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Study of the interaction of an anticancer drug with human and bovine serum albumin: Spectroscopic approach

P.B. Kandagal [a], S. Ashoka [a], J. Seetharamappa [a,*], S.M.T. Shaikh [a], Y. Jadegoud [b], O.B. Ijare [b]

[a] *Department of Chemistry, Karnatak University, Dharwad 580003, India*
[b] *Center of Biomedical Magnetic Resonance, Sanjay Gandhi Post Graduate Institute of Medical Science, Lucknow 226014, India*

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Abstract

The interactions between gemcitabine hydrochloride (GEM) and bovine serum albumin (BSA) or human serum albumin (HSA) have been studied by spectroscopic techniques. By the analysis of fluorescence spectrum and fluorescence intensity, it was observed that the GEM has a strong ability to quench the intrinsic fluorescence of both BSA and HSA through a static quenching procedure. The association constants of GEM with BSA and HSA were determined at different temperatures based on fluorescence quenching results. The negative ΔH° and positive ΔS° values in case of GEM–BSA and GEM–HSA complexes showed that both hydrogen bonds and hydrophobic interactions play a role in the binding of GEM to BSA or HSA. Experimental results showed that the binding of GEM to BSA or HSA induced conformational changes in BSA and HSA. From the quantitative analysis data of CD spectra, the α-helix of 57.58% and 34.82% in free BSA and free HSA decreased to 40.82% and 29.84% in BSA–GEM and HSA–GEM complexes, respectively, and hence confirmed that the secondary structure of protein was altered by GEM. The interactions of BSA and HSA with GEM were also confirmed by UV absorption spectra. The distance, *r*, between donor (BSA or HSA) and acceptor (GEM) was obtained according to the Förster's theory of non-radiation energy transfer. The effects of common ions on the binding constants of both BSA–GEM and HSA–GEM complexes were also investigated.

Keywords: Protein; Gemcitabine hydrochloride; Fluorescence quenching; Fluorescence resonance energy transfer; Thermodynamic parameters

1. Introduction

Gemcitabine hydrochloride (GEM), a synthetic pyrimidine nucleoside (Fig. 1) is an antineoplastic agent [1]. The nucleoside analog consists of the pyrimidine base diflurocytidine, and the sugar moiety deoxyribose. The cytotoxic effect of gemcitabine is attributed to the combined actions of its diphosphate and triphosphate nucleoside, which lead to inhibition of DNA synthesis. GEM can interfere with the growth of rapidly growing cells, like cancer cells and eventually causes cell death. GEM is used alone or in combination with other chemotherapy agents. This medicine, along with another anticancer drug, may also be used to treat non-small cell lung cancer or breast cancer. Common side effects include fatigue and temporary drop in bone marrow function.

HSA is a major circulatory protein of well-known structure. The crystal structure analyses have revealed that the drug binding sites are located in subdomains IIA and IIIA [2]. A large hydrophobic cavity is present in IIA subdomain. The geometry of the pocket in IIA is quite different from that found for IIIA. HSA has one tryptophan (Trp-214) in subdomain IIA, whereas BSA has two tryptophan moieties (Trp-135 and Trp-214) located in subdomains IA and IIA, respectively [2].

The present study focused on biophysical interactions of GEM with serum albumins that play an important role in drug transport and storage in vertebrates [3]. Drug interactions at protein binding level will in most cases significantly affect the apparent distribution volume of the drugs and also affect the elimination rate of drugs. Therefore, the studies on this aspect can provide information of the structural features that determine the therapeutic effectiveness of drug, and have been an interesting research field in life sciences, chemistry and clinical medicine [4].

The molecular interactions are often monitored by spectroscopic techniques because these methods are sensitive and

* Corresponding author. Tel.: +91 836 2215286; fax: +91 836 2747884.
E-mail address: jseetharam@yahoo.com (J. Seetharamappa).

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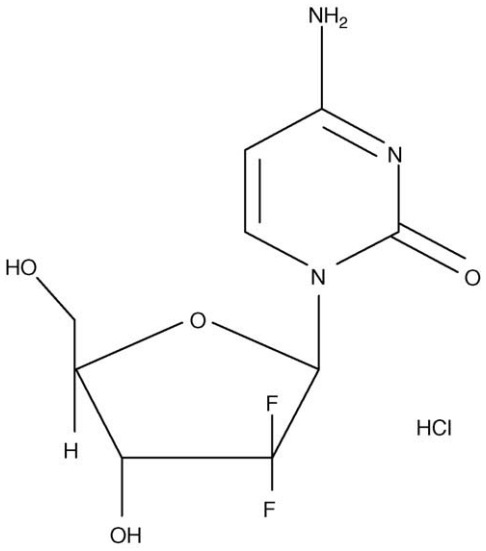

Fig. 1. Structure of gemcitabine hydrochloride.

relatively easy to use. They have advantages over conventional approaches such as affinity and size exclusion chromatography, equilibrium dialysis, ultrafiltration and ultracentrifugation, which suffer from lack of sensitivity, long analysis time or both and use of protein concentrations far in excess of the dissociation constant for the drug–protein complex [5,6] and for drug–protein interaction studies. In the present paper, we are reporting the mechanism of interaction of GEM with BSA and HSA using three spectral methods for the first time.

2. Experimental

2.1. Materials

Bovine serum albumin (BSA) and human serum albumin (HSA) were obtained from Sigma Chemical Company, St. Louis, USA. Gemcitabine hydrochloride was obtained as a gift sample from the Lifecare Laboratories Pvt. Ltd., India, in pure form. The solutions of GEM, BSA and HSA were prepared in 0.1 M phosphate buffer of pH 7.4 containing 0.15 M NaCl. BSA and HSA solutions were prepared based on their molecular weights of 65,000 and 66,000, respectively. All other materials were of analytical reagent grade and double distilled water was used throughout.

2.2. Apparatus

Fluorescence measurements were performed on a spectrofluorimeter Model F-2000 (Hitachi, Japan) equipped with a 150 W Xenon lamp and a slit width of 10 nm. A 1.00 cm quartz cell was used for measurements. The CD measurements were made on a JASCO-J-715 spectropolarimeter (Tokyo, Japan) using a 0.1 cm cell at 0.2 nm intervals, with three scans averaged for each CD spectrum in the range of 200–250 nm. The absorption spectra were recorded on a double beam CARY 50-BIO UV–vis spectrophotometer (Varian, Australia) equipped with a 150 W Xenon lamp and a slit width of 10 nm. A quartz cell of 1.00 cm was used for measurements.

2.3. Procedures

2.3.1. GEM–protein interactions

Based on preliminary experiments, the BSA and HSA concentrations were fixed at 12 μM and the drug concentration was varied from 25 to 200 μM for BSA and from 12.5 to 75 μM for HSA. Fluorescence spectra were recorded at three temperatures (288, 298 and 309 K) in the range of 300–500 nm upon excitation at 296 nm for BSA and at 280 nm for HSA.

2.3.2. UV measurements

The UV measurements of BSA and HSA in the presence and absence of GEM were made in the range of 200–310 nm. BSA and HSA concentrations were fixed at 12 μM while the drug concentration was varied from 12 to 44 μM for BSA and from 12 to 50 μM for HSA.

2.3.3. Circular dichroism (CD) measurements

The CD measurements of BSA and HSA in presence and absence of GEM were made in the range of 200–250 nm using a 0.1 cm cell at 0.2 nm intervals with three scans averaged for each CD spectra. A stock solution of each of 150 μM BSA and HSA was prepared in 0.1 M phosphate buffer containing 0.15 M NaCl. The molar ratio of BSA to drug concentration was 1:6, 1:14 and 1:26 while that of HSA to drug was maintained at 1:2 and 1:8 for recording CD spectra.

2.3.4. Energy transfer between GEM and protein

The absorption spectrum of GEM (12 μM) was recorded in the range of 300–500 nm. The emission spectrum of BSA/HSA (12 μM) was also recorded in the range of 300–500 nm. Then, the overlap of the UV absorption spectrum of GEM with the fluorescence emission spectrum of protein was used to calculate the energy transfer.

2.3.5. Effects of some common ions

The fluorescence spectra of GEM–BSA, GEM–HSA were recorded in presence and absence of various common ions, viz., SO_4^{2-}, F^-, NO_3^-, CH_3COO^-, I^-, Mg^{2+}, Cu^{2+}, K^+, Ca^{2+} and V^{5+} in the range of 300–500 nm upon excitation at 296 and 280 nm for BSA and HSA, respectively. The concentration of BSA and HSA was fixed at 12 μM and that of common ion was maintained at 5 μM.

3. Results and discussion

3.1. Binding property of the GEM to the serum albumins

For macromolecules, the fluorescence measurements can give some information of the binding of small molecule substances to protein, such as the binding mechanism, binding mode, binding constants, binding sites and intermolecular distances. Fluorescence intensity of a compound can be decreased

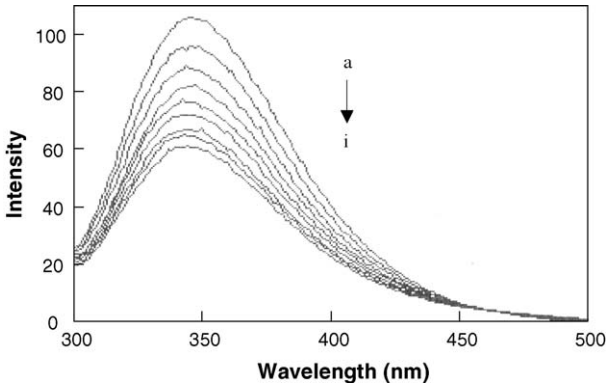

Fig. 2. Fluorescence spectra of BSA in the presence of GEM. BSA concentration was 12 μM (a). GEM concentration was at 25 μM (b), 50 μM (c), 75 μM (d), 100 μM (e), 125 μM (f), 150 μM (g), 175 μM (h) and 200 μM (i).

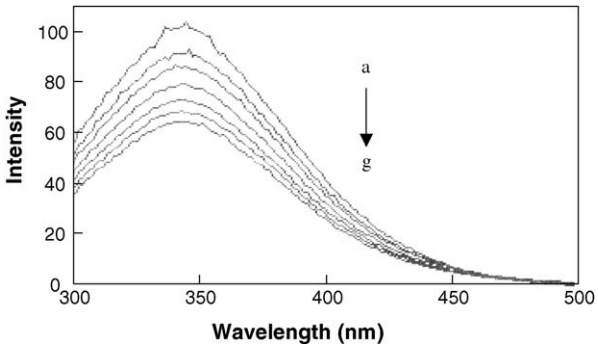

Fig. 3. Fluorescence spectra of HSA in the presence of GEM. HSA concentration was 12 μM (a). GEM concentration was at 12.5 μM (b), 25 μM (c), 37.5 μM (d), 50 μM (e), 62.5 μM (f) and 75 μM (g).

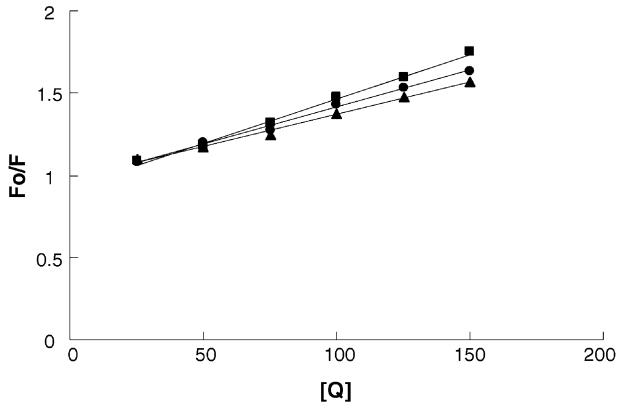

Fig. 4. Stern–Volmer plot for the binding of GEM with BSA at 288 K (■), 298 K (●) and 309 K (▲).

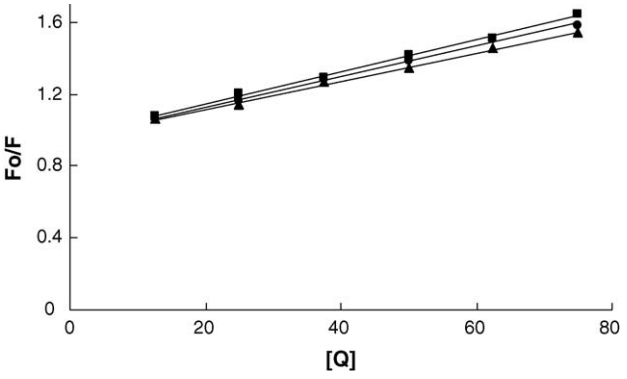

Fig. 5. Stern–Volmer plot for the binding of GEM with HSA at 288 K (■), 298 K (●) and 309 K (▲).

by a variety of molecular interactions, viz., excited-state reactions, molecular rearrangements, energy transfer, ground state complex formation and collisional quenching. Such decrease in intensity is called quenching.

The fluorescence spectra of BSA and HSA in presence of different amounts of GEM were recorded in the range of 300–500 nm upon excitation at 296 and 280 nm, respectively. GEM causes a concentration dependent quenching of the intrinsic fluorescence of both BSA (Fig. 2) and HSA (Fig. 3) without changing the emission maximum and shape of the peaks. These results indicated that there were interactions between GEM and BSA or HSA and the binding reactions resulted in non-fluorescent complexes. The interactions of GEM to BSA and HSA were further confirmed by UV–vis absorption and CD techniques.The fluorescence quenching data were analyzed by the Stern–Volmer equation:

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

where F_0 and F are the steady-state fluorescence intensities in the absence and presence of quencher, respectively, K_{SV} the Stern–Volmer quenching constant and [Q] is the concentration of quencher (GEM). The values of K_{SV} and R^2 at different temperatures are shown in Table 1. The linearity of the F_0/F versus [Q] (Stern–Volmer) plots for both BSA (Fig. 4) and HSA (Fig. 5) revealed the quenching type, the static or dynamic, since the characteristic Stern–Volmer plot of combined quenching (both static and dynamic) is an upward curvature. A linear Stern–Volmer plot, however, does not define per se

Table 1
Thermodynamic parameters of BSA–GEM and HSA–GEM systems

System	T (K)	$K_{SV} \times 10^{-3}$ (L mol^{-1})	$K_q \times 10^{-11}$ (L mol^{-1} s^{-1})	R^2	ΔG° (kJ mol^{-1})	ΔH° (kJ mol^{-1})	ΔS° (J mol^{-1} K^{-1})
BSA	288	5.46 ± 0.051	5.46 ± 0.051	0.9975	−25.03 ± 0.003		
	298	4.85 ± 0.043	4.85 ± 0.043	0.9986	−25.39 ± 0.005	−15.76 ± 0.081	32.68 ± 0.072
	309	4.62 ± 0.024	4.62 ± 0.024	0.9982	−25.67 ± 0.002		
HSA	288	8.13 ± 0.046	8.13 ± 0.046	0.9985	−21.68 ± 0.006		
	298	7.91 ± 0.028	7.91 ± 0.028	0.9976	−22.01 ± 0.002	−13.96 ± 0.068	26.92 ± 0.061
	309	7.74 ± 0.039	7.74 ± 0.039	0.9980	−22.22 ± 0.004		

the quenching type, and additional information is required for this determination. One way to distinguish dynamic from static quenching is to examine the temperature effect on the interaction of the drug to BSA and HSA. The K_{SV} values decrease with an increase in temperature for static quenching, but the reverse effect will be observed for dynamic quenching. The results of the present study indicate that the probable quenching mechanism of fluorescence of BSA and HSA by GEM is a static quenching procedure and revealed the formation of a complex between GEM and BSA/HSA. The formation of complex was further confirmed from the values of quenching rate constants, K_q, which are evaluated using the equation:

$$K_q = \frac{K_{SV}}{\tau_0} \quad (2)$$

where τ_0 is the average lifetime of the protein without the quencher. The value of τ_0 of the biopolymer is $10^{-8}\,s^{-1}$ [7] and hence the values of K_q were of the order of $10^{11}\,L\,M^{-1}\,s^{-1}$ (Table 1). The maximum scatter collision quenching constant, K_q of various quenchers with the biopolymer is $2 \times 10^{10}\,L\,M^{-1}\,s^{-1}$. Obviously, the K_q values of protein quenching procedure initiated by GEM are greater than the K_q of the scattered procedure. This indicated that the quenching is not initiated by dynamic collision but from the formation of a compound [8].

3.2. Analysis of binding equilibria

When small molecules bind independently to a set of equivalent sites on a macromolecule, the equilibrium between free and bound molecules is given by the equation [9,10]:

$$\log \frac{F_0 - F}{F} = \log K + n\ \log[Q] \quad (3)$$

where K and n are the binding constant and the number of binding sites, respectively. Thus, a plot of $\log(F_0 - F)/F$ versus log [Q] can be used to determine K as well as n. The values of K were found to be $(3.47 \pm 0.032) \times 10^4$, $(2.81 \pm 0.055) \times 10^4$ and $(2.20 \pm 0.062) \times 10^4\,M^{-1}$ for BSA and $(8.5 \pm 0.027) \times 10^3$, $(7.21 \pm 0.046) \times 10^3$ and $(5.71 \pm 0.058) \times 10^3\,M^{-1}$ for HSA at 288, 298 and 309 K, respectively. The values of n were noticed to be (1.16 ± 0.02), (1.10 ± 0.04) and (1.09 ± 0.05) for BSA and (1.01 ± 0.04), (0.96 ± 0.06) and (0.90 ± 0.03) for HSA, respectively, at 288, 298 and 309 K. It was found that the binding constant decreased with an increase in temperature, resulting in a reduction of the stability of the GEM–BSA and GEM–HSA complexes. Meanwhile, from the data of n it may be inferred that there was one independent class of binding sites on BSA and HSA for GEM.

3.3. Types of interaction force between BSA and HSA with GEM

Considering the dependence of binding constant on temperature, a thermodynamic process was considered to be responsible for the formation of a complex. Therefore, the thermodynamic parameters dependent on temperatures were analyzed in order to further characterize the acting forces between GEM and HSA or BSA. The acting forces between a small molecule and macromolecule mainly include hydrogen bonds, van der Waals forces, electrostatic forces and hydrophobic interaction forces. The thermodynamic parameters, enthalpy change (ΔH°), entropy change (ΔS°) and free energy change (ΔG°) are the main evidences to determine the binding mode. The thermodynamic parameters were evaluated using the following equations:

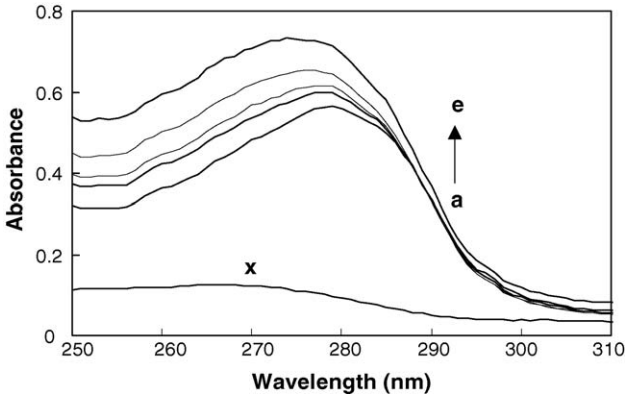

Fig. 6. Absorbance spectra of BSA, GEM and BSA–GEM system. BSA concentration was 12 μM (a). GEM concentration for GEM–BSA system was at 12 μM (b), 19 μM (c), 31 μM (d) and 44 μM (e). A concentration of 12 μM GEM (x) was used for GEM only.

$$\log K = -\frac{\Delta H^\circ}{2.303RT} + \frac{\Delta S^\circ}{2.303R} \quad (4)$$

$$\Delta G^\circ = \Delta H^\circ - T\,\Delta S^\circ \quad (5)$$

where K and R are the binding constant and gas constant, respectively. The results obtained are shown in Table 1. The positive entropy change occurs because the water molecules that are arranged in an orderly fashion around the ligand and protein acquire a more random configuration as a result of hydrophobic interactions. Negative ΔH° value cannot be attributed to electrostatic interactions since for electrostatic interactions, ΔH° is very small, almost zero [11,12]. Negative ΔH° value is observed whenever there is hydrogen bonding in the binding [12]. The negative ΔH° and positive ΔS° values in case of GEM, therefore, showed that both hydrogen bonds and hydrophobic interactions play a role in the binding of GEM to BSA and HSA [12–14].

3.4. UV–vis absorption studies

The complex formation between GEM–HSA and GEM–BSA was also evident from UV–vis absorption spectral data (Figs. 6 and 7). The UV absorption intensity of BSA and HSA increased with the variation of GEM concentration. Further, blue shift of maximum peak position was also noticed possibly due to complex formation between GEM and HSA or BSA [15,16].

3.5. Circular dichroism studies

CD, a sensitive technique to monitor the conformational change in the protein was employed in the present study. In this

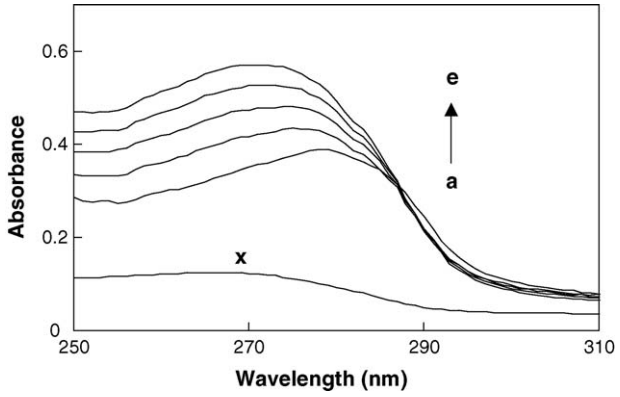

Fig. 7. Absorbance spectra of HSA, GEM and HSA–GEM system. HSA concentration was at 12 μM (a). GEM concentration for GEM–HSA system was at 12 μM (b), 25 μM (c), 37 μM (d) and 50 μM (e). A concentration of 12 μM GEM (x) was used for GEM only.

work, the molar ratios of 1:6, 1:14 and 1:26 for BSA:GEM, and 1:2 and 1:8 for HSA:GEM were used for the CD measurements. The CD spectra of BSA in the absence (line a) and presence (lines b–d) of GEM and that of HSA in the absence (line a) and presence (lines b and c) of GEM are shown in Figs. 8 and 9, respectively. The CD spectra of HSA exhibited two negative bands in the UV region at 208 and 220 nm, while that of BSA showed bands at 208 and 222 nm, characteristic of an α-helical structure of protein [17]. The CD results were expressed in terms of mean residue ellipticity (MRE) in deg cm^2 $dmol^{-1}$ according to the following equation:

$$\text{MRE} = \frac{\text{observed CD (mdeg)}}{C_p n l \times 10} \quad (6)$$

where C_p is the molar concentration of the protein, n the number of amino acid residues and l is the path length. The α-helical contents of free and combined BSA and HSA were calculated from MRE values at 208 nm using the equation [11]:

$$\alpha\text{-helix } (\%) = \frac{-\text{MRE}_{208} - 4000}{33000 - 4000} \times 100 \quad (7)$$

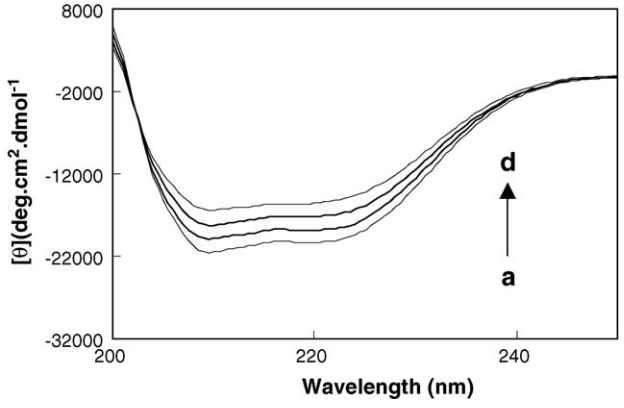

Fig. 8. The CD spectra of the BSA–GEM system obtained in 0.1 M phosphate buffer of pH 7.4 at room temperature. BSA concentration was kept fixed at 5 μM (a). In BSA–GEM system, the GEM concentration was 30 μM (b), 70 μM (c) and 130 μM (d).

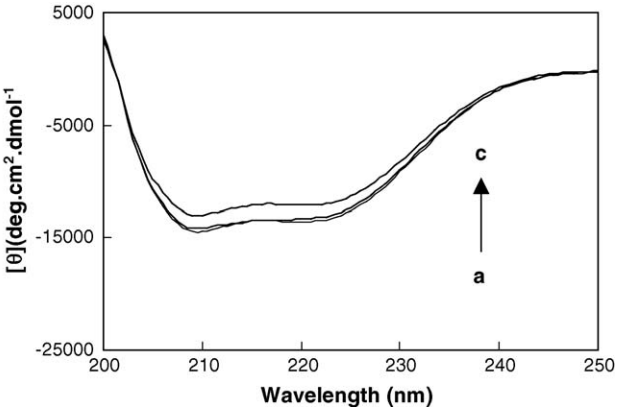

Fig. 9. The CD spectra of the HSA–GEM system obtained in 0.1 M phosphate buffer of pH 7.4 at room temperature. HSA concentration was kept fixed at 5 μM (a). In HSA–GEM system, the GEM concentration was 10 μM (b) and 40 μM (c).

where MRE_{208} is the observed MRE value at 208 nm, 4000 is the MRE of the β-form and random coil conformation cross at 208 nm and 33,000 is the MRE value of a pure α-helix at 208 nm. From the above equation, the α-helicity in the secondary structure of BSA and HSA were determined. They differed from that of 57.58% in free BSA to 40.82% in the BSA–GEM complex and 34.82% in free HSA to 29.84% in the HSA–GEM complex, which was indicative of the loss of α-helicity upon interaction. Further, the CD spectra of HSA/BSA in presence and absence of GEM are observed to be similar in shape, indicating that the structure of HSA/BSA is also predominantly α-helical [17].

3.6. Energy transfer between GEM and protein

The spectral studies suggested that both HSA and BSA form complexes with GEM. HSA has a single tryptophan residue (Trp-214) while BSA has two tryptophan residues (Trp-135 and Trp-214). The distance r between the Trp-214 in HSA and the bound GEM could be determined using fluorescence resonance energy transfer (FRET). However, the r calculated for BSA–GEM complex is actually the average value between the bound GEM and the two tryptophan residues [18]. Generally, FRET occurs whenever the emission spectrum of a fluorophore (donor) overlaps with the absorption spectrum of another molecule (acceptor). The overlap of the UV absorption spectrum of GEM with the fluorescence emission spectra of BSA and HSA are shown in Figs. 10 and 11. The distance between the donor and acceptor and extent of spectral overlaps determines the extent of energy transfer. The distance between the donor and acceptor can be calculated according to Förster's theory [19]. The efficiency of energy transfer, E, is calculated using the equation:

$$E = 1 - \frac{F}{F_0} = \frac{R_0^6}{R_0^6 + r^6} \quad (8)$$

where F and F_0 are the fluorescence intensities of HSA in the presence and absence of GEM, r the distance between acceptor and donor and R_0 is the critical distance when the transfer

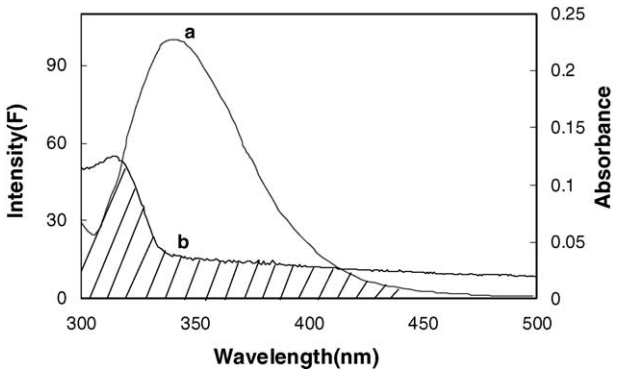

Fig. 10. The overlap of the fluorescence spectrum of BSA (a) and the absorbance spectrum of GEM (b) [λ_{ex} = 296 nm, λ_{em} = 344 nm, c(BSA)/c(GEM) = 1:1].

efficiency is 50%.

$$R_0^6 = 8.8 \times 10^{-25} k^2 N^{-4} \Phi J \quad (9)$$

where k^2 is the spatial orientation factor of the dipole, N the refractive index of the medium, Φ the fluorescence quantum yield of the donor and J is the overlap integral of the fluorescence emission spectrum of the donor and the absorption spectrum of the acceptor. J is given by:

$$J = \frac{\sum F(\lambda)\varepsilon(\lambda)\lambda^4 \Delta\lambda}{\sum F(\lambda)\,\Delta\lambda} \quad (10)$$

where $F(\lambda)$ is the fluorescence intensity of the fluorescent donor of wavelength, λ, and $\varepsilon(\lambda)$ is the molar absorption coefficient of the acceptor at wavelength, λ. In the present case, $k^2 = 2/3$, $N = 1.36$ and $\Phi = 0.15$ for BSA [20], and $k^2 = 2/3$, $N = 1.336$ and $\Phi = 0.118$ for HSA [6]. From Eqs. (8) to (10), we were able to calculate that $J = 1.32 \times 10^{-15}$ cm^3 L mol^{-1}, $R_0 = 1.79$ nm, $E = 0.26$ and $r = 2.13$ nm for BSA and $J = 1.2 \times 10^{-15}$ cm^3 L mol^{-1}, $R_0 = 1.69$ nm, $E = 0.24$ and $r = 2.05$ nm for HSA. The donor-to-acceptor distance, $r < 8$ nm [16,20] indicated that the energy transfer from BSA and HSA to GEM occurs with high possibility. Larger BSA/HSA–GEM distance, r compared to that of R_0 values observed in the present study also reveals the presence of static type quenching mechanism [21,22].

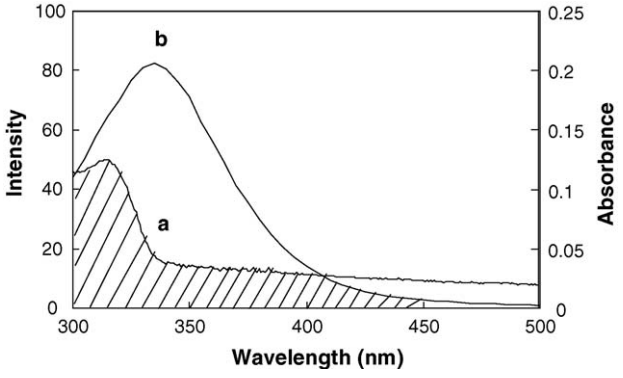

Fig. 11. The overlap of the fluorescence spectrum of HSA (a) and the absorbance spectrum of GEM (b) [λ_{ex} = 280 nm, λ_{em} = 342 nm, c(HAS)/c(GEM) = 1:1].

Table 2
Effects of common ions on binding constants of BSA–GEM and HSA–GEM systems

System	Association constant (M^{-1})
BSA + GEM	$2.81 \times 10^3 \pm 0.068$
BSA + GEM + V^{5+}	$5.22 \times 10^2 \pm 0.054$
BSA + GEM + K^+	$3.48 \times 10^2 \pm 0.046$
BSA + GEM + Cu^{2+}	$1.83 \times 10^3 \pm 0.071$
BSA + GEM + Ca^{2+}	$1.65 \times 10^3 \pm 0.066$
BSA + GEM + Mg^{2+}	$1.52 \times 10^3 \pm 0.043$
BSA + GEM + SO_4^{2-}	$4.21 \times 10^2 \pm 0.035$
BSA + GEM + CH_3COO^-	$7.52 \times 10^2 \pm 0.059$
BSA + GEM + NO_3^-	$4.09 \times 10^2 \pm 0.051$
BSA + GEM + I^-	$5.24 \times 10^2 \pm 0.029$
BSA + GEM + F^-	$4.82 \times 10^2 \pm 0.073$
HSA + GEM	$7.90 \times 10^3 \pm 0.062$
HSA + GEM + V^{5+}	$1.08 \times 10^2 \pm 0.039$
HSA + GEM + K^+	$2.28 \times 10^2 \pm 0.051$
HSA + GEM + Cu^{2+}	$2.70 \times 10^2 \pm 0.030$
HSA + GEM + Ca^{2+}	$6.52 \times 10^2 \pm 0.060$
HSA + GEM + Mg^{2+}	$3.05 \times 10^2 \pm 0.052$
HSA + GEM + SO_4^{2-}	$6.86 \times 10^2 \pm 0.076$
HSA + GEM + CH_3COO^-	$3.99 \times 10^2 \pm 0.080$
HSA + GEM + NO_3^-	$2.81 \times 10^2 \pm 0.063$
HSA + GEM + I^-	$4.39 \times 10^2 \pm 0.053$
HSA + GEM + F^-	$5.52 \times 10^2 \pm 0.072$

3.7. The effect of ions on the binding constant of GEM–protein

In plasma, there are some metal ions, which can affect the reactions of the drugs and the serum albumins. The effects of common ions, viz., SO_4^{2-}, F^-, NO_3^-, I^-, CH_3COO^-, Mg^{2+}, Cu^{2+}, K^+, Ca^{2+} and V^{5+} on the binding constants of both GEM–BSA and GEM–HSA system were investigated at 298 K by recording the fluorescence intensity in the range of 300–500 nm upon excitation at 296 and 280 nm for BSA and HSA systems, respectively. As evident from Table 2, the presence of common ions reduced the binding constants of GEM–BSA and GEM–HSA systems, causing GEM to be quickly cleared from the blood, which may lead to the need for more doses of GEM to achieve the desired therapeutic effect [23,24].

4. Conclusions

This paper provided an approach for studying the interactions of fluorescent protein with GEM using absorption, fluorescence and CD techniques for the first time. Common side effects of GEM include fatigue and temporary drop in bone marrow function. Hence, we have investigated the interactions of GEM with serum albumins as the binding (of the drug to serum albumins) influences the drug availability at the site of action. GEM quenched the fluorescence of both BSA and HSA through static quenching mechanism. This work gave a more comprehensive study and the distance between the donor (protein) and the acceptor (GEM) was also calculated using FRET. The biological significance of this work is evident since albumin serves as a carrier molecule for multiple drugs and the interac-

tions of GEM with albumin are not characterized so far. Hence, this report has a great significance in pharmacology and clinical medicine as well as methodology.

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