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Interaction of solifenacin succinate with bovine serum albumin by spectroscopic techniques and molecular modeling

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The interaction of Solifenacin succinate (SFS) with bovine serum albumin (BSA) has been investigated by UV-visible spectrophotometric, spectrofluorometric viscometric and molecular modeling methods. Fluorescence spectra suggested that the quenching mechanism of the interaction of SFS to BSA was a static quenching type. By the analysis of UV/vis spectra, it was observed that SFS had a high affinity with BSA which was indicated by the large binding constant. The thermodynamic parameters, ΔH and ΔS for the interaction of SFS and BSA have been calculated at different temperatures. The thermodynamic studies suggested that the interaction processes were endothermic disfavoured ($\Delta H > 0$) and entropy favoured ($\Delta S > 0$), which indicated that the SFS might interact with BSA by a non-traditional intercalation mode of binding *via* hydrophobic force. Moreover, the results obtained from molecular docking corroborate the experimental results obtained from spectroscopic investigations.

Keywords: BSA, Fluorescence, Molecular modeling, Solifenacin succinate, UV-visible

Solifenacin succinate is an orally administered urinary antispasmodic anticholinergic drug. The chemical name of Solifenacin succinate is 1-Azabicyclo[2.2.2]octan-8yl (1S)-1-phenyl-3,4-dihydro-1H-isoquinoline-2-carboxylate butanedioic acid (Fig. 1)[1]. Solifenacin is a competitive muscarinic acetylcholine receptor antagonist. The binding of acetylcholine to these receptors, particularly the M_3 receptor sub type, plays a critical role in the contraction of the smooth muscle. By preventing the binding of acetylcholine to these receptors, solifenacin reduces the smooth muscle tone in the bladder, allowing the bladder to retain larger volumes of urine and reducing the number of micturition, urgency and incontinence episodes[2,3]. A literature survey reveals one HPLC[4], one mass spectrometry[5] and one spectrophotometric method[6] for the assay of SFS. There has not any report about the interaction study of SFS with BSA based on spectroscopic behaviour and molecular modeling.

The binding mechanism of proteins with ligands from last few years have shed light on different areas of research and its important applications in numerous fields of science, such as the development of new biomaterials, biochemistry, food chemistry or pharmaceutical sciences[7,8]. The nature of interaction between the drug molecule and protein gives new opportunity for the development of new drugs. Since drugs are the compounds which are carried by albumin, it is necessary to study the interaction of new drug with protein[9]. Serum albumins are a model globular protein which plays a key role for acting as a carrier for several endogenous compounds. It has proved the most valuable invented globular proteins, used for transportation and metabolism of many biologically active compounds in the body[10].

Serum albumin has a well-established structure, having physicochemical properties, a versatile binding capacity stability and water solubility[11]. In the present scenario bovine serum albumin (BSA) has created an extensive area of research because of its

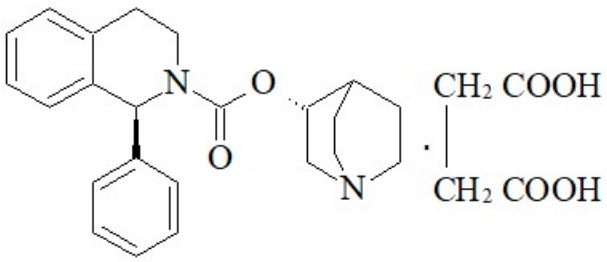

Fig. 1 — Chemical structure of Solifenacin Succinate

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presence in blood plasma of animals. It consist a single polypeptide chain of 583 amino acids and contains 17 cysteine residues (eight disulfide bonds and one free thiol). It is divided into three specific binding sites (I, II and III) for high-affinity of drugs. Every sites is consists of two subdomains (A and B). Human serum albumin (HSA) contributes about 80% of the osmotic pressure of blood[12]. It bears residue of 585 amino acids with molar mass of 66,411 g mol^{-1} having 17 disulfide bridges and free thiol (SH) group. Moreover, HSA consist of single tryptophan (TRP 214) present in subdomain IIA[13]. The theme has a better insight for research fields such as clinical medicine, chemistry and life sciences.

In the present work, the interaction of SFS with BSA has been investigated under imitated physiological conditions (pH 7.4) *via* ultraviolet-visible, fluorescence, viscometric and molecular modeling techniques. The binding constants, number of binding sites and thermodynamic parameters have been calculated and the binding site of SFS on BSA was identified by fluorescence displacement experiments. Moreover, the molecular modeling was used to improve the understanding of the interaction of SFS with BSA. These results should be useful for understanding the interaction of SFS with BSA at the molecular level and be useful for the design of new drugs.

Materials and Methods

Chemicals and Materials

A stock solution 1×10^{-3} mol L^{-1} of SFS was prepared by dissolving its powder in double distilled water. BSA was purchased from S. D. fine-chem Limited and its stock solution was prepared by dissolving an appropriate amount in distilled water and stored at 4°C. A buffer solution of pH 7.4 was prepared by following the standard methods. All other chemicals used were of analytical reagent grade. Doubly distilled deionised water was used throughout.

Instrumentation

UV-vis absorption spectra were measured by using Ellico UV-visible spectrophotometer (INDIA) and Fluorescence measurements were performed by using Hitachi F4500 spectrofluorimeter (JAPAN) equipped with a 150W Xenon lamp and 1 cm quartz cell. The widths of excitation and emission slits were set at 5.0 and 10 nm, respectively, and the scan rate was 1200 nm min^{-1}. The viscosity measurements were carried out using Ostwald viscometer. The pH measurements were made with Scott Gerate pH meter CG 804. An electronic thermostat water-bath was used for controlling the temperature. Molecular Docking Study of Solifenacin with Bovine serum albumin was performed with flexible molecular docking program Surflex-Dock implemented in sybyl-X v1.2. Target protein structure of Bovine serum albumin was retrieved from protein database of PDB ID:4F5S. The protein preparation was carried out by two steps, preparation and refinement. After ensuring chemical correctness, water molecules in the crystal structures were deleted and hydrogens were added where hydrogen atoms were missing. Using the AMBER FF99 force field energy of crystal structure was minimized. Protomol were defined centering them on the ligand in the crystal structure using the default box size. The solifenacin ligands were built using Sybyl build panel and minimized using tripos force field then docked into binding site of Bovine serum albumin protein.

Results and Discussion

UV-Visible Spectroscopic study

Figure 2 showed the UV/vis absorption spectra of SFS in the absence and presence of different concentrations of BSA. The maximum absorbance of SFS was located at around 256 nm. It was observed that on the addition of BSA, SFS showed an increase in molar absorptivity with a red shift. This hypochromic effect is thought to be due to the properties of BSA-drug interaction which are closely related with double helix structure[14].

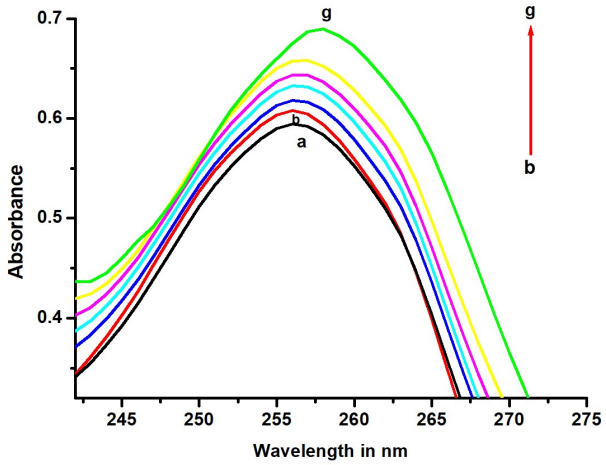

Fig. 2 — UV-visible spectra of (a) 1×10^{-4} M SFS in the absence of BSA and in presence of C_{BSA}= 2.0, 4.0, 6.0, 8.0, 10.0, 12.0 μML^{-1} of BSA (b to g) in PBS buffer of pH 7.4

The hyperchromism which indicates a strong interaction between the electronic ground states of SFS with BSA and changes in the absorption bands and small bathochromic shifts shows that the binding between SFS and BSA was intercalation mode[15]. Generally, the red shift (or blue shift), hyperchromic (or hypochromic) changes in absorbance and wavelength shifts of this characteristic band reflect the corresponding structural changes of the BSA, including changes of stacking pattern, disruption of the hydrogen bonds between complementary strands, covalent binding of BSA bases, intercalation between aromatic rings of molecules, *etc*[16-19]. The hyperchromic effect and bathochromic shift indicates that to confirm the changes of BSA double helix structure after interacting with the SFS and observing the non-traditional intercalating mode.

Based on the variations in the absorbance spectra of SFS upon binding to BSA, the binding constant (K) was calculated according to the Eq. 1.

$$\frac{A_0}{A-A_0}=\frac{\varepsilon_G}{\varepsilon_{H-G}-\varepsilon_G}+\frac{\varepsilon_G}{\varepsilon_{H-G}-\varepsilon_G}\times\frac{1}{K[BSA]} \qquad \ldots (1)$$

Where, A_0 and A are the absorbances of drug in the absence and presence of BSA, ε_G and ε_{H-G} are the absorption coefficients of drug and its complex with BSA, respectively. The plot of $A_0/(A-A_0)$ versus 1/[BSA] was constructed using the data from the absorbance titrations was shown in Fig. 3)

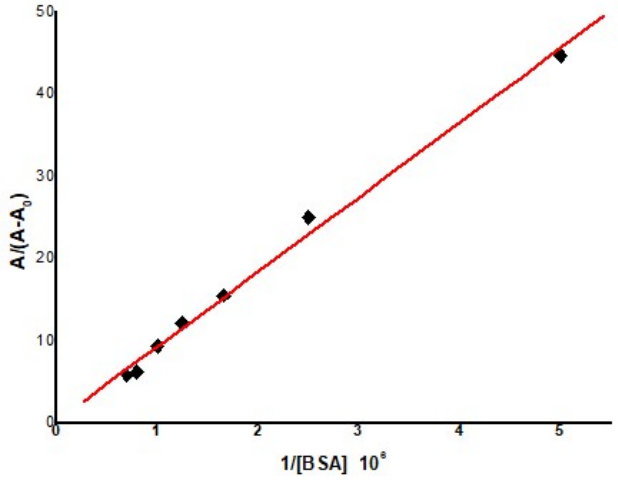

Fig. 3 — Plot of $(A_0 / (A-A_0)$ *versus* 1/ [BSA] for SFS-BSA system

From the linear fitting, the binding constant (K) can be estimated from the ratio of the intercept to the slope. With this procedure, we calculated K = 2.253 × 10^5 M^{-1}. It is seen that the fitting is good linear and the value of K determined here is consistent with that reported for the BSA binding to SFS (K ≈ 10^3 - 10^5)[20]. This indicates that the SFS display a relative high affinity with BSA[21].

Thermodynamic parameters and nature of Binding forces

The interactions forces between drugs and biomolecules may include electrostatic interactions, multiple hydrogen bonds, van der Waals interactions, hydrophobic, steric contacts within the antibody-binding site, *etc*. In order to elucidate the interaction of SFS with BSA, the thermodynamic parameters were calculated from the van't Hoff plots.

Thermodynamic parameters are employed in different temperatures to analyze the acting forces between SFS and BSA. The binding forces between drugs and biomolecules mainly include electrostatic interaction, hydrogen bond, van der Waals interaction, and hydrophobic interaction[22]. The different drugs may have different types of binding forces on interaction of protein. Thermodynamic parameters, free energy change (ΔG), enthalpy change (ΔH) and entropy change (ΔS) of interaction are essential to interpret the binding mode of BSA-drug complexes. When the temperature does not vary significantly, the reaction enthalpy can be considered as a constant. Its value can be evaluated from the Clausius-Clapeyron Eqs:

$$\ln (K_2/K_1) = - \Delta H /R [(1/T_2) - (1/T_1)] \qquad \ldots (2)$$

$$\Delta G = - RT \ln K \qquad \ldots (3)$$

$$\Delta G = \Delta H - T\Delta S \qquad \ldots (4)$$

where, R is the gas constant, T is the experimental temperature, and K is the binding constant at the corresponding T. The Gibbs free energy change (ΔG) and the entropy change (ΔS) can be obtained from Eqs. 3 and 4, respectively. The value of ΔH can be calculated from the slope and intercept of the regression curve of *ln* K *vs*. 1/T, ΔG and ΔS values are consequently obtained according to (3) and (4), respectively. The values of ΔH, ΔS and ΔG for the interaction of SFS with BSA are presented in Table 1. The value of free energy (ΔG) of the

Table 1 — Binding constants and thermodynamic parameters for the interaction of SFS and BSA

Temperature in K	Binding constant (K_b)	ΔH (J mol^{-1})	ΔG (J mol^{-1})	ΔS (J mol^{-1} K^{-1})	R^2
287	1.1025×10^5		-1.205×10^3	133.38	0.99673
297	6.3822×10^5	37.077×10^3	-22.888×10^3	195.32	0.99681
307	2.2528×10^5		-11.452×10^3	163.39	0.99773

interaction between SFS and BSA at different temperature (287, 297 and 307 K) were all negative, which revealed that the processes of the interaction between SFS and BSA was spontaneous. The values of ΔH and ΔS suggested that the interaction process was endothermic disfavoured ($\Delta H > 0$) and entropy favoured ($\Delta S > 0$). When $\Delta H < 0$ or $\Delta H \approx 0$, $\Delta S > 0$, the mainly acting force is electrostatic; when $\Delta H < 0$, $\Delta S < 0$, the mainly acting force is van der Waals or hydrogen bond and when $\Delta H > 0$, $\Delta S > 0$, the mainly force is hydrophobic. For the binding system of SFS with BSA, the ΔH and ΔS values were positive. Therefore, in the case of the present system, we presumed that hydrophobic interaction might be the main acting force in the binding of the SFS and BSA. If $\Delta G < 0$ indicates the binding process is spontaneous, $\Delta S > 0$ is an evidence of hydrophobic interactions[23,24]. Therefore, the hydrophobic interaction might play a major role in the interaction of SFS with BSA.

Spectrofluorimetric study

The fluorescence spectra of SFS in presence of different concentrations of BSA are shown in (Fig. 4). SFS shows strong fluorescence emission peak at 460 nm and the fluorescence intensity increases gradually with increasing concentration of BSA, which indicates that BSA can interact with SFS and quench its intrinsic fluorescence. Furthermore, there is a slight red shift of maximum emission wavelength occurring with the addition of BSA, implying that the microenvironment around the chromophore of SFS is changed. The fluorescence intensity increases due to increase in the molecular planarity of the complex and decreases the collision frequency of solvent molecules with SFS. This is due to diffusion which occurs between adjacent base pairs of BSA[25]. The binding constant was calculated according to Stern-Volmer Eq.:

$$F_o/F = 1 + k_q \tau_o [Q] = 1 + K_{sv}[Q] \quad \ldots (5)$$

where, F_o and F are the fluorescence intensities in absence and presence of BSA, respectively, [Q] is the concentration of quencher, k_q is the quenching rate constant, τ_o is the average life time of biomolecule without quencher and its value 10^{-8} s and K_{sv} is the Stern-Volmer quenching constant. The values of K_{sv} and K_q can be determined from the slope of regression curve F_o/F *vs.* [Q] (Fig. 5).

The binding constant K_{sv} and K_q values calculated were found to be 7.273×10^4 L mol^{-1} and 7.273×10^{12} L mol^{-1} s^{-1} ($R^2 = 0.99876$), respectively. The maximum rate constant of collisional quenching K_q of various quenchers with biopolymers[26] is about 2.0×10^{10} L mol^{-1} s^{-1}, which suggests that the fluorescence quenching process may be mainly controlled by static quenching mechanism rather than dynamic.

The Binding Constant and Number of Binding Sites

The binding constant and number of binding sites for SFS-BSA were determined by the following Eq[26].

$$\log\left[\frac{(F_0 - F)}{F}\right] = \log K_b + n\log[Q] \quad \ldots (6)$$

where, K_b is binding constant and n is number of binding sites in base pair units, respectively. The

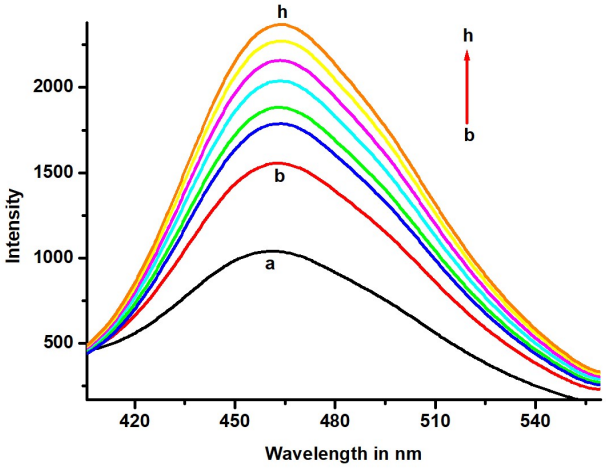

Fig. 4 — Fluorescence spectra of a) 1.5×10^{-4} M SFS in the absence of BSA and the presence of C_{BSA}= 5.0, 10.0, 15.0, 20.0 25.0, 30.0, 35.0 μM L^{-1} of BSA (b to h) in PBS buffer of pH-7.4

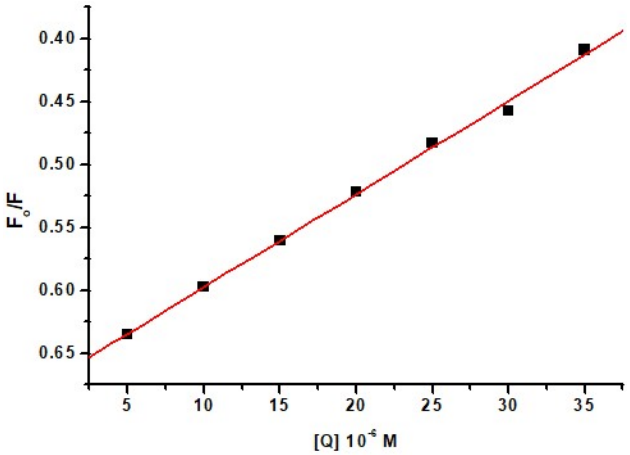

Fig. 5 — Stern-volmer plot of (F_0 / F) *vs* [Q] for SFS-BSA system

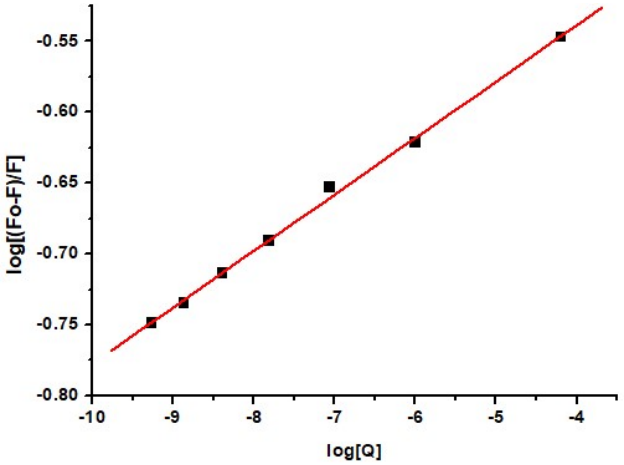

Fig. 6 — Plot of log [(F_0 - F)/F] *vs* log [Q] for SFS-BSA system

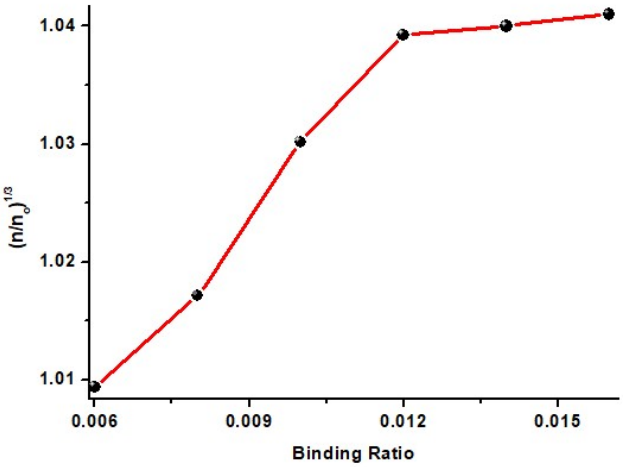

Fig. 7 — Effect of increasing the concentration of BSA on the relative viscosity of SFS

values of n and Kb can be determined from the slope and intercept of the double logarithm regression curve (log [(F_0 - F)/F] *vs* log [Q]) (Fig. 6). The binding constant value 3.794 × 10^4 L mol^{-1} (R^2 = 0.99879) and values of n approximately equal to unity, indicating that there is one independent class of binding sites in BSA for SFS.

Viscosity Measurements

Viscosity experiment is an effective tool to study the binding mode of small molecules to BSA. The interaction between SFS and BSA, we carried out viscosity measurements at room temperature. A classical intercalation binding demands the space adjacent base pairs to be large enough to accommodate the bound ligand and elongate the double helix, resulting in an increase of BSA viscosity while a non-classical intercalation or a groove mode would reduce the BSA viscosity[28]. The viscosity measurements were taken by varying the concentration ratio of BSA and SFS. The values of relative specific viscosity $(\eta/\eta_0)^{1/3}$ *vs*. [SFS] / [BSA] were plotted in the absence and presence of BSA as shown in (Fig. 7).

It was observed that, the relative specific viscosities of BSA exhibited a dependence on the concentration of SFS, which increases with the value of [SFS]/[BSA]. The behaviour indicates that non-classical intercalation mode of binding and possibly a groove binding *via* hydrophobic interaction between SFS with BSA.

Molecular modeling of Solifenacin succinate with Bovine serum albumin

To investigate the detailed intermolecular interactions between the ligand and the target protein

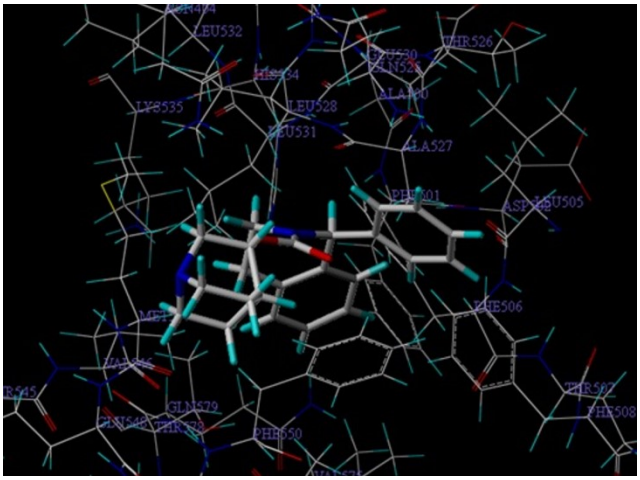

Fig. 8 — Amino acid residues involved in the binding of SFS to BSA

docking studies was carried out using Sybyl-X v1.2. Molecular Docking Study of SFS with BSA was performed with flexible molecular docking program Surflex-Dock implemented in sybyl-X v1.2. Surflex-Dock uses an empirical scoring function (based on the Hammerhead docking system) that has been updated and re-parameterized with additional negative training data5, along with a search engine that relies on a surface based molecular similarity method.

The Docked conformation of the most active pose of SFS in binding site shows total docking score of 4.0428. Docking pose of compound showing hydrophobic interactions with receptor active site residues PHE 501, LEU505, LEU531, ALA527, GLN 579 (Fig. 8). Hydrophobic interaction play important role in binding of SFS with BSA (Fig. 9).

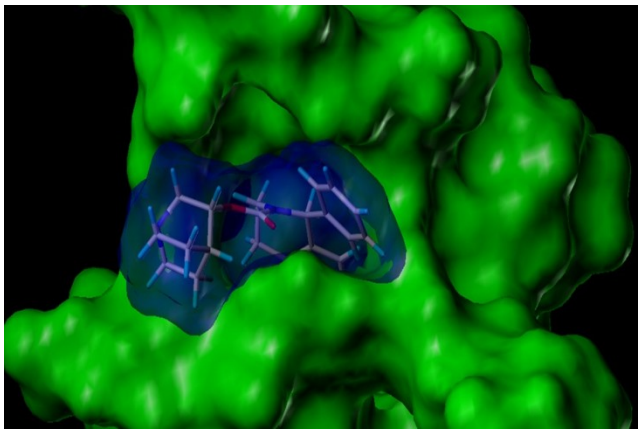

Fig. 9 — Molecular model of the surface and the active site of SFS (stick) and BSA (green surface)

Conclusion

Interaction mechanism of SFS with BSA has been investigated by fluorescence spectroscopic, UV-vis spectroscopic, Viscometric methods and molecular modeling techniques. The results show that SFS interact with BSA by a non-traditional intercalation mode via hydrophobic force. The thermodynamic studies suggested that the binding between BSA and SFS is spontaneous ($\Delta G < 0$). The binding process is endothermic disfavoured ($\Delta H > 0$) and entropy is favoured ($\Delta S > 0$), this is the evidence of main interaction forces are hydrophobic forces accompanied by hydrogen bonds and electrostatic interactions. The molecular modeling results revealed that intercalated mechanism is followed by SFS to bind with BSA and thus will be very helpful to the design of new drug.

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Conflict of interest

All authors declare no conflict of interest.

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