Synthesis, crystal structure and spectroscopic investigations on organic complex salt and its interaction with bovine serum albumin

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A new organic complex salt based on chlorogenic acid with 1,10-phenanthroline has been synthesized and characterized. The complex adopts 1:1 stoichiometry in the solid state. The proton transfer occurs from the carboxyl oxygen to the aromatic nitrogen atom to form the salt. The Hphen molecule individually occupies the pseudo-tetragonum that is formed with chlorogenic acid. The self-assembly of the compound in solid state is caused by a hydrogen-bonding interaction. The interaction of the organic complex salt with bovine serum albumin molecules has been studied by fluorescence spectroscopy. The bimolecular quenching constant, apparent quenching constant and number of binding sites have been obtained by fluorescence quenching and the binding distance calculated according to Foster’s non-radioactive energy transfer theory. The corresponding thermodynamic parameters $\Delta H$, $\Delta G$ and $\Delta S$ have also been calculated. The conformational changes of BSA have been observed from synchronous fluorescence spectra.

Keywords: Organic complex salts, Chlorogenic acid, Fluorescence spectroscopy, Bovine serum albumin

Cocrystals are a well-known, but relatively unexplored, class of compounds. The study of cocrystals is of growing interest in organic chemistry and complex molecular research because they are an important tool for the modification of the physicochemical properties of an active complex ingredient, such as solubility, hygroscopicity, thermal stability and processability without changing the complex activity. A cocrystal is a multicomponent solid form, usually in stoichiometric ratio, with each component being an atom, ionic compound, or molecule. Cocrystals rely primarily on the use of non-covalent interactions such as hydrogen bonds to form molecular complexes in a solid state. Although the practical applications of crystal engineering and supramolecular synthesis in complex sciences are still emerging areas of research, synthesizing cocrystals is becoming an accepted approach for creating solid dosage forms.

Similar to cocrystals, organic complex salts are also multi-component systems that can have different physical and chemical properties. The difference between cocrystal and salt is that the protons are transferred between the components in a complex salt.

Serum albumin is the most abundant plasma protein in human and other mammals and several studies have consistently confirmed that human albumins and BSA are homologous proteins. In view of its importance in medicine, stability and binding and transport properties, in the present studies we have chosen BSA as our protein model.

Previous studies have investigated the binding of small organic molecules and metal-organic complexes to BSA, by fluorescence quenching, ultrafiltration, isothermal titration calorimetry, heteronuclear 2D-NMR and reversed-phase liquid chromatography. These small molecules are stabilized when binding to BSA through a series of weak interactions, such as the $\pi$-stacking interactions, hydrogen-bonding, and van der Waals interactions. It is important to study the interactions of drugs with this protein since the effectiveness of the drugs depends on their binding ability.

Herein, we have used the quenching measurements of albumin fluorescence to determine the interaction between the organic complex salts and biomacromolecule BSA, which is expected to reveal the accessibility of quenchers to albumin’s fluorophores, help to better understand albumin binding mechanisms to compounds, and, provide clues to the nature of the binding phenomenon.

Chlorogenic acid (HAC), an important active component in traditional Chinese medicine, exists widely in numerous plant species and may interact covalently or non-covalently with BSA. To the best
of our knowledge, very few studies on crystals of chlorogenic acid have been reported. We have recently reported the interaction between an organic complex salt based on chlorogenic acid and biological macromolecules. To get more information about the binding and the mechanism of the interactions between organic complex salts and the biological macromolecules, we have synthesized an organic complex salt based on chlorogenic acid with 1,10-phenanthroline (phen) and studied its interaction with BSA. By analyzing the fluorescence parameters, we have obtained information about the interactive features and the structural changes in the biomacromolecules.

Materials and Methods

BSA was obtained from Shanghai Yuanju Biological Technology Co. Ltd. (China). Chlorogenic acid was extracted from the Blumea riparia (Bl) DC. BSA stock solution (1.0×10^{-4} mol L^{-1}) was prepared in 0.1 mol L^{-1} Tris-HCl buffer solution (pH = 7.4) and then stored in a refrigerator prior to use. Stock solutions of the compounds were prepared by dissolving the complex (CA·Hphen), HCA and phen in the same buffer. These solutions (1×10^{-4} mol L^{-1}) are stable when kept under 2-5 °C. All other chemicals were commercial products of analytical grade and were used without purification. Doubly distilled water was used throughout.

The X-ray diffraction intensity data for the crystal were collected on a Bruker SMART CCD diffractometer equipped with a graphite-monochromated Mo-Kα radiation source (λ = 0.71073 Å) at 298(2) K. All absorption corrections were performed with SADABS program. The structure was solved by direct methods and refined with full matrix least-squares and expanded using Fourier techniques. Anisotropic thermal parameters were assigned to all non-hydrogen atoms. All calculations were performed using SHELXTL-97 program. The crystallographic data are summarized in Table S1 (Supplementary Data).

The fluorescence measurements were made on a spectrofluorometer (model RF-5301PC) equipped with a xenon lamp source and a 1.0 cm cell. Fluorescence spectra were obtained at excitation wavelength of 295 nm, with the excitation and emission slit widths set at 3 nm. The range of synchronous scanning was Δλ = 15 nm and Δλ = 60 nm, with the excitation and emission slit widths at 3 nm and 3 nm respectively. All pH measurements were made with a pHs-3C digital pH meter (Shanghai Leici Device Works, China) with a combined glass-calomel electrode. The temperature was controlled by a water bath and temperatures were kept in a certain range (T±0.1 °C) throughout the experiment.

Results and Discussion

Structure of CA-Hphen

The organic complex salt of 1,10-phenanthroline (phen) and chlorogenic acid (HCA) (CA-Hphen) forms in the space group P21/n with the asymmetric unit, comprising one Hphen molecule and one CA molecule. Single crystals of the CA-Hphen were obtained from an ethanol solution via slow evaporation of the solvent. The proton transfer occurs from the carboxyl oxygen to the aromatic nitrogen atom (Fig. 1) and extended left-handed helical chains are formed (Fig. 2) by hydrogen bonds [O2···O8, H2O···O8, O2-H2O···O8 (1-x, 0.5+y, 1-z): 2.684(3), 1.56(5) Å, 161.(4)°; O1···O8, H1O···O8(1-x, 0.5+y, 1-z): 2.684(3), 1.56(5) Å, 161.(4)°; O1···O8, H1O···O8(1-x, 0.5+y, 1-z): 2.684(3), 1.56(5) Å, 161.(4)°].

Fig. 1 — Atomic numbering scheme for CA-Hphen with displacement ellipsoids at the 50% probability level. The highlighted region depicts the proton transfer region in the molecular complex.

Fig. 2 — (a) View of the 1D supramolecular helical chain formed by multiple O-H···O hydrogen bonds in the crystal structure of CA-Hphen. (b) Space-filling representation of the helices.
0.5+y, 1-z): 2.917(3), 2.13(5) Å, 135.(4)°; O1⋯O7, H1O⋯O7 (1-x, 0.5+y, 1-z): 2.813(3), 1.93(5) Å, 146.(4)°]. The adjacent chains are connected and aligned along the b-axis via O-H⋯O hydrogen bonding [O5⋯O9, H5O⋯O9, O5-H5O⋯O9(-1+x, y, z): 2.743(3), 1.73(6) Å, 171.(5)°; O6⋯O8, H6O⋯O8, O6-H6O⋯O8(-1+x, y, z): 2.668(3), 1.66(5) Å, 164.(4)°] interactions, forming a two-dimensional supramolecular sheet (Fig. 3a). An interesting structural feature of the crystal is that each pseudotetragonum in the 2D sheet is filled by one Hphen molecule and each Hphen molecule within it is fixed by only one N-H⋯O [N2⋯O9, H2N⋯O9, N2-H2N⋯O9 (x, y, -1+z): 2.664(3), 1.75(4) Å, 141.(3)°] hydrogen bond between the carboxyl oxygen and the phen nitrogen atom (Fig. 3b).

Fluorescence quenching spectra

Proteins are considered to have intrinsic fluorescence due to the presence of amino acids, mainly tryptophan (Trp), tyrosine (Tyr) and phenylalanine (Phe)\(^2\). BSA solutions excited at 295 nm emit fluorescence attributable mainly to the tryptophan residues. Figure 4 shows the fluorescence quenching spectra of solutions containing a fixed BSA concentration and varying concentrations of each of the studied complex (CA-Hphen, HCA\(^{18}\) and phen).

Fig. 3 — (a) Supramolecular sheet formed by inter-chain O-H⋯O hydrogen bonding between the CA. (b) Phen fill of the pseudotetragonum of the 2D sheet.

Fig. 4 — The quenching effect of (a) CA-Hphen, (b) HCA, and, (c) phen on BSA fluorescence intensity. \(\lambda_{ex} = 295\) nm; BSA = 1.00×10\(^{-5}\) mol L\(^{-1}\); CA-Hphen and HCA (a-g) = 0.00, 2.50, 5.00, 7.50, 10.00, 12.50 and 15.00 ×10\(^{-5}\) mol L\(^{-1}\); phen (a-i) = 0.00, 2.50, 5.00, 7.50, 10.00, 12.50, 15.00 and 17.5×10\(^{-5}\) mol L\(^{-1}\).
The fluorescence intensity of BSA decreased regularly with an increase in the concentration of the complexes. There was a weak red shift of emission wavelength due to the effect of the complexes on the fluorescence spectra of the BSA. This suggests that conformational changes induced by the interaction leads to an increase in the polarity around the Trp residues and decrease in hydrophobicity, which agrees with a recent study that the tertiary structure of proteins and decrease in hydrophobicity, which agrees with a recent study that the tertiary structure of proteins.

Fluorescence quenching is described by the Stern-Volmer equation,

$$F_0/F = 1 + K_q \tau_0 [Q] = 1 + K_{SV}[Q];$$

where $F_0$ and $F$ are the fluorescence intensities before and after the addition of the quencher, respectively, and $K_q$, $K_{SV}$, $\tau_0$, and $[Q]$ are the quenching rate constant, the Stern-Volmer dynamic quenching constant, the average lifetime of the biomolecule without the quencher ($\tau_0 = 10^{-8} s$), and the concentration of the quencher respectively. Fluorescence quenching proceeds via different mechanisms, usually classified as dynamic quenching and static quenching. For dynamic quenching, the maximum scatter collision quenching constant of various quenchers with the biopolymer is $2.0 \times 10^{10}$ L mol$^{-1}$ s$^{-1}$. If $K_{SV}$ is much greater than $2.0 \times 10^{10}$ L mol$^{-1}$ s$^{-1}$, it can be concluded that the quenching is not initiated by dynamic quenching, but probably partly by static quenching due to the formation of the drug-BSA complex.

For the complex-BSA systems, the Stern-Volmer graphs are presented in Fig. 5 and the values of $K_{SV}$ and $K_q (=K_{SV}/\tau_0)$ obtained from the plots at $24^\circ$C are shown in Table 1. The values of $K_q$ are larger than $2.0 \times 10^{10}$ L mol$^{-1}$ s$^{-1}$ for a variety of quenchers with biopolymers, which suggests that the fluorescence quenching mechanism mainly arises from complex formation and is governed by a static quenching mechanism, while dynamic collision is negligible in the concentration range studied$^{27,28}$.

For reconfirming the static fluorescence quenching mechanisms of CA-Hphen (HCA and phen) to BSA, the fluorescence quenching data were also analyzed according to the Lineweaver-Burk (modified Stern-Volmer or double reciprocal) equation, $F_0/\Delta F = 1/(f_s K_q [Q]) + 1/f_s$, where $\Delta F$ is the fluorescence intensity difference in the absence and presence of the quencher at concentration $[Q]$, $f_s$ is the fraction of initial fluorescence which is accessible to quencher and $K_q$ is the static fluorescence quenching association constant. The dependence of $F_0/\Delta F$ on the reciprocal value of the quencher concentration $[Q]^{-1}$ is linear with the intercept equal to the value of $f_s^{-1}$. The $f$ and $K$ values at $24^\circ$C have been listed in Table 2, which shows that all the Lineweaver-Burk plots have a better linear relationship than the corresponding Stern-Volmer plots at $24^\circ$C. This further confirms that the fluorescence quenching mechanism of CA-Hphen, HCA$^{18}$ and phen to BSA is mainly a static quenching procedure$^{29}$.

**Binding constant and binding site number**

For static quenching, the relationship between fluorescence quenching intensity and concentration of quenchers can be described by the double logarithm equation$^{30}$, $\log(F_0 - F)/F = \log K_q + n \log [Q]$. Thus, fluorescence intensity data can also be used to obtain the binding constant and the number of binding sites.

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**Table 1** — Stern-Volmer quenching constants for the interaction of compounds with BSA at $24^\circ$C

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CA-Hphen</th>
<th>HCA</th>
<th>phen</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_{SV}$ (L mol$^{-1}$)</td>
<td>6.286$x$10$^3$</td>
<td>4.653$x$10$^3$</td>
<td>3.985$x$10$^3$</td>
</tr>
<tr>
<td>$K_q$ (L mol$^{-1}$ s$^{-1}$)</td>
<td>6.286$x$10$^{11}$</td>
<td>4.653$x$10$^{11}$</td>
<td>3.985$x$10$^{11}$</td>
</tr>
<tr>
<td>$R^2$</td>
<td>0.9940</td>
<td>0.9936</td>
<td>0.9479</td>
</tr>
</tbody>
</table>

**Table 2** — The fraction of initial fluorescence ($f$) and the static fluorescence quenching association constant ($K$) according to Lineweaver-Burk plots at $24^\circ$C

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CA-Hphen</th>
<th>HCA</th>
<th>phen</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K$ (L mol$^{-1}$)</td>
<td>1.333$x$10$^4$</td>
<td>3.5294$x$10$^3$</td>
<td>1.923$x$10$^4$</td>
</tr>
<tr>
<td>$f$</td>
<td>0.75</td>
<td>0.17</td>
<td>0.26</td>
</tr>
<tr>
<td>$R^2$</td>
<td>0.9968</td>
<td>0.9939</td>
<td>0.9624</td>
</tr>
</tbody>
</table>
The values of $K_b$ and $n$ at 24 ºC, 30 ºC and 35 ºC obtained from the double logarithm regression curve (Supplementary Data, Fig. S1) are listed in Table 3.

The $n$ values are nearly 1, indicating the existence of a single binding site in BSA for the complex. The mutual dependence of binding constant and temperature for interaction of HCA with BSA decreased with increasing temperature, indicating formation of an unstable compound. This indicates that higher temperatures would weaken the binding and the reaction is exothermic\textsuperscript{18}. However, for the interaction of CA.Hphen and phen with BSA, the binding constants at 30 ºC is greater than that at 24 ºC and 35 ºC, which suggests that 30 ºC is the optimum temperature for the binding and the reaction is exothermic. The thermodynamic parameters, $\Delta H$ and $\Delta S$, for the binding reaction are the main evidence to confirm binding modes. When both $\Delta H$ and $\Delta S$ are positive, it implies a hydrophobic interaction and when both are negative, it indicates van der Waals forces and hydrogen bond formation while very low positive or negative $\Delta H$ ($\Delta H \approx 0$) and positive $\Delta S$ values are observed in electrostatic interactions\textsuperscript{32, 33}. For this reason, the temperature dependence on the binding constant was studied at three different temperatures (24, 30 and 35 ºC). The thermodynamic parameters, $\Delta G$, $\Delta H$ and $\Delta S$, calculated on the basis of the van’t Hoff equation are presented in Table 4. The negative $\Delta G$ indicates that the binding processes are spontaneous for the three compounds. The formation of HCA-BSA coordination compounds are spontaneous and exothermic reactions accompanied by a negative $\Delta H$ value. The negative $\Delta H$ and $\Delta S$ values show that both hydrogen bond and van der Waals forces play a role in the binding process to BSA. For the formation of CA.Hphen-BSA and phen-BSA complexes, both $\Delta H$ and $\Delta S$ are positive, suggesting that a strong contribution of the hydrophobic effect.

### Binding modes

The acting forces between a compound and a biomolecule are composed of weak interactions of molecules such as hydrogen bond formation, van der Waals forces, electrostatic forces, and hydrophobic interaction\textsuperscript{31}. The thermodynamic parameters, $\Delta H$ and $\Delta S$, for the binding reaction are the main evidence to confirm binding modes. When both $\Delta H$ and $\Delta S$ are positive, it implies a hydrophobic interaction and when both are negative, it indicates van der Waals forces and hydrogen bond formation while very low positive or negative $\Delta H$ ($\Delta H \approx 0$) and positive $\Delta S$ values are observed in electrostatic interactions\textsuperscript{32, 33}. For this reason, the temperature dependence on the binding constant was studied at three different temperatures (24, 30 and 35 ºC). The thermodynamic parameters, $\Delta G$, $\Delta H$ and $\Delta S$, calculated on the basis of the van’t Hoff equation are presented in Table 4. The negative $\Delta G$ indicates that the binding processes are spontaneous for the three compounds. The formation of HCA-BSA coordination compounds are spontaneous and exothermic reactions accompanied by a negative $\Delta H$ value. The negative $\Delta H$ and $\Delta S$ values show that both hydrogen bond and van der Waals forces play a role in the binding process to BSA. For the formation of CA.Hphen-BSA and phen-BSA complexes, both $\Delta H$ and $\Delta S$ are positive, suggesting that a strong contribution of the hydrophobic effect.

### Binding distances between BSA and organic complex salt

The spectral studies suggest that the interaction of CA.Hphen, HCA and phen with BSA molecules can be deduced from the regular fluorescence quenching of BSA solutions with the increase of complex concentration. The binding distances between BSA and the complex can be determined according to Föster’s non-radioactive energy transfer theory\textsuperscript{34, 35}. Figure 6 shows the overlapping of the absorption spectra of 1.00 x 10^{-5} mol L^{-1} CA.Hphen, HCA and phen with the fluorescence emission spectra of 1.00 x 10^{-5} mol L^{-1} BSA solutions in the wavelength range of 300–450 nm. The efficiency ($E$) of energy transfer can be determined as $1-F/F_0$, where $F$ is the fluorescence intensity of BSA in the presence of the complex and the concentration ratio of BSA to complex is 1:1. $E$, $R_0$ and $J$ can be calculated from the Föster’s non-radioactive energy transfer theory and the corresponding results were given in Table 5. The binding distance ($r$) between BSA and CA.Hphen, HCA and phen are found to be 3.9565, 4.6253 and 3.4298 nm, respectively. The values of $r$ are less than the reference value (8 nm), which indicates that there is a high possibility of energy transfer from BSA to the complex\textsuperscript{36}. This suggests that the

### Table 3 — Binding constants and binding sites at various temperatures for the complex-BSA system

<table>
<thead>
<tr>
<th>Comp.</th>
<th>$K_b$ (mol L\textsuperscript{-1})</th>
<th>$n$</th>
<th>$K_b$ (mol L\textsuperscript{-1})</th>
<th>$n$</th>
<th>$K_b$ (mol L\textsuperscript{-1})</th>
<th>$n$</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA.Hphen</td>
<td>3.396 x 10\textsuperscript{5}</td>
<td>0.9355</td>
<td>8.735 x 10\textsuperscript{5}</td>
<td>1.0222</td>
<td>8.224 x 10\textsuperscript{5}</td>
<td>1.0115</td>
</tr>
<tr>
<td>HCA</td>
<td>3.314 x 10\textsuperscript{5}</td>
<td>1.44</td>
<td>1.692 x 10\textsuperscript{5}</td>
<td>1.5677</td>
<td>1.044 x 10\textsuperscript{5}</td>
<td>1.3219</td>
</tr>
<tr>
<td>phen</td>
<td>1.847 x 10\textsuperscript{5}</td>
<td>1.3993</td>
<td>3.535 x 10\textsuperscript{5}</td>
<td>1.4543</td>
<td>2.369 x 10\textsuperscript{5}</td>
<td>1.3878</td>
</tr>
</tbody>
</table>

### Table 4 — Thermodynamic parameters of the benzodiazepines-BSA system

<table>
<thead>
<tr>
<th>Comp.</th>
<th>$\Delta G$ (kJ mol\textsuperscript{-1})</th>
<th>$\Delta H$ (kJ mol\textsuperscript{-1})</th>
<th>$\Delta S$ (J K\textsuperscript{-1} mol\textsuperscript{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 ºC</td>
<td>-11.442</td>
<td>-15.944</td>
<td>110.16</td>
</tr>
<tr>
<td>30 ºC</td>
<td>-13.898</td>
<td>-19.859</td>
<td>409.23</td>
</tr>
<tr>
<td>35 ºC</td>
<td>-15.944</td>
<td>-23.859</td>
<td>767.03</td>
</tr>
</tbody>
</table>

The acting forces between a compound and a biomolecule are composed of weak interactions of molecules such as hydrogen bond formation, van der Waals forces, electrostatic forces, and hydrophobic interaction. The thermodynamic parameters, $\Delta H$ and $\Delta S$, for the binding reaction are the main evidence to confirm binding modes. When both $\Delta H$ and $\Delta S$ are positive, it implies a hydrophobic interaction and when both are negative, it indicates van der Waals forces and hydrogen bond formation while very low positive or negative $\Delta H$ ($\Delta H \approx 0$) and positive $\Delta S$ values are observed in electrostatic interactions. For this reason, the temperature dependence on the binding constant was studied at three different temperatures (24, 30 and 35 ºC). The thermodynamic parameters, $\Delta G$, $\Delta H$ and $\Delta S$, calculated on the basis of the van’t Hoff equation are presented in Table 4. The negative $\Delta G$ indicates that the binding processes are spontaneous for the three compounds. The formation of HCA-BSA coordination compounds are spontaneous and exothermic reactions accompanied by a negative $\Delta H$ value. The negative $\Delta H$ and $\Delta S$ values show that both hydrogen bond and van der Waals forces play a role in the binding process to BSA. For the formation of CA.Hphen-BSA and phen-BSA complexes, both $\Delta H$ and $\Delta S$ are positive, suggesting that a strong contribution of the hydrophobic effect.

The spectral studies suggest that the interaction of CA.Hphen, HCA and phen with BSA molecules can be deduced from the regular fluorescence quenching of BSA solutions with the increase of complex concentration. The binding distances between BSA and the complex can be determined according to Föster’s non-radioactive energy transfer theory. Figure 6 shows the overlapping of the absorption spectra of 1.00 x 10^{-5} mol L^{-1} CA.Hphen, HCA and phen with the fluorescence emission spectra of 1.00 x 10^{-5} mol L^{-1} BSA solutions in the wavelength range of 300–450 nm. The efficiency ($E$) of energy transfer can be determined as $1-F/F_0$, where $F$ is the fluorescence intensity of BSA in the presence of the complex and the concentration ratio of BSA to complex is 1:1. $E$, $R_0$ and $J$ can be calculated from the Föster’s non-radioactive energy transfer theory and the corresponding results were given in Table 5. The binding distance ($r$) between BSA and CA.Hphen, HCA and phen are found to be 3.9565, 4.6253 and 3.4298 nm, respectively. The values of $r$ are less than the reference value (8 nm), which indicates that there is a high possibility of energy transfer from BSA to the complex. This suggests that the
binding of CA-Hphen, HCA and phen to BSA molecules was formed through energy transfer and the fluorescence quenching of BSA was a non-radiation transfer process.

**Conformation investigations**

The synchronous fluorescence spectra provide information about the molecular environment in the vicinity of the fluorophore functional groups. The spectra were obtained through the simultaneous scanning of the excitation and the emission monochromators of a spectrofluorimeter, with a fixed wavelength difference ($\Delta\lambda$) between them. When the $\Delta\lambda$ between excitation wavelength and emission wavelength was stabilized at 15 or 60 nm, the synchronous fluorescence was characteristic of tyrosine residues or tryptophan residues.

In the present work, the synchronous fluorescence spectra of BSA at various complex concentrations show that when $\Delta\lambda = 60$ nm, the addition of the complexes leads to a decrease in the synchronous fluorescence intensity with the red-shifts of the spectral peak (Supplementary Data, Fig. S2). This indicates that the polarity around the Trp residues increases and the hydrophobicity decreases with increase in concentration of the complexes. On the other hand, the synchronous fluorescence spectra for $\Delta\lambda = 15$ nm, the emission peaks show essentially no shift over the investigated concentration range, which indicates that CA-Hphen, HCA and phen have little effect on the micro-environment of tyrosine residues in the BSA (Supplementary Data, Fig. S2).

**Conclusions**

In summary, we have determined the crystal structure of the complexes formed by CA and 1,10-phenanthroline with a 1:1 stoichiometry. The complex unit contains one 1,10-phenanthroline molecule and one CA molecule, and the CA molecules are connected to each other through hydrogen bonds to form a 2D supramolecular sheet. The Hphen is adjunction to the 2D sheet through the hydrogen bonds formed by carboxyl oxygen and a phen nitrogen atom. The results indicate that the fluorescence quenching mechanism of BSA by CA-Hphen, CA and phen are consistent with the static model, and the binding reaction is spontaneous. Hydrogen bonds and van der Waals forces play a major role in the complex-BSA interaction. The bindings of compounds to BSA molecules are formed through non-radioactive energy transfer.

<table>
<thead>
<tr>
<th>Comp.</th>
<th>$E$ (%)</th>
<th>$R_0$ (nm)</th>
<th>$J$ (cm$^3$ L mol$^{-1}$)</th>
<th>$r$ (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA-Hphen</td>
<td>6.80</td>
<td>2.5578</td>
<td>1.03×10$^{-14}$</td>
<td>3.9565</td>
</tr>
<tr>
<td>HCA</td>
<td>3.03</td>
<td>2.5959</td>
<td>1.11×10$^{-14}$</td>
<td>4.6253</td>
</tr>
<tr>
<td>phen</td>
<td>2.92</td>
<td>1.9129</td>
<td>1.79×10$^{-15}$</td>
<td>3.4298</td>
</tr>
</tbody>
</table>

Fig. 6 — Spectral overlap of fluorescence ($\lambda_{em} = 295$ nm) of BSA solution and absorption of (a) CA-Hphen, (b) HCA and (c) phen.
energy transfer. Synchronous fluorescence spectra show that the secondary structure of the BSA molecules is affected significantly in the presence of the complexes.

Supplementary Data
CCDC 760962 contains the supplementary crystallographic data for CA-Hphen. The data can be obtained free of charge from The Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB21EZ, UK. Email: deposit@ccdc.com.ac.uk; fax (+44) 1223-336-033. Other supplementary data associated with this article, viz., Table S1 and Figs S1 and S2, are available in the electronic form at http://www.niscair.res.in/jinfo/ijca/IJCA_52A(02)192-199.SupplData.pdf.

Acknowledgement
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22 Synthesis of CA-Hphen: To an ethanol aqueous solution of 1,10-phenanthroline (10 mL) and the mixture was left for evaporating at room temperature to obtain yellow block crystals after several days.


