diluted to 10.0 mL with distilled water and then shaken harmoniously. After a 20 min lay-aside at experiment temperatures, fluorescence measurements were carried out at 344 nm keeping

the excitation wavelength at 286 nm. Both excitation and emission slits were at 10 nm. Meanwhile, make use of the fluorescence intensity of BSA without any drugs as  $F_0$ , to study the quenching effects of single drug to BSA.

### The interaction of OFX to CFP

At 293, 303 and 308 K, 1.0 mL 10  $\mu\text{mol/L}$  BSA aqueous solution was added into a 10.0 mL colorimetric tube, followed by 1.0 mL Tris-HCl buffer solution, 0.5 mL 200  $\mu\text{mol/L}$  OFX aqueous solution and a certain amount of 200  $\mu\text{mol/L}$  CFP aqueous solution. The mixture was diluted to 10.0 mL with distilled water and then shaken harmoniously. After a 20 min lay-aside at experiment temperatures, fluorescence measurements were carried out at 344 nm keeping the excitation wavelength at 286 nm. Both excitation and emission slits were at 10 nm. Meanwhile, make use of the fluorescence intensity of BSA-OFX without CFP as  $F_0$ , to study the quenching effects of CFP to BSA in the presence of OFX. Then, fluorescence spectra were measured as previously described with different concentration of OFX, to discuss the influence of their concentration on drugs' binding.

### The interaction of CFP to OFX

At 293, 303 and 308 K, 1.0 mL 10  $\mu\text{mol/L}$  BSA aqueous solution was added into a 10.0 mL colorimetric tube, followed by 1.0 mL Tris-HCl buffer solution, 0.5 mL 200  $\mu\text{mol/L}$  CFP aqueous solution and a certain amount of 200  $\mu\text{mol/L}$  OFX aqueous solution. The mixture was diluted to 10.0 mL with distilled water and then shaken harmoniously. After a 20 min lay-aside at experiment temperatures, fluorescence measurements were carried out at 344 nm keeping the excitation wavelength at 286 nm. Both excitation and emission slits were at 10 nm. Meanwhile, make use of the fluorescence intensity of BSA-CFP without OFX as  $F_0$ , to study the quenching effects of OFX to BSA in the presence of CFP. Then, fluorescence spectra were measured as previously described with different concentration of CFP, to discuss the influence of their concentration on drugs' binding.

### Site marker competitive experiments

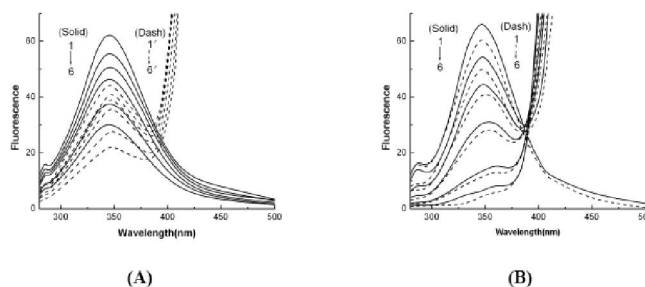
1.0 mL 10  $\mu\text{mol/L}$  BSA aqueous solution was added into a 10.0 mL colorimetric tube, followed by

1.0 mL Tris-HCl buffer solution, 1.0 mL 10  $\mu\text{mol/L}$  site marker I(WF), II(IB) and III(DG) for different series and a certain amount of 200  $\mu\text{mol/L}$  CFP or OFX aqueous solution. The mixture was diluted to 10.0 mL with distilled water and then shaken harmoniously. After a 20 min lay-aside at experiment temperatures (298 K), fluorescence measurements were carried out.

## RESULTS AND DISCUSSION

### Fluorescence quenching of BSA and drugs

Proteins are considered to have intrinsic fluorescence due to the presence of amino acids, mainly tryptophan, tyrosine and phenylalanine. Fluorescence quenching is the decrease of the quantum yield of fluorescence from a fluorophore induced by a variety of molecular interactions. In order to find out if the interaction between a drug and serum albumin changes in the presence of the second drug, we analyzed the quenching of serum albumin by one and then by two drugs. The fluorescence emission spectra of BSA in the absence and presence of drugs are shown in Figure 1. As shown in Figure 1, with the excitation wavelength at 286 nm, the maximum emission wavelength of BSA was 344 nm. The relative fluorescence intensity of BSA decreased with increasing concentration of drugs. These data indicates that CFP and OFX can interact with BSA and quench its intrinsic fluorescence<sup>[14]</sup>.



**Figure 1** • Quenching fluorescence spectra of BSA-OFX • ABSA-CFP-OFX (A), BSA-CFP • ABSA -OFX-CFP (B) at 298K

(A): BSA-CFP (Solid line),  $C_{\text{BSA}}=1.0 \mu\text{mol/L}$ , 1~6:  $C_{\text{CFP}}/(\mu\text{mol/L})$ : 0, 5, 10, 20, 40, 60 respectively

BSA-OFX-CFP (Dash line),  $C_{\text{BSA}}=1.0 \mu\text{mol/L}$ ,  $C_{\text{OFX}}=10 \mu\text{mol/L}$ , 1~6':  $C_{\text{CFP}}/(\mu\text{mol/L})$ : 0, 5, 10, 20, 40, 60 respectively

(B): BSA-OFX (Solid line),  $C_{\text{BSA}}=1.0 \mu\text{mol/L}$ , 1~6:  $C_{\text{OFX}}/(\mu\text{mol/L})$ : 0, 5, 10, 20, 40, 60 respectively

BSA-CFP-OFX (Dash line),  $C_{\text{BSA}}=1.0 \mu\text{mol/L}$ ,  $C_{\text{CFP}}=10 \mu\text{mol/L}$ , 1~6':  $C_{\text{OFX}}/(\mu\text{mol/L})$ : 0, 5, 10, 20, 40, 60 respectively

## Regular Paper

### Fluorescence quenching mechanism of BSA

Fluorescence quenching can occur by different mechanisms, which are usually classified as static and dynamic quenching. The mechanism can be distinguished from the differing dependence on temperature and viscosity on the Stern–Volmer constant values. Dynamic quenching depends upon diffusion. Since higher temperatures results larger diffusion coefficients, the bimolecular quenching constants are expected to increase with increasing temperature. In contrast, increased temperature is likely to result in decreased stability of complexes, and thus lower values of the static quenching constants<sup>[15]</sup>. In order to confirm the quenching mechanism, calculations are performed as follows: according to dynamic quenching, the mechanism can be described by the Stern-Volmer equation<sup>[16]</sup>:

$$F_0 / F = 1 + K_q \tau_0 [C] = 1 + K_{sv} [C] \quad (2)$$

$F_0$  and  $F$  are the fluorescence intensities in the absence and presence of quencher, respectively.  $K_q$  is the quenching rate constant of bio-molecule.  $K_{sv}$  is the dynamic quenching constant.  $\tau_0$  is the average lifetime of the bio-molecule without quencher, which is about  $10^{-8}$  s and  $[C]$  is the concentration of the quencher. In such an analysis, a plot of  $F_0/F$  versus  $[C]$  will give a straight line. By approximating the slope of quenching curve,  $K_q$  was obtained. The calculated results are shown in TABLE 1.  $K_q$  decreased with increasing temperature, suggesting that quenching is static quenching. For dynamic quenching, maximum scattering collision quenching constant of various quenchers with bio-molecules,  $K_{diff}$  is  $2.0 \times 10^{10} \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$ . Obviously, the rate constant of the protein quenching by drug  $K_q$  is much larger than the limiting diffusion coefficient  $K_{diff}$  of the bio-molecule. This means that the assumption is wrong and quenching is not initiated by dynamic collision, but static quenching by the formation of a complex compound<sup>[17]</sup>.

For static quenching, the relationship between the fluorescence intensity and the concentration of quenchers can be described by the following equation<sup>[18]</sup>:

$$F_0 / (F_0 - F) = 1/n + 1/(nK_A [C]) \quad (3)$$

Where  $F_0$  and  $F$  are the fluorescence intensities of BSA-drug in the absence and presence of drug, respectively, and  $[C]$  is the concentration of drug.  $n$  is the number of binding sites and  $K_A$  is the binding constant of BSA and drug. The slope and intercept were obtained from the  $F_0/(F_0 - F)$  versus  $[C]^{-1}$  curve. Therefore, we obtained the number of binding sites  $n$ , the binding constants  $K_A$  of BSA-drug and the linear relative coefficients  $r$  at different temperature (TABLE 2).

### The Hill's coefficient of the systems

In biochemistry, the binding of a ligand to a macromolecule often influences the affinity for other sites or ligands on the same macromolecule, this is known as cooperative binding. Hill's coefficient provides a way to quantify this effect and is calculated graphically on the basis of the following equation<sup>[19]</sup>:

$$\lg \frac{Y}{1-Y} = \lg K + n_H \lg [L] \quad (4)$$

where  $Y$  is the fractional binding saturation;  $K$  is the binding constant and  $n_H$  is the Hill's coefficient. If binding to one ligand molecule promotes the affinity for other ligand molecules with protein, the binding exhibits positive cooperativity. Hill's coefficient is greater than one. Conversely, if binding to one ligand molecule inhibits the binding to other ligand molecules on the protein, this is negative cooperativity ( $n_H < 1$ ). A coefficient of 1 indicates noncooperative reaction, in which the affinity of the protein for a ligand molecule is not dependent on whether or not other ligand molecules are already bound. Hill's coefficient in the binary system reflects the interaction for the same kind of drug binding at dif-

TABLE 1 : Stern-Volmer quenching rate constants of the systems of Drug-BSA at different temperatures

| System      | 298 K                                       |        | 303 K                                       |        | 308 K                                       |        |
|-------------|---|--------|---|--------|---|--------|
|             | $K_{q1}/(\text{L}/\text{mol}\cdot\text{s})$ | $r_1$  | $K_{q2}/(\text{L}/\text{mol}\cdot\text{s})$ | $r_2$  | $K_{q3}/(\text{L}/\text{mol}\cdot\text{s})$ | $r_3$  |
| BSA-CFP     | $1.38 \times 10^{12}$                       | 0.9950 | $1.37 \times 10^{12}$                       | 0.9989 | $1.35 \times 10^{12}$                       | 0.9972 |
| BSA-OFX     | $1.22 \times 10^{13}$                       | 0.9851 | $1.21 \times 10^{13}$                       | 0.9788 | $1.12 \times 10^{13}$                       | 0.9805 |
| BSA-OFX-CFP | $1.29 \times 10^{12}$                       | 0.9993 | $1.21 \times 10^{12}$                       | 0.9943 | $1.20 \times 10^{12}$                       | 0.9931 |
| BSA-CFP-OFX | $1.03 \times 10^{13}$                       | 0.9803 | $9.22 \times 10^{12}$                       | 0.9843 | $9.07 \times 10^{12}$                       | 0.9907 |

$r_1$ ,  $r_2$  and  $r_3$  are the linear relative coefficients at 298 K, 303 K and 308 K respectively.

The system of BSA-OFX-CFP was fixed  $C_{\text{OFX}} = 10 \mu\text{mol/L}$ ; and system of BSA-CFP-OFX was fixed  $C_{\text{CFP}} = 10 \mu\text{mol/L}$ .

ferent sites of protein, while in the ternary system it reflects the influence of one kind of drug binding on the affinity for the other kind of drug. For fluorescence measurement:

$$\frac{Y}{1-Y} = \frac{Q}{Q_m - Q} \quad (5)$$

where  $Q = (F_0 - F)/F_0$ ;  $1/Q_m$  = intercept of the plot  $1/Q$  vs.  $1/[L]$ . The binding constants of systems  $K_A$  were obtained according to Eq. (3) and presented in TABLE 2.

The results indicated: (1) The number of binding sites is 1 in various systems. (2) Compared with the BSA-CFP system, the binding constants of BSA-OFX-CFP system does not change significantly. Compared with the BSA-OFX system, the binding constants of BSA-CFP-OFX system does not change significantly. It indicated no cooperativeness in the drug's binding with BSA. (3) the values of  $n_H$  were approximately equal to 1 in the systems, which indicated no cooperativeness between CFP and OFX simultaneously bound to BSA.

## The type of interaction force between drug and BSA

Generally, there are hydrogen bond, Van Der Waals force, electrostatic attraction and hydrophobic interaction force when the pharmaceutical mini-molecule binds with biomacromolecule<sup>[20]</sup>. If the temperature does not vary significantly, the enthalpy change ( $\Delta H$ ) and entropy change ( $\Delta S$ ) can be regarded as constants. In order to elucidate the interaction between drug and BSA, the thermodynamic parameters were calculated from the Eq. (6) and (7)<sup>[21]</sup>:

$$R \ln K = \Delta S - \Delta H / T \quad (6)$$

$$\Delta G = \Delta H - T\Delta S \quad (7)$$

where  $K$  is the binding constant at the corresponding temperature (TABLE 2) and  $R$  is the gas constant. The  $\Delta H$  and  $\Delta S$  were obtained from the slope and intercept of the linear van't Hoff plot based on  $\ln K$  versus  $1/T$ . The free energy change ( $\Delta G$ ) was estimated from Eq. (7). Therefore, the values of  $\Delta H$ ,  $\Delta S$  and  $\Delta G$

TABLE 2 : The binding constants, number of binding sites and Hill's coefficients of the systems of Drug-BSA at different temperatures

| Drug<br>/(10 <sup>-5</sup> mol/L) | 298 K       |                      |          |       | 303 K                |          |       | 308 K                |          |  |
|-----------------------------------|-------------|----------------------|----------|-------|----------------------|----------|-------|----------------------|----------|--|
|                                   | $n_1$       | $K_{A1}/(L/mol)$     | $n_{H1}$ | $n_2$ | $K_{A2}/(L/mol)$     | $n_{H2}$ | $n_3$ | $K_{A3}/(L/mol)$     | $n_{H3}$ |  |
| OFX/                              | BSA-OFX-CFP |                      |          |       |                      |          |       |                      |          |  |
| 0.0                               | 0.788       | 2.03×10 <sup>4</sup> | 1.04     | 0.849 | 1.79×10 <sup>4</sup> | 1.05     | 1.03  | 1.29×10 <sup>4</sup> | 1.06     |  |
| 0.5                               | 0.667       | 3.12×10 <sup>4</sup> | 1.06     | 1.32  | 8.25×10 <sup>3</sup> | 0.992    | 1.13  | 1.06×10 <sup>4</sup> | 1.10     |  |
| 1.0                               | 1.15        | 1.03×10 <sup>4</sup> | 0.991    | 0.660 | 2.44×10 <sup>4</sup> | 1.17     | 0.774 | 2.20×10 <sup>4</sup> | 1.14     |  |
| 2.0                               | 0.766       | 1.83×10 <sup>4</sup> | 1.05     | 0.919 | 1.53×10 <sup>4</sup> | 1.05     | 1.19  | 1.05×10 <sup>4</sup> | 0.967    |  |
| 3.0                               | 1.56        | 7.26×10 <sup>3</sup> | 0.931    | 1.55  | 6.13×10 <sup>3</sup> | 0.965    | 0.825 | 2.15×10 <sup>4</sup> | 1.05     |  |
| 4.0                               | 0.935       | 1.48×10 <sup>4</sup> | 1.12     | 1.23  | 8.07×10 <sup>3</sup> | 1.00     | 0.889 | 1.50×10 <sup>4</sup> | 1.17     |  |
| 5.0                               | 0.771       | 2.07×10 <sup>4</sup> | 1.01     | 0.922 | 1.22×10 <sup>4</sup> | 1.02     | 0.846 | 2.01×10 <sup>4</sup> | 0.948    |  |
| 6.0                               | 0.910       | 9.82×10 <sup>3</sup> | 1.02     | 0.749 | 2.48×10 <sup>4</sup> | 1.07     | 1.20  | 5.34×10 <sup>3</sup> | 1.14     |  |
| CFP /                             | BSA-CFP-OFX |                      |          |       |                      |          |       |                      |          |  |
| 0.0                               | 1.39        | 3.66×10 <sup>4</sup> | 0.92     | 1.35  | 3.48×10 <sup>4</sup> | 0.960    | 1.35  | 3.45×10 <sup>4</sup> | 0.936    |  |
| 0.5                               | 1.31        | 3.77×10 <sup>4</sup> | 0.983    | 1.34  | 3.31×10 <sup>4</sup> | 0.983    | 1.28  | 3.67×10 <sup>4</sup> | 1.01     |  |
| 1.0                               | 1.19        | 4.57×10 <sup>4</sup> | 1.06     | 1.40  | 3.06×10 <sup>4</sup> | 0.957    | 1.28  | 3.79×10 <sup>4</sup> | 0.992    |  |
| 2.0                               | 1.43        | 2.85×10 <sup>4</sup> | 0.983    | 1.31  | 3.40×10 <sup>4</sup> | 1.00     | 1.48  | 2.71×10 <sup>4</sup> | 0.931    |  |
| 3.0                               | 1.34        | 3.32×10 <sup>4</sup> | 0.987    | 1.20  | 4.12×10 <sup>4</sup> | 1.04     | 1.25  | 4.18×10 <sup>4</sup> | 1.05     |  |
| 4.0                               | 1.17        | 4.89×10 <sup>4</sup> | 1.06     | 1.33  | 3.48×10 <sup>4</sup> | 0.981    | 1.34  | 1.29×10 <sup>4</sup> | 0.938    |  |
| 5.0                               | 1.14        | 5.29×10 <sup>4</sup> | 1.09     | 1.61  | 2.27×10 <sup>4</sup> | 0.906    | 1.24  | 3.56×10 <sup>4</sup> | 1.02     |  |
| 6.0                               | 1.27        | 3.70×10 <sup>4</sup> | 1.02     | 1.36  | 2.97×10 <sup>4</sup> | 1.04     | 1.21  | 3.97×10 <sup>4</sup> | 1.03     |  |

$n_1$ ,  $n_2$  and  $n_3$  are the number of binding sites at 298, 303 and 308 K respectively.

$K_{A1}$ ,  $K_{A2}$  and  $K_{A3}$  are the binding constants at 298, 303 and 308 K respectively.

$n_{H1}$ ,  $n_{H2}$  and  $n_{H3}$  are the Hill's coefficients at 298, 303 and 308 K respectively.

## Regular Paper

**TABLE 3 : Thermodynamic parameters of binding for BSA and the drugs**

| System  | $\Delta H / (\text{kJ/mol})$ | $\Delta S / (\text{J/mol}\cdot\text{K})$ | $\Delta G_{298\text{K}} / (\text{kJ/mol})$ |
|---------|------------------------------|--|--|
| BSA-CFP | -34.51                       | -33.06                                   | -24.66                                     |
| BSA-OFX | -4.526                       | 72.12                                    | -26.02                                     |

were shown in TABLE 3.

The negative  $\Delta H$  and  $\Delta S$  values indicate that the binding power is mainly hydrogen bond and van der Waals; the positive  $\Delta H$  and  $\Delta S$  values generally represent hydrophobic interactions<sup>[22]</sup>. Moreover, a specific electrostatic interaction between ionic species in an aqueous solution is characterized by the positive  $\Delta S$  value and negative  $\Delta H$  value<sup>[23]</sup>. The results showed that: (1) The negative value of  $\Delta G$  clarified there was an automatic reaction happened between CFP(OFX) and BSA. (2) The negative value of  $\Delta H$  and negative value of  $\Delta S$  showed that hydrogen bond and van der Waals played an important role in the binding of CFP to BSA. The negative value of  $\Delta H$  and positive value of  $\Delta S$  showed that electrostatic attraction played an important role in the binding of OFX to BSA.

### Effect of site markers on the binding of drug to BSA

Binding constants determined based on Eq. (3) show the effect of WF, IB and DG on the BSA-CFP or BSA-OFX at 298 K (TABLE 4).

It can be obtained that binding constants for the ternary system (TABLE 4) are lower than that for the binary system of BSA-drug ( $K_{\text{BSA-CFP}}=2.03 \times 10^4 \text{ L}\cdot\text{mol}^{-1}$  •  $AK_{\text{BSA-OFX}}=3.66 \times 10^4 \text{ L}\cdot\text{mol}^{-1}$ ). It can be seen that the binding constant for the ternary system of BSA-WF-drug was of the most extensive change. It indicated that WF hinders the formation of BSA-drug and can compete for the same binding site from sub-domain IIA (site I).

**TABLE 4 : The binding constant of the systems of Drug-BSA with adding three specific sites**

| System  | WF    |   |        | IB    |   |        | DG    |   |        |
|---------|-------|---|--------|-------|---|--------|-------|---|--------|
|         | $n_1$ | $K_{A1} / (\text{L}\cdot\text{mol}^{-1})$ | $r_1$  | $n_2$ | $K_{A2} / (\text{L}\cdot\text{mol}^{-1})$ | $r_2$  | $n_3$ | $K_{A3} / (\text{L}\cdot\text{mol}^{-1})$ | $r_3$  |
| BSA-CFP | 0.954 | $9.96 \times 10^3$                        | 0.9974 | 0.847 | $1.28 \times 10^4$                        | 0.9976 | 1.02  | $1.65 \times 10^4$                        | 0.9950 |
| BSA-OFX | 1.11  | $2.64 \times 10^4$                        | 0.9995 | 1.37  | $3.37 \times 10^4$                        | 0.9999 | 1.46  | $2.86 \times 10^4$                        | 0.9963 |

$n_1, n_2$  and  $n_3$  are the number of binding sites with adding WF, IB and DG respectively.  
 $r_1, r_2$  and  $r_3$  are the linear relative coefficients with adding WF, IB and DG respectively.

## CONCLUSIONS

Using the quenching fluorescence method we studied the antibacterial drugs (CFP and OFX) binding to BSA respectively, as well as of the two drugs together to BSA. In addition, the cooperativity of drugs on BSA was quantitatively investigated by Hill's coefficients. The results suggested the quenching mechanism of the combination for BSA and drugs was a static procedure and the primary binding site for both CFP and OFX was located at site I in sub-domain IIA of BSA. The electrostatic interactions played a major role in the interaction of drugs with BSA. The values of  $n_H$  in the systems were approximately equal to 1, suggesting no cooperativeness for binding sites when drugs bound to BSA. Co-administration of CFP and OFX do not affect each other's transportation with serum albumin. This method is simple, rapid, and provides a theoretical basis for research of combination therapy.

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