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Interaction characteristics of lomefloxacin and/or cefazolin with bovine serum albumin

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ABSTRACT

In this paper, the interaction of lomefloxacin (LMF) and/or cefazolin (CFZ) with bovine serum albumin (BSA) was studied by fluorescence quenching in combination with UV-vis spectroscopic method under simulative physiological conditions. The fluorescence quenching constants, binding distance, and binding constants for BSA–LMX and/or CFZ systems were determined. The fluorescence quenching of BSA by addition of LMF and/or CFZ is due to static quenching and energy transfer. The ratio of binding constants (K_a) for BSA–LMF to BSA–CFZ equals to 22.41. In the presence of CFZ (LMF), the binding distance of BSA–LMF (BSA–CFZ) decreased from 4.44 to 2.45 nm (1.94 to 1.48 nm), and the binding constant (K_a) of BSA–LMF (BSA–CFZ) increased from 1.21×10^4 to 2.72×10^4 L mol^{-1} (5.40×10^2 to 1.20×10^3 L mol^{-1}). Two-coexisting CFZ and LMF may lead to the need for more doses to achieve therapeutic effect. Circular dichroism spectra, synchronous fluorescence, and three-dimensional fluorescence studies showed that the presence of LMF or CFZ could change the conformation of BSA during the binding process, and the presence of coexisting LMF and CFZ could change the conformation of BSA further, here LMF was reigning.

Key words: Bovine serum albumin, Lomefloxacin, Cefazolin, Fluorescence quenching, Interaction

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INTRODUCTION

Protein–drug binding greatly influences absorption, distribution, metabolism and excretion properties of typical drugs [1,2]. It is important to study the interaction of drug with serum albumins at molecular level. Cefazolin (CFZ) is administered parenterally and mainly used as prophylactic agent for surgical procedures in children and adults. Lomefloxacin (LMF) has wide antibiotic spectra, high activity, low side effect, and high clinical effect. Combination of CFZ and some fluoroquinolones was suggested to improve purpose for clinical treatment [3,4]. Protein binding has long been considered one of the most important physicochemical characteristics of drugs, playing a potential role in distribution, excretion and therapeutic effectiveness.

A few of protein binding studies of CFZ to bovine serum albumin (BSA) have been carried out by UV difference spectrophotometry [5], chemiluminescence method [6], and fluorescence method [7]. Effect of protein binding on the disposition of CFZ and cephalexin in a simultaneous perfusion system of rat liver and kidney was investigated [8]. Several works have studied the interaction of LMF and BSA using capillary electrophoresis [9,10], and fluorescence method [11,12]. Recently, the synergism effect of LMF and ofloxacin for BSA in solution was studied by spectroscopic techniques [13]. However, one of the major problems associated with measurement of fluorescent organic matter in natural samples is the inner-filter effect (IFE)[14]. To our knowledge, the effect of two-coexisting CFZ and LMF on pharmacodynamics is seldom studied for drug–serum albumin systems.

In this work, CFZ and LMF were selected as model drug because of their clinical compatibility. This study examined, for the first time, the interaction between BSA and two-coexisting CFZ and LMF under near

physiological conditions by the fluorescence quenching in combination with UV-vis spectroscopic method.

EXPERIMENTAL SECTION

Reagents and chemicals

Commercially available bovine serum albumin (BSA, catalog no. A-7030, purity: 98%, M×10^{-4} M) was prepared by dissolving an appropriate amount of BSA with 0.1 M Tris–HCl (pH 7.4) buffer solution, and kept in the dark at 4 ˚C. BSA working solutions were prepared by diluting the stock solution with water. Cefazolin sodium was purchased from the North China Pharmaceutical Co. Ltd (Shijiazhuang, China). A stock solution (1.0×10^{-3} M) of CFZ was prepared in water, and stored in refrigerator at –4 ˚C. Stock solution of LMF (1.0×10^{-3} M) was prepared by dissolving the standards with 67 mM phosphate buffer (pH 7.4, I = 0.17). Working solutions were obtained by dilution with the phosphate buffer from the corresponding stock solution. Tris–HCl buffer (pH 7.40) consists of Tris (0.1 M) and HCl (0.1 M), and NaCl solution (0.1 M) was used to maintain the ion strength. All chemicals were of analytical reagent grade or better. Purified water was prepared by an XGJ-30 highly pure water machine (Yongcheng purification Science & Technology Co. Ltd., Beijing, China).

Equipment

All fluorescence measurements were performed on an F-7000 Fluorescence spectrophotometer (Hitachi, Japan) which was equipped with a 1 cm quartz cell and thermostat bath. The spectrum data points were collected from 280 to 500 nm. The widths of the excitation and the emission slit were both set at 5 nm. Fluorescence measurements were carried out at room temperatures.

Circular dichroism (CD) spectra were obtained on a J-810 circular dichroism chiroptical spectrometer (JASCO Co. Ltd, Japan). The absorption spectra were performed on a TU-1900 double light Spectrophotometer (Beijing TAYASAF Science & Technology Co., Ltd, China) using a 1-cm quartz cell in the wavelength range of 250–450 nm. All pH measurements were performed with a pHS-3C pH meter (Shanghai, China).

Determination of fluorescence intensity

Five 10-ml clean and dried test tubes were taken, and 2 ml of 0.5 M NaCl, 2.0 ml Tris–HCl buffer (pH 7.40), 0.25 ml of 4.0×10^{-5} M BSA, and different volumes (0.5–2.5 ml) of LMF or CFZ standard solution of 1.0×10^{-4} M were added in each test tube, and diluted to the mark with water. The concentration of BSA was 1.0×10^{-6} M, and that of LMF or CFZ was 0.5, 1.0,1.5, 2.0, and 2.5×10^{-5} M. Otherwise, using same method BSA test solutions with CFZ or LMF (1.0×10^{-7} M) were prepared, which contain 0.5, 1.0, 1.5, 2.0, and 2.5×10^{-5} M for LMF or CFZ. Sixth test-tube containing only BSA solution at pH 7.4 was marked as ‘‘control’’. After mixing the solutions, these were allowed to stand for 15 min for maximum binding of LMF (and/or CFZ) to BSA. The fluorescence intensity after the correction of inner-filter effect was calculated by the equation [15]: $F_{cor}=F_{obs}$ exp (½A_{ex}+½A_{em}), where F_{obs} is fluorescence intensity measured before the correction of inner-filter effect, A_{ex} and A_{em} are absorbance of quencher in the test solution at excitation and emission wavelengths of BSA, respectively. The corrected fluorescence intensity was used for studying on the interaction of DEX-P and BSA. After corrected inner-filter effect, the fluorescence intensity (F_0) in the absence of quencher LMF (and/or CFZ) and the fluorescence intensity (F) in the presence of quencher LMF and/or CFZ were measured at a wavelength of λex 280 nm and λem 340 nm under temperature of 25˚C for estimating the interaction of LMF and/or CFZ with BSA.

RESULTS AND DISCUSSION

Fluorescence quenching mechanism

Fluorescence quenching refers to any process that decreases the fluorescence intensity of a sample. Figure 1 shows the fluorescence spectra of BSA in the absence and presence of LMF and/or CFZ after corrected inner-filter effect.

The fluorescence spectra of BSA show a broad band with maximum at ~340 nm. It is observed that the fluorescence intensity of BSA decreases with increasing concentration of LMF or CFZ. The quenching degree of LMF to fluorescence of BSA was higher than CFZ. Otherwise, the fluorescence spectra of LMF show a broad band with maximum at ~412 nm, its intensity increased with increasing concentration of LMF. The maximum fluorescence emission of BSA underwent spectral shift from 340 to 350 nm in the presence of LMF, and no spectral shift was observed in the presence of CFZ. The fluorescence spectra of BSA-LMF-CFZ system show similar spectra with BSA-LMF system. The quenching degree of LMF plus CFZ to fluorescence of BSA was higher than LMF or CFZ alone. It is suggested that an energy transfer between LMF/CFZ and BSA occurred.

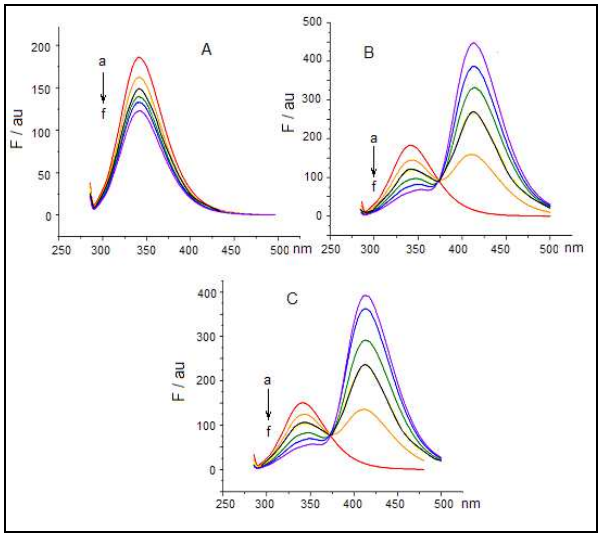

Fig. 1: Quenching fluorescence spectra of BSA–CFZ (A) and BSA–LMF (B) and BSA –LMF (C) in the presence of 1.0×10^{-5} M CFZ after corrected inner-filter effect. BSA, 1.0×10^{-6} M; LMF or CFZ ($\times10^{-5}$ M) (a—f): 0, 0.5, 1.0, 2.0, and 2.5

The fluorescence quenching data are analyzed by the Stern–Volmer equation [16]:

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

where F_0 and F are the fluorescence intensity in the absence and presence of quencher, respectively. K_q is the quenching rate constant, τ_0 is the fluorescence life time of biopolymer BSA ($\tau_0=10^{-8}$ s)[17], K_{SV} and [Q] are the Stern–Volmer quenching constant and concentration of quencher, respectively. In this work, the Stern–Volmer plots of F_0/F vs concentration of LMF and/or CFZ were obtained. The estimated values of kinetic data along with correlation coefficient are given in Table 1.

Table 1 Quenching reactive parameter of BSA–LMF and/or CFZ systems at 25˚C

System	Equation	r	K_q(L· mol^{-1}·s^{-1})	SD(10^{12}L· mol^{-1}·s^{-1})
BSA–CFZ	Y=1.005+1.276×10^4X	0.9942	1.28×10^{12}	0.021
BSA–LMF–CFZ[a]	Y=1.013+1.257×10^4X	0.9939	1.26×10^{12}	0.015
BSA–LMF	Y=1.004+2.646×10^4X	0.9988	2.65×10^{12}	0.053
BSA–CFZ–LMF[b]	Y=1.001+2.503×10^4X	0.9973	2.50×10^{12}	0.062

a, LMF，1.0×10^{-7} M; CFZ：0，0.5, 1.0, 1.5, 2.0, and 2.5×10^{-5} M
b, CFZ，1.0×10^{-7} M; LMF：0，0.5, 1.0, 1.5, 2.0, and 2.5×10^{-5} M

Obviously, the rate constants K_q of the tested four systems are greater than the maximum scatter collision quenching constants of various quenchers with the biomolecule (2.0×10^{10} L· mol^{-1}·s^{-1})[18], which suggests that the quenching is not initiated by dynamic quenching but by static quenching resulted from the formation of a complex. No shift in emission wavelength of BSA was observed in the presence of CFZ, it is due to that the complex of BSA and CFZ has no fluorescence characteristic. The shift in emission wavelength from 340 to 337 nm for BSA indicates the formation of complex with fluorescence characteristic by binds of LMF with BSA sites.

Binding constant and binding site number

For static quenching, the following equation was used to calculate the binding constant and binding sites [19,20]:

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

where K_a and n are the binding constant and binding site number, respectively. The plots of log [$(F_0–F)/F$] vs log [Q] presented in Fig. 2 are linear. Binding constant (K_a) and the binding site number (n) could be calculated from the intercept and slope.

The obtained binding constant K_a and binding site number n were $(5.40\pm0.056)\times10^2$ L mol^{-1} and 0.70 for BSA–CFZ, $(1.21\pm0.021)\times10^4$ L mol^{-1} and 0.93 for BSA–LMF, $(1.20\pm0.012)\times10^3$ L mol^{-1} and 0.78 for BSA–LMF–CFZ, and $(2.72\pm0.040)\times10^4$ L mol^{-1} and 1.01, respectively. The K_a value in this work was in the middle of the literature values [5-7, 9, 10, 13]. There was stronger combination action between LMF (and/or CFZ) and BSA. The K_a ratio of BSA–LMF to BSA–CFZ equals to 22.4, showing higher binding of LMF to BSA than CFZ. It is shown that

concentration of free CFZ in blood is higher than LMF. The K_a ratio of BSA–CFZ–LMF system to BSA–LMF system equals to 2.25, suggesting that free LMF in blood in the presence of CFZ decreased. The K_a ratio of BSA–LMF–CFZ to BSA–CFZ equals to 2.22, suggesting that free CFZ in blood in the presence of LMF decreased. The results showed that there was a contradict effect for LMF to binding of CFZ with BSA and for CFZ to binding of LMF with BSA. This may lead to the need for more doses of LMF and CFZ to achieve therapeutic effect.

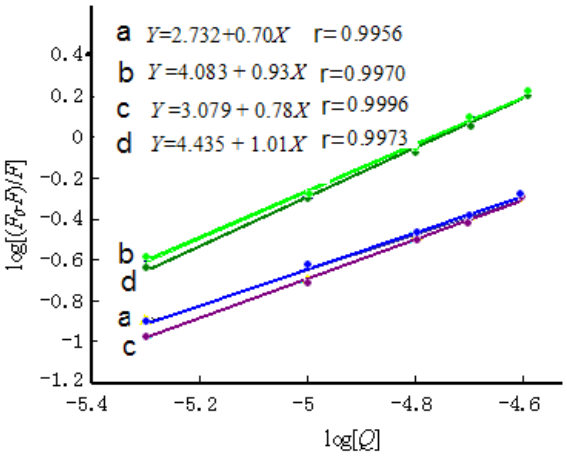

Fig. 2: Plot of log[(F_0–F)/F] versus log[Q] at 25˚C

a—BSA–CFZ; b—BSA–LMF; c—BSA–LMF–CFZ (LMF，1.0×10^{-7} M；CFZ：0，0.5, 1.0, 1.5, 2.0, and 2.5×10^{-5} M); d—BSA-CFZ–LMF (CFZ，1.0×10^{-7} M；LMF：0，0.5, 1.0, 1.5, 2.0, and 2.5×10^{-5} M)

Energy transfer from BSA to CFZ/LMF

Fluorescence resonance energy transfer is an important technique for investigating a variety of biological phenomena including energy transfer processes [21]. Here the donor and acceptor are BSA and CFZ/LMF, respectively. It was observed that there is spectral overlap between fluorescence emission of BSA and absorption spectra of LMF and CFZ in the wavelength range of 260–500 nm, as shown in Fig. 3.

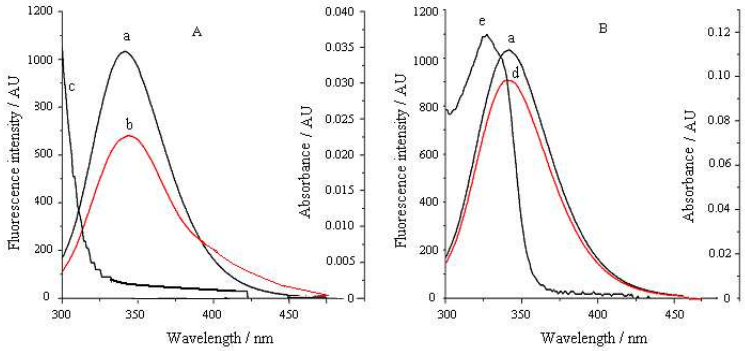

Fig. 3: Quenching fluorescence spectra and UV absorption spectra. A: a—fluorescence spectrum of BSA, b—fluorescence spectra of BSA–LMF, c—absorption spectrum of CFZ；B: a—fluorescence spectrum of BSA, b—fluorescence spectra of BSA–CFZ, c—absorption spectrum of LMF; BSA, CFZ, and LMF：each 1.0×10^{-5} M

The fluorescence emission (330 nm) of BSA–CFZ solution at an excitation wavelength of 280 nm is from BSA only since CFZ is a non-fluorescence drug molecule. However, at this wavelength CFZ has weak absorption. The fluorescence emission (337 nm) of BSA–LMF solution at an excitation wavelength of 280 nm is from the complex of BSA and LMF, which is a fluorescence molecule. However, at this wavelength LMF has stronger absorption. It suggested the possibility of fluorescence resonance energy transfer from BSA to LMZ/CFZ molecules in solution.

The region of integral overlap is used to calculate the critical energy transfer distance (R_0) between BSA (donor) and CFZ/LMF (acceptor) according to Foster's non-radioactive energy transfer theory using Förster's equation [19, 22]. Based on this theory, the efficiency (E) of energy transfer between donor (BSA) and acceptor (CFZ/LMF) can be calculated by Equation:

$$E = R_0^6/(R_0^6 + r^6) \qquad (3)$$

Where, r is the binding distance between donor and acceptor, and R_0 is the critical binding distance. When the efficiency (E) of energy transfer is 50%, R_0 can be calculated by Equation:

$$R_0^6=8.8\times10^{-25}k^2n^{-4}\Phi_D J \quad (4)$$

Where, the k^2 is the spatial orientation factor of the dipole, n is the refractive index of medium, Φ_D is the quantum yield of the donor in the absence of acceptor and J is the overlap integral of the emission spectrum of the donor and the absorption spectrum of the acceptor. The J can be calculated by Equation:

$$J = \sum F(\lambda)\varepsilon(\lambda)\lambda^4\Delta\lambda/\sum F(\lambda)\Delta\lambda \quad (5)$$

Where, $F(\lambda)$ is the fluorescence intensity of the fluorescent donor of wavelength, λ, $\varepsilon(\lambda)$ is the molar absorption coefficient of the acceptor at wavelength, λ. In the present case, k^2, n and Φ_D are 2/3, 1.336 and 0.118, respectively [23].

The efficiency (E) of energy transfer can be determined by Equation:

$$E = 1 - F/F_0 \quad (6)$$

Where, F_0 and F are the fluorescence intensities of BSA solutions in the absence and presence of DES, respectively. From the overlapping R_0 was found based on Eq. 4 using K^2 = 2/3, n = 1.336 and Φ_D =0.118 (tryptophan residue) for the aqueous solution of BSA. J and E could be calculated from Eq. 5 and Eq. 6, respectively. At the same time, the binding distance (r) between BSA and LMF/CFZ is obtained by Eq. 3. Their values are listed in Table 2.

Table (2) Energy transfer parameters for the interactions of the drugs with BSA at 25 ˚C

System	E (%)	J / cm^3 L mol^{-1}	R_0 / nm	r / nm
BSA–CFZ	12.1	3.97×10^{-15}	1.40	1.94
BSA–LMF–CFZ[a]	40.4	3.31×10^{-16}	1.39	1.48
BSA–LMF	34.1	6.81×10^{-15}	2.3	4.44
BSA–CFZ–LMF[b]	40.6	6.80×10^{-15}	2.3	2.45

a, LMF，1.0×10^{-7} M； CFZ：0，0.5, 1.0, 1.5, 2.0, and 2.5×10^{-5} M

b, CFZ，1.0×10^{-7} M； LMF：0，0.5, 1.0, 1.5, 2.0, and 2.5×10^{-5}

For BSA-CFZ in the presence of LMF the critical binding distance (R_0) did not change, but the binding distance (r) decreased from 1.94 to 1.48 nm, and the efficiency (E) of energy transfer increased from 12.1 to 40.4%. For BSA-LMF in the presence of CFZ the critical binding distance (R_0) did not change, but the binding distance (r) decreased from 4.44 to 2.45 nm, and the efficiency (E) of energy transfer increased from 34.1 to 40.6%. These data indicate that the nonradiative energy transfer from BSA to CFZ/LMF occurred, with high possibility. It is suggested that the bindings of CFZ/LMF to BSA molecules were occurred through energy transfer, which quenched the fluorescence of BSA molecules, showing the presence of static quenching interaction between BSA and CFZ/LMF.

Effect of CFZ and/or LMF on the conformation of BSA

Circular dichroism studies. Circular dichroism (CD) is a sensitive technique to monitor conformational changes in protein structure [24]. CD spectra of BSA, BSA-LMF, BSA-CFZ, and BSA- CFZ-LMF are shown in Fig. 4.

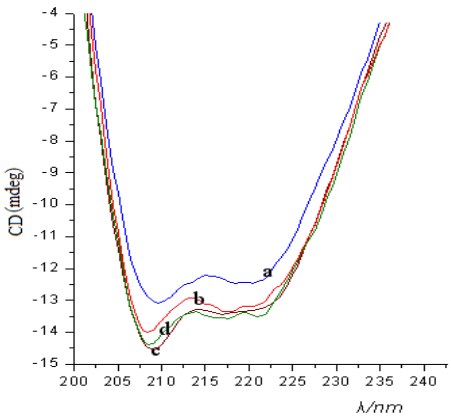

Fig. 4: CD spectra of (a) BSA, (b) BSA-LMX, (c) BSA-CFZ, and (d) BSA-CFZ-LMX systems. BSA,1.0×10^{-6} M; LMX, 5.0×10^{-6} M; CFZ, 5.0×10^{-6} M

In BSA spectrum, there are negative peaks in the ultraviolet region, one at 209 nm and the other at 222 nm, which are characteristic of the α-helical structure of a protein [25]. Trynda-Lemiesz et al. explained that both of the negative peaks between 208-209 and 222-223 nm contribute to the transfer for the peptide bond of the α-helix[26]. In the presence of LMX, the intensity of both the negative peaks increased, proving the change of the α-helical structure of BSA due to the formed complex of BSA and LMX. Change of the secondary structure of BSA caused the fluorescence quenching. However, the shape and position of the peaks did not obviously change. The CD spectra of BSA in the presence and absence of CFZ and/LMX are observed to be similar in shape, indicating that the structure of BSA is also predominantly α-helical [27].

Synchronous fluorescence. Synchronous fluorescence is a kind of simple and sensitive method to measure the fluorescence quenching. When Δλ is 15 nm, synchronous fluorescence is characteristic of tyrosine residue, while when Δλ is 60 nm, it provided the characteristic information of tryptophan residues [28]. In this work, the synchronous fluorescence spectra of tyrosine residue and tryptophan residues in BSA with addition of CFZ and/or LMF were observed, as shown in Fig. 5. When wavelength interval Δλ is 15 nm, the spectrum characteristic of tyrosine residues in BSA was observed. When Δλ= 60 nm, the spectrum characteristic of tryptophan residues in BSA was manifested.

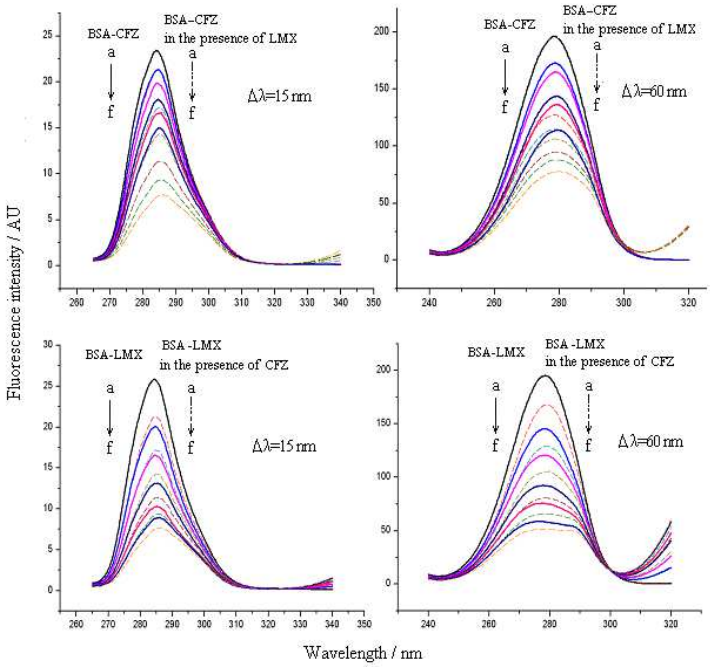

Fig. 5: Synchronous fluorescence of BSA–LMF and BSA-CFZ in the absence (solid line) and presence (dashed line) of CFZ or LMF. BSA, 1.0×10^{-6} M in all cases; CFZ, LMF (10^{-5} M), a→f: 0, 0.5, 1.0, 1.5, 2.0, and 2.5; BSA–LMF in the presence of CFZ (1.0×10^{-5}M); BSA–CFZ in the presence of 1.0×10^{-5}M LMF

When the drug CFZ was gradually added, the fluorescence intensity decreased regularly, and the main peak of tyrosine residues (Δλ= 15 nm) and tryptophan residues (Δλ=60 nm) in BSA did not change. It is indicated that the presence of CFZ did not change the conformation of BSA. It is also shown that the microenvironment around the tyrosine residue and tryptophan residue did not change during the binding process. In the presence of LMF, the fluorescence intensity of both tyrosine residues and tryptophan residues decreased regularly with increasing in CFZ content, and the main peak of tyrosine residues (Δλ= 15 nm) was red-shifted slightly (1 nm), and the main peak of tryptophan residues (Δλ= 60 nm) was not red-shifted. When the drug LMF was gradually added, the main peak of tyrosine residues (Δλ= 15 nm) in BSA was red-shifted (2 nm), but the main peak of tryptophan residues (Δλ= 60 nm) was blue-shifted (3 nm). It may be due to the changes of tyrosine and tryptophan residues microenvironment with the insertion of LMF. In the presence of CFZ, the similar results were gained. Above result indicated that the presence of coexisting LMF and CFZ could change the conformation of BSA, here LMF was reigning.

Three-dimensional fluorescence studies. The three-dimensional fluorescence spectrum is another powerful method for studying conformation change of BSA. In this work, the three-dimensional fluorescence spectra of BSA, BSA–CFZ, BSA–LMX, and BSA–CFZ–LMX systems were observed, as shown in Fig. 6.

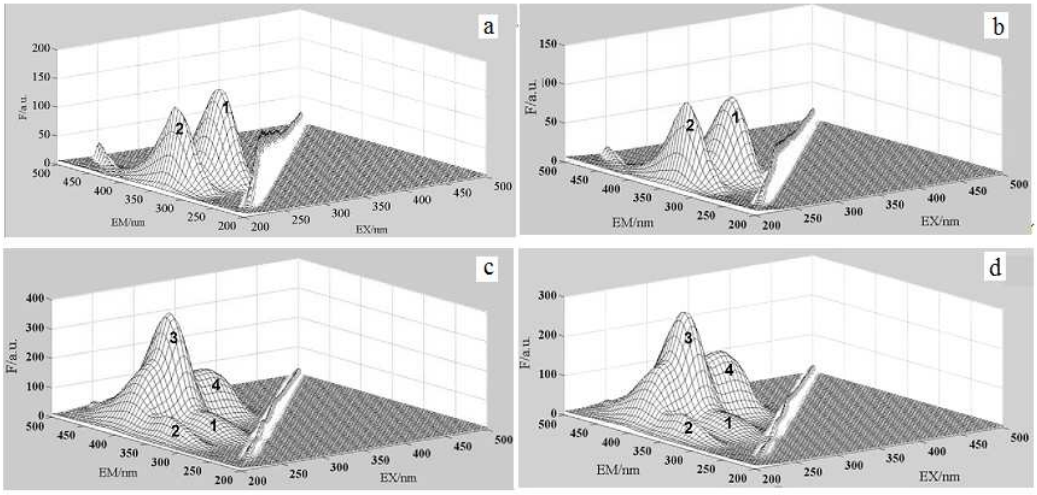

Fig. 6: Three-dimensional fluorescence spectra of (a) BSA, (b) BSA–CFZ, (c) BSA–LMX and (d) BSA–CFZ–LMX systems. BSA, 1×10^{-6} M, CFZ, 2.5×10^{-5}M, LMX, 2.5×10^{-5}M

From Fig. 6, peak 1 (λex/λem = 275.0/340.0 nm) reveals the spectral characteristic of tryptophan and tyrosine residues. After the addition of CFZ or LMX in BSA, the fluorescence intensity of BSA decreased from 158.7 to 103.6 or to 67.55, and the maximum emission wavelength of BSA was shifted. In addition, fluorescence spectrum (peak 3) of LMX was observed. After the addition of CFZ and LMX in BSA, peak 1 could not be observed due to serious fluorescence quenching. This suggests a less polar environment of both residues and almost all the hydrophobic amino acid residues of BSA were buried in the hydrophobic pocket. Less polar environment means that the binding position of LMX and/or CFZ with BSA located within this hydrophobic pocket, the addition of them changed the polarity of this hydrophobic microenvironment and the conformation of BSA [29]. In Fig. 6, peak 2 (λex/λem=225.0/345.0 nm) reveals the fluorescence spectra behavior of polypeptide backbone structures, which is caused by the transition of π-π* of the characteristic polypeptide backbone structure C=O of BSA [29].

After the addition of CFZ and/or LMX, the fluorescence intensity decreased and the maximum emission wavelength of BSA was shifted. These revealed that the microenvironment and conformation of BSA were changed in the binding reaction. The interaction of CFZ and/or LMX with BSA induced the unfolding of the polypeptides chains of BSA and conformational change of BSA.

CONCLUSION

All the results indicated that the fluorescence quenching of bovine serum albumin by lomefloxacin and/or cefazolin is a static process. In the presence of cefazolin or lomefloxacin, the interaction of lomefloxacin or cefazolin with bovine serum albumin increased markedly. This may lead to the need for more doses of lomefloxacin and cefazolin to achieve therapeutic effect. The conformation of bovine serum albumin has been changed with the addition of lomefloxacin or lomefloxacin–cefazolin. The results are of great importance in pharmacy, pharmacology and biochemistry, and are expected to provide important insight into the interactions of the physiologically important protein BSA with drugs.

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REFERENCES

[1] B Zhou; Z D Qi; Q Xiao; JX Dong; Y Z Zhang; Y Liu, *J. Biochem. Biophys. Meth.*, **2007**,70, 743–747.
[2] PB Kandagal; SMT Shaikh; DH Manjunatha; J Seetharamappa; BS Nagaralli, *J. Photochem. Photobiol., A* **2007**,189, 121–127.
[3] L Bai; XL Wang; XC Lv; CP Gao, *Pharm. J. Chin. PLA*, **2004**, 20, 200**–**203.
[4] S Erjongmanee; N Kasetsuwan; N Phusitphoykai; V Puangsricharern; L Pariyakanok, *J. Med. Assoc. Thailand*., **2004**, 87, S83–90.
[5] C K Kim; YS Lim; J S Yang, *Arch. Pharm. Res*., **1984**, 7, 87–93.
[6] Z Wang; Z Song; D Chen, *Talanta*, **2010**, 83, 312–319.
[7] M M Yang; XL Xi; P Yang, *Chin. J. Chem*., **2006**, 24, 642–648.
[8] K Okumura; H Katayama; M Yasuhara; R Hori, *Chem. Pharm. Bulletin*., (Tokyo) **1989**, 37, 3150–3152.

[9] M Guo; QS Yu; JW Yan; F Tan; GZ Ma, *Chin. Chem. Let.,* **2004**,15, 1331–1334.
[10] S Liu; LW. Zhang; XX Zhang, *Anal. Sci*., **2006**, 22, 1515–1518.
[11] H He; HY Ye; L Dai; QC Jiao; PH Chuong, *Spectros. Spec. Anal*., **2006**, 26, 480–483.
[12] JQ Lu; F Jin; TQ Sun; XW Zhou, *Int. J. Biolog. Macromol*., **2007**, 40, 299–304.
[13] XY Yu; RH Liu; DH Ji; F Yang; X Li; J Xie; J Zhou; P Yi, *J. Solut. Chem*., **2011**, 40, 521–531.
[14] T Lon; M Wedborg; D Turner, *Anal. Chim. Acta*, **2007**, 583, 357–363.
[15] M Dan; D Dasgupta, *Indian J.Biochem. Biophysics*, **2001**, 38, 78–83.
[16] LN Zhang; F Y Wu; AH Liu, *Spectrochim. Acta, A* **2011**, 79, 97–103.
[17] JR Lakowicz; G Weber, *Biochem*., **1973**, 12, 4161–4170.
[18] RE Maurice; AG Camillo, *Anal. Biochem.*, **1981**, 114, 199–212.
[19] YJ Hu; Y Liu; RM Zhao; JX Dong; S S Qu, *J. Photochem. Photobiol., A* **2006**,179, 324–329.
[20] N Barbero; E Barni; C Barolo; P Quagliotto; G Viscardi; L Napione; S Pavan; F Bussolino, *Dyes Pigments*, **2009**, 80, 307–313.
[21] A Mallick; B Haldar; N Chattopadhyay, *J. Phys. Chem., B* **2005**, 109, 14683–14690.
[22] SL Bhattar; GB Kolekar; SR Patil, *J. Lumin*., **2008**, 128, 306–310.
[23] SMT Shaikh; J Seetharamappa; PB Kandagal; S Ashoka, *J. Mol. Struct*., **2006**, 786, 46–52.
[24] XC Zhao; RT Liu; ZX Chi; Y Teng; P F Qin, *J. Phys. Chem., B* **2010**, 114, 5625–5631.
[25] YY Pi; YZ Shang; CJ Peng; H L Liu; Y Hu; JW Jiang, *Biopolym*., **2006**; 83, 243–249.
[26] L Trynda-Lemiesz; A Karaczyn; B K Keppler; H Kozlowski, *Inorg. Biochem*., **2000**, 78, 341–346.
[27] YJ Hu; Y Liu; XS Shen; XY Fang; SS Qu, *J. Mol. Struct*., **2005**, 738, 143–147.
[28] M Guo; WJ Lue; MH Li; W Wang, *Eur. J. Med. Chem*., **2008**, 43, 2140–2148.
[29] Q Xiao; S Huang; Y Liu; FF Tian; JC Zhu, *J. Fluores*., **2009**, 19, 317–326.