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The Effect of Caffeine on the interaction between Bovine Serum Albumin and Losartan potassium by Spectroscopic Methods

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ABSTRACT

Losartan potassium (LP) is an antihypertensive drug, and its binding to plasma proteins determines its pharmacokinetic properties. An exogenous substance like Caffeine (CAF) may affect the binding of drugs to proteins. Accordingly, we aim to characterize LP and bovine serum albumin (BSA) interactions in CAF presence employing spectroscopic techniques (UV-vis) absorption and fluorescence measurements) under physiological circumstances. Our findings indicated that LP's quenching effect on BSA decreased by adding CAF by calculating the Stern-Volmer constant. Therefore, the binding constants for the BSA-LP and BSA-CAF-LP systems were determined using the Modified Stern-Volmer equation; the results were 4.47×103 and 0.23×103, respectively.

Thermodynamic parameters, the Gibbs free energy (∆G), enthalpy (∆H), and entropy (∆S) calculated for each system show spontaneous interaction for both systems and suggest that the binding interaction between BSA and LP is exothermic and entropy is unfavorable, while for BSA-CAF-LP is endothermic and entropy is favorable.

1. Introduction

The drug and protein interactions within the bloodstream significantly affect the pharmacodynamics and pharmacokinetics of therapeutic molecules(1,2). The degree to which a pharmaceutical compound interacts with a protein determines its stability and potential for toxicity(1). About 60% of the total protein content in the human body is made up of albumin, so it is considered the main blood plasma protein(3). Albumin transports unesterified fatty acids, metabolites, organic compounds, dyes, and drugs(4) thereby modulating their delivery, distribution, and efficiency(5). Human serum albumin (HSA) and bovine serum albumin (BSA) share structural similarities (6,7). Both proteins possess three unique drug binding sites: I, II, and III in subdomains IIA, IIIA, and IB, respectively(8–11). In addition to its homogeneity, this substance exhibits other notable attributes, including its exceptional affinity for ligand binding, accessibility, and affordability(12). In our work, we have chosen BSA as a protein model.

Losartan Potassium (LP) is a pharmacological agent utilized to treat hypertension. Its mechanism of action involves the selective and competitive inhibition of type 1 (AT1) angiotensin II receptors, resulting in a reduction of blood pressure(13,14).

LP exhibits a strong affinity for serum albumin, with a binding capacity of around 98.8% (15,16). At a lower drug-to-BSA ratio, LP exhibits a greater affinity for subdomain IIIA in site II of the BSA molecule compared to subdomain IIA in site I. In contrast, when the ratio is increased, it exhibits binding affinity towards both locations (I &II)(15–17).CAF is a natural substance found in the seeds and fruits of more than 63 plant species globally(18,19), coffee(20), cocoa beans(21,22), chocolate(23), cola nuts(24), tea(25), and pharmacologically active substance(26). According to existing literature, it has been established that CAF is the most extensively ingested psychoactive compound on a global scale(27,28). CAF has been revealed to exhibit binding affinity towards site I, corresponding to subdomain IIA of BSA(29–32). As with any exogenous substances, it can impact the pharmacokinetic characteristics of medicines through their reversible binding to serum albumin(33,34). The present paper aims to explore the potential interaction of caffeine with bovine serum albumin (BSA) in the presence of losartan potassium through spectroscopic techniques (UV-visible and fluorescence measurements), to find if there is any hindrance effect of caffeine on the binding of the antihypertensive drug with BSA. There are no previous data on the effect of caffeine on the binding of LP with BSA.

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2. Materials and methods

2.1. Chemicals and reagents

Our study obtained lyophilized BSA powder from Sigma Aldrich (www.sigmaaldrich.com) with a purity of at least 96%, ensuring it was free from any fatty acids. The LP sample was received as a complimentary sample from Mepaco-Pharma (www.mepaco-pharma.net). The CAF compound was purchased from Sisco Research Laboratories (www.srlchem.com). The structures of these compounds are provided in Figure 1. Tris-HCl buffer was obtained from Sigma Aldrich (www.sigmaaldrich.com).

2.2. Sample preparation

Herein, we dissolved 70 mg of BSA in a Tris-HCl buffer (0.1 M, pH 7.4) to prepare a BSA stock solution (0.062 mM), stored at 4°C. The molar concentration of BSA was calculated using a spectrophotometric approach, utilizing the molar extinction coefficient of BSA (43,824 M^{-1} cm^{-1}) at 280 nm(35,36). Stock solutions of (1mM) for each LP and CAF were prepared by dissolving 4.61 and 1.94 mg in Tris-HCl buffer (0.1 M, pH 7.4).

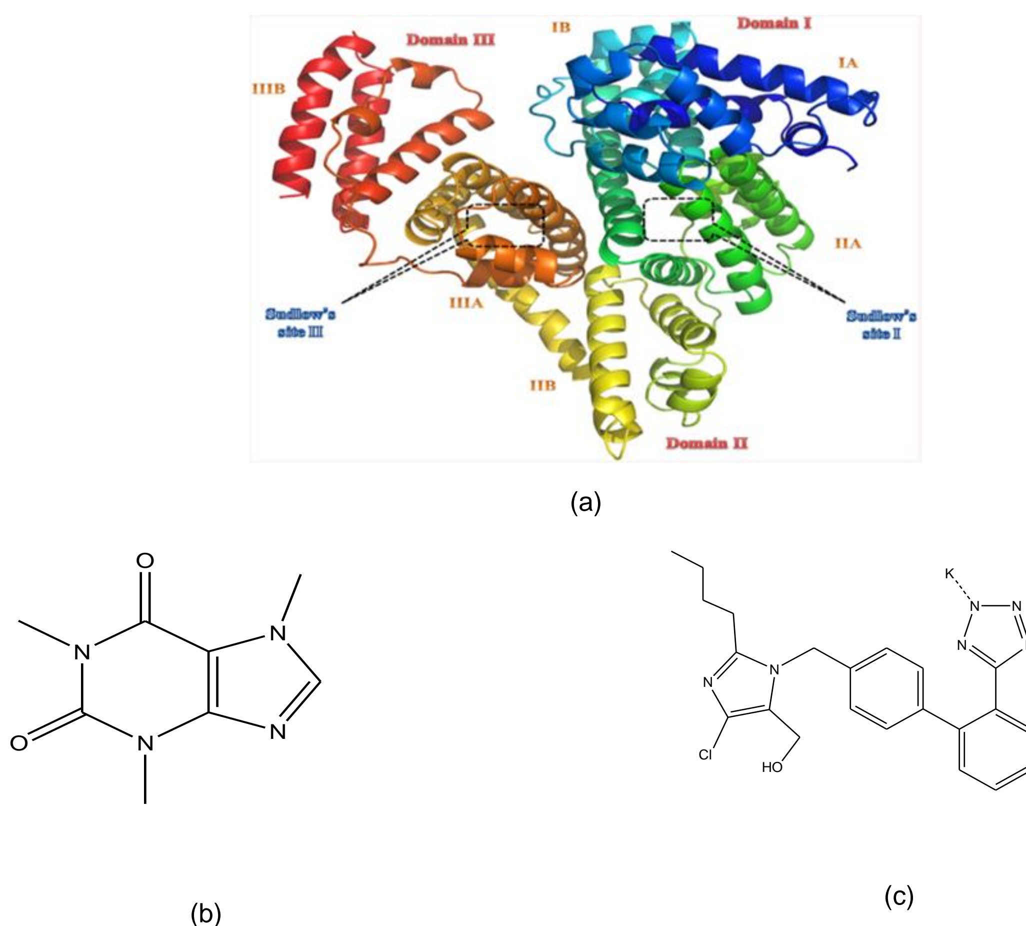


Fig. 1. Chemical structure of (a) BSA structure (PDB ID: 3 V03), (b) Caffeine, and (c) Losartan potassium.

2.3. Spectroscopic studies

2.3.1. Fluorescence measurements

The fluorescence measurements were carried out using a Jasco FP-6300 spectrofluorometer (https://jscoinc.com) with a 150 W Xenon lamp and a quartz cell with a 1.00 cm path length. The aim was to monitor the influence of CAF on the binding of LP with BSA. BSA was kept at a constant concentration of 1 μM and was titrated with various concentrations of LP (0-24 μM) in Tris-HCl buffer at room temperature. Then a solution consisting equimolar of BSA and CAF was titrated by increasing the LP concentration from 0-24 μM in Tris-HCl buffer at RT. Subsequently, every sample was stimulated at 280 nm, measuring the resulting emission spectra within 290–420 nm. The measurements were conducted using medium response, high sensitivity, and a bandwidth of 5 nm for excitation and emission. Scanning curves were created for each experiment. The data underwent analysis

and processing using Origin Lab 2019 software. Moreover, the temperature effect was observed through repeated steady-state fluorescence measurements at 298, 303, 308, 313, and 318 K.For the BSA-LP system, BSA of 1 μM was incubated with and without various LP concentrations (1, 2, 3, 4, 5, 6, and 7 μM). In contrast, for BSA-CAF-LP interactions, BSA-CAF was incubated alone and with different amounts (1 μM to 7 μM) of LP. Then, the samples were subjected to excitation at 280 nm, followed by recording the emission spectra at 345nm. Stern-Volmer equations were employed for analyzing the data(12,37).

$$\frac{Fo}{F} = 1 + Ksv[Q] \quad (1)$$

$$Kq = \frac{Ksv}{\tau o} \quad (2)$$

F_0 and F refer to fluorescence intensity without and with a quencher, respectively; K_{SV} is the Stern–Volmer quenching constant; Kq refers to the bimolecular rate constant of the quenching reaction; [Q] is quencher concentration; and τo is the protein average integral fluorescence lifetime without quencher, which is in the order of 10^{-8}s(38). The binding constant and the number of binding sites were studied by the modified Stern-Volmer equation (39,40).

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

F_0 and F refer to fluorescence intensity without and with a quencher, respectively; K is the binding constant; n is the number of binding sites; and [Q] is the quencher concentration.

$$lnK_b = -\left(\frac{\Delta H}{RT}\right) + \left(\frac{\Delta S}{R}\right) \quad (4)$$

$$\Delta G = \Delta H - T\Delta S = -RlnK_b \quad (5)$$

ΔH and ΔS are the enthalpy and entropy changes, respectively; K is the binding constant at the corresponding temperature; and R is the gas constant (41).

2.3.2. UV-Vis studies

Ultraviolet-visible (UV-vis) absorption spectroscopy is used for understanding protein-ligand interactions(42). Conformational changes of the protein can be checked through UV-vis spectral examinations.UV-1800 Shimadzu double-beam UV-Vis spectrophotometer with a 1.00 cm quartz cell (https://www.shimadzu.com) was employed to measure the absorption spectra. BSA (0.01mM) was titrated by LP (0.01mM) and followed by the same concentration of CAF. The absorption of free CAF and LP are also taken into account. The samples were prepared in a Tris-HCl buffer with a pH of 7.4. Measurements were estimated within the wavelength range of 220-320.

3. Results and Discussion

3.1. Fluorescent measurements

3.1.1 Binding of BSA with LP in the absence and presence of CAF

Previous studies have demonstrated the binding of both LP and CAF to BSA in subdomain IIA at site I. Therefore, our objective was to investigate any possible influence of CAF on LP-BSA binding. LP was found to reduce the fluorescence of BSA when CAF is not present with a quenching constant value of 12.7 × 10^4 mol^{-1}L at 298 K. On the other hand, when CAF is present the quenching constant value was 6.27 × 10^4 mol^{-1}L at 298 K (Figure 2.3). The decrease in the quenching ability of LP, as shown by the significant decrease in the Ksv value, when CAF is present compared to BSA-LP alone, suggests that CAF and LP compete for binding at site I in domain IIA. As a result, the interaction of LP with BSA in the presence of caffeine is reduced, likely due to the probable binding of caffeine with losartan potassium. This greatly decreases the binding of the LP drug with BSA, especially at room temperature. To conduct a more comprehensive analysis of the impact of CAF on the interaction between BSA and LP, the binding constant values were determined by employing a modified Stern-Volmer equation. The binding constant was found to be 4.47×10^3 for BSA-LP and 0.23×10^3 for BSA-CAF-LP Figure 4, suggesting that CAF hinders the binding of LP to BSA.

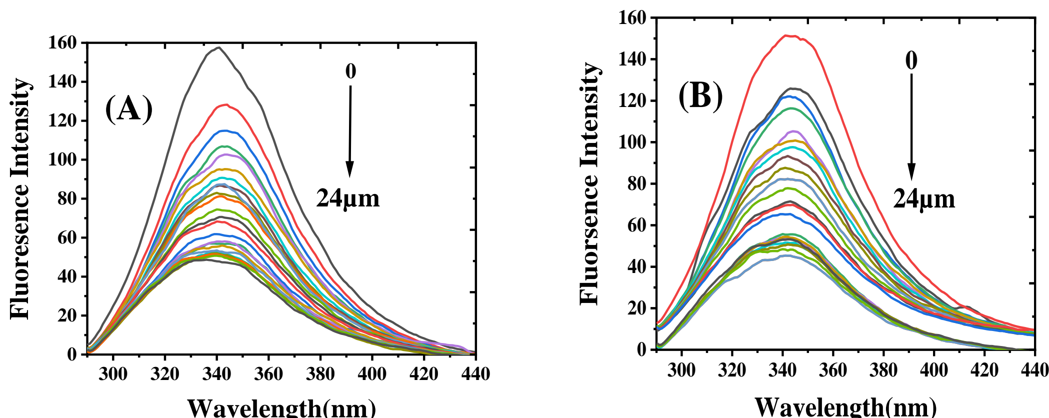
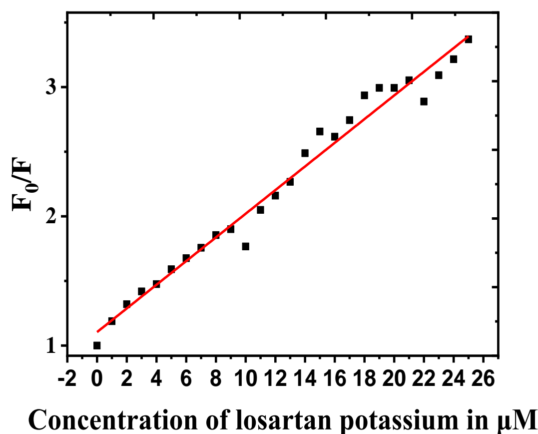
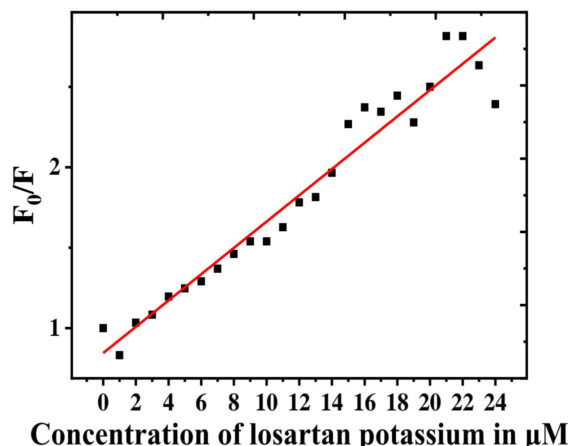


Fig. 2. Emission spectra of (A) 1μM BSA in the presence of various concentrations of Losartan Potassium and (B) BSA-CAF system through various Losartan Potassium concentrations in Tris-HCl buffer=7.4.

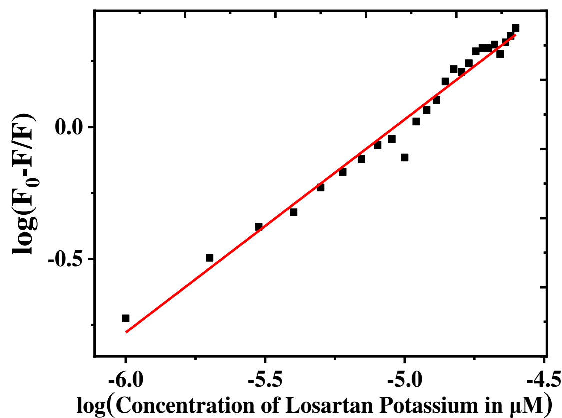


(A)

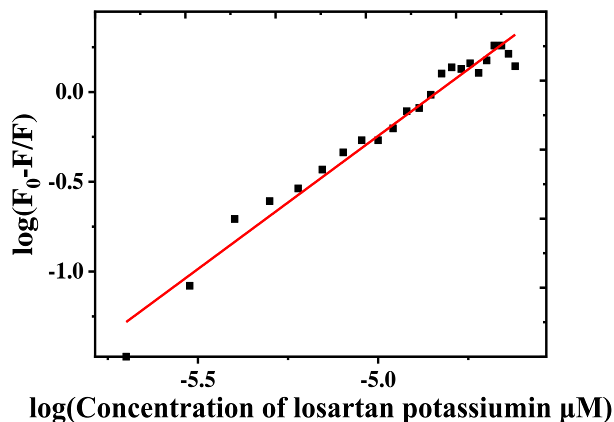


(B)

Fig. 3. Stern Volmer plot for (A) the binding of LP with BSA in Tris-HCl buffer=7.4. (B) the binding of BSA with losartan potassium in the presence of caffeine in Tris-HCl buffer=7.4.



(A)



(B)

Fig. 4. Modified Stern Volmer plot for (A) the binding of LP with BSA Tris-HCl buffer pH=7.4 and (B) binding of BSA with losartan potassium in the presence of caffeine in Tris-HCl buffer=7.4.

3.1.2 Determination of the quenching mechanism of BSA-LP and BSA-CAF-LP system

The decrease in fluorescence intensity results from various molecular interactions, including the formation of ground state complexes, rearrangement of excited state molecules, and energy transfer. Dynamic quenching, closely tied to diffusion, occurs due to these interactions. Higher temperatures accelerate diffusion, resulting in elevated quenching constants. Conversely, in static quenching, increased temperatures prompt the breakdown of complexes formed between drugs and proteins, consequently reducing quenching constant values. (43). Fluorescence spectroscopy was performed at 298 K, 303 K, 308 K, and 313 K to investigate the quenching mechanism related to the interaction between LP and BSA, as well as LP with BSA-CAF based on their observed fluorescence quenching. The linearity of the Stern-Volmer plots for the BSA-LP and BSA-CAF-LP systems at five different temperatures can be shown in Figure 5, namely in the relationship between F_0/F and LP concentration [M]. The obtained Ksv values exhibited an inverse correlation with temperature for both systems (Table 1) revealing that quenching is static. Additionally, a graphical representation was generated to illustrate the relationship between the logarithm of the expression [(F0-F)/F] and the logarithm of

LP concentration [M]. Subsequently, the intercepts of these graphs were employed to calculate the apparent binding constant (K) for the BSA-LP and BSA-CAF-LP systems across five different temperatures. Figure 6 and Table 2 indicate a significant decrease as temperature increases in the case of the BSA-LP system, indicating that a temperature rise disrupts the complex formation between BSA and LP. While K values exhibited notable increases with higher temperatures for the interaction of LP with BSA-CAF, implying that the interaction becomes stronger under elevated temperature conditions.

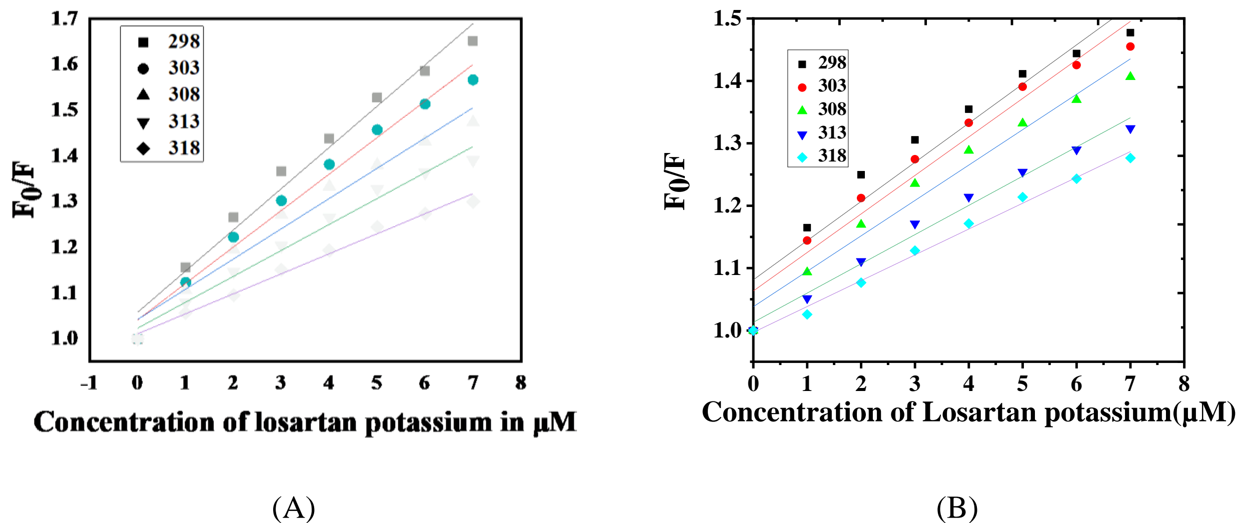


Fig. 5. Stern–Volmer plot for (A) LP with BSA binding and (B) BSA binding with losartan potassium in caffeine presence at different temperatures.

Table 1 Stern–Volmer Quenching constant for BSA-LP and BSA-CAF-LP at different temperatures.

System	Temperature (K)	Ksv×10^4 ($mol^{-1}L$)	R^2
BSA-LP	298 ° K	12.7	0.968
	303 ° K	12.3	0.972
	308° K	11.7	0.974
	313 ° K	11.2	0.979
	318 ° K	10.6	0.988
BSA-CAF-LP	298 ° K	6.27	0.914
	303 ° K	6.17	0.942
	308° K	5.67	0.966
	313 ° K	4.68	0.986
	318 ° K	4.13	0.991

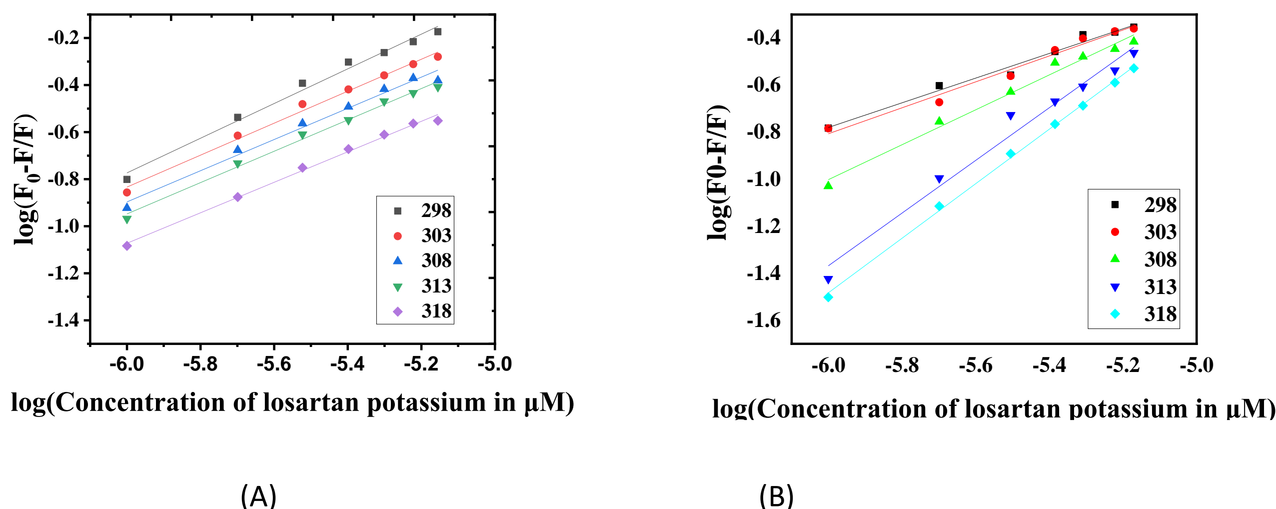


Fig. 6. Modified Stern–Volmer plot for (A) LP with BSA binding and (B) BSA binding with losartan potassium in caffeine presence in Tris-HCl buffer=7.4 at different temperatures.

Table 2 Binding constant of BSA-LP and BSA-CAF-LP at different temperatures.

System	Temperature (K)	$K \times 10^3$ ($mol^{-1}L$)	n	R^2
BSA-LP	298 ° K	4.47	0.73	0.986
	303 ° K	1.74	0.67	0.990
	308° K	1.21	0.66	0.980
	313 ° K	1.12	0.66	0.991
	318 ° K	0. 65	0.64	0.993
BSA-CAF-LP	298 ° K	0.02	0.52	0.976
	303 ° K	0.03	0.55	0.975
	308° K	0.28	0.74	0.981
	313 ° K	22.3	1.11	0.974
	318 ° K	29.6	1.15	0.997

3.1.3 Determination of thermodynamic parameters and nature of binding forces involved in BSA-LP and BSA-CAF-LP systems

The thermodynamic parameters (e.g., free energy (G), entropy (S), enthalpy (H)) for the BSA-LP and BSA-CAF-LP systems were studied at five different temperatures: 303K, 308K, 313K, and 318K. A plot (Fig. 7) of the natural logarithm of the binding constant (ln Kb) against the reciprocal of temperature (1/T) demonstrates that ΔG is consistently negative across all five temperatures for the binding of LP to BSA and BSA-CAF.This negative value suggests that the interaction is spontaneous for both systems. Similarly, ΔH and ΔS values for BSA-LP were -67.84 **kJ** mol^{-1} and -159.76 J K^{-1} mol^{-1}, respectively. The presence of negative values for both ΔS and ΔH suggests that the binding interaction between BSA and LP is exothermic and driven mainly by enthalpy, as the entropy is unfavorable here showing that the van der Waals forces

and hydrogen bonds are crucial in the BSA-LP interaction. In contrast, ΔH and ΔS values were 328.09 kJ $mol^{-1}$and 1139.07 J K^{-1} mol^{-1}, respectively, suggesting that the binding interaction between BSA-CAF and LP is endothermic and driven mainly by enthalpy and the acting force is hydrophobic interaction.

3.2. UV–vis spectrophotometric measurements

UV–Vis absorption spectroscopy is a straightforward and widely utilized technology frequently employed for examining structural alterations and investigating complex development(44). The alterations, such as the hypochromic or hyperchromic impact and the red or blue shift in the UV spectra throughout titration, can indicate the prevailing interaction mode between chemicals and BSA. Dynamic (collisional) quenching exclusively affects the fluorophore-excited states, leaving the absorption spectra unaffected. Nonetheless, developing a ground-state complex leads to modifications in the fluorophore absorption spectrum (45). BSA's absorption peak of nearly 280 nm is attributed to the aromatic amino acid residue tryptophan (46,47). Figure 8 shows the absorption spectra of free CAF and LP at identical concentrations (0.01mM) and their interaction with BSA. The observed increase in absorbance at approximately 280 nm is mainly attributed to the π–π* transition of aromatic amino acid residues present in BSA (48,49) which means that both alter the environment of BSA tryptophan residues.

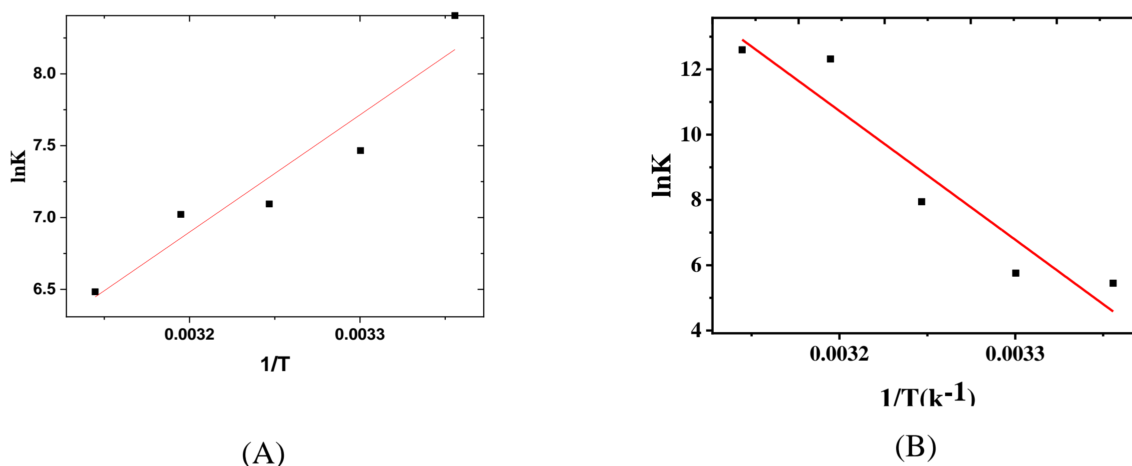


Fig. 7. Van›t Hoff equation for binding (A) LP with BSA and (B) LP with BSA-CAF.

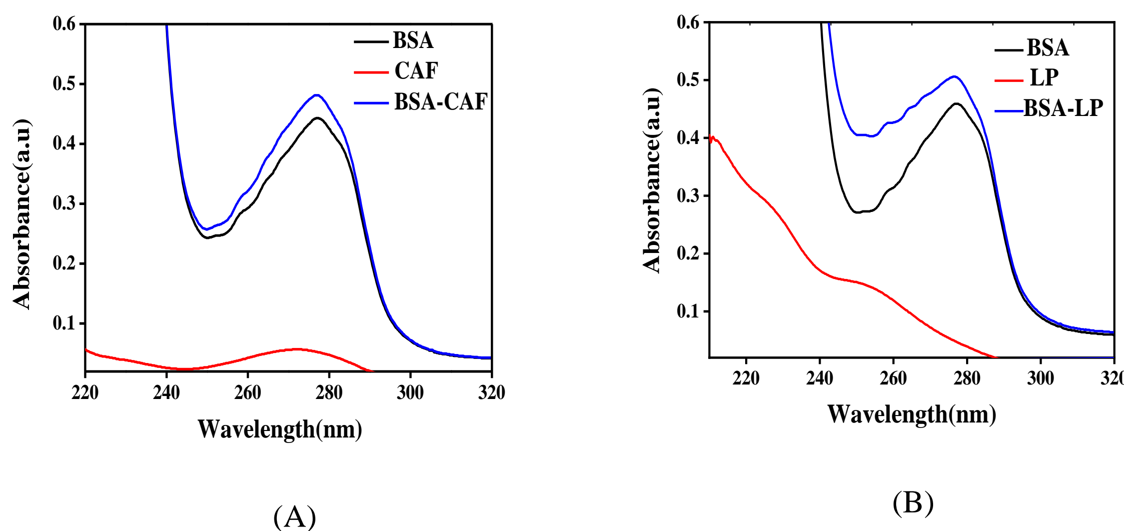


Fig. 8. Absorption spectra of BSA(0.01mM) in (A) caffeine and (B) losartan potassium presence each of Tris-HCl buffer.

The absorption spectra of BSA with LP were accurately measured, and then 0.01 mM of CAF was introduced Figure 9.Our findings indicate that adding LP, CAF, or a combination of both heightens BSA absorbance values. This suggests the formation of stable complexes in all instances, further supported by a similar observation made using the fluorescence method. The UV-Vis spectral results demonstrate that adding CAF to BSA-LP increases absorbance, providing evidence for developing novel protein-drug-drug complexes (BSA-LP-CAF).

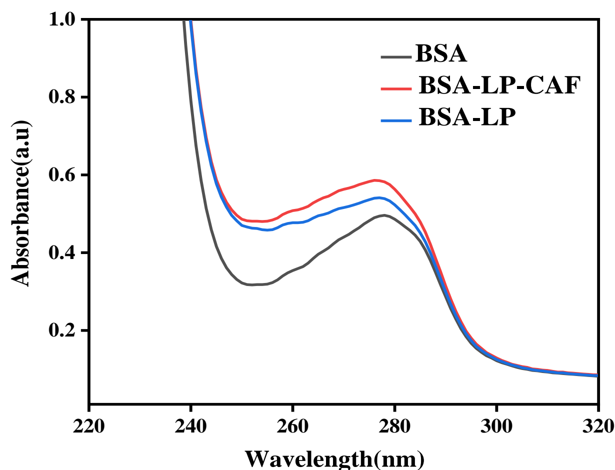


Fig. 9. Absorption spectra of 0.01m BSA, (1:1) BSA–LP system of 0.01mM, and (1:1:1) BSA-LP-CAF of 0.01mM in Tris-HCl buffer.

Table 3 Thermodynamic parameters for interaction of BSA-LP and BSA-CAF-LP complex at different temperatures.

System	Temperature (K)	∆ G (kJ mol^{-1})	∆ H (kJ mol^{-1})	∆ S (J K^{-1} mol^{-1})
BSA-LP	298 ° K	-20.24	-67.84	-159.76
	303 ° K	-19.44		
	308° K	-18.64		
	313 ° K	-17.84		
	318 ° K	-17.04		
BSA-CAF-LP	298 ° K	-11.35	328.09	1139.07
	303 ° K	-17.04		
	308° K	-22.74		
	313 ° K	-28.43		
	318 ° K	-34.13		

Conclusion

Serum albumins possess binding sites for many medications, with Sudlow sites I and II being the primary pharmaceutical binding sites (11,50). This study aims to investigate the impact of pharmacological medicines, including CAF, on the binding of LP to BSA, specifically at subdomain IIA, where LP is known to bind. Our findings indicate a significant impact of CAF on LP binding to BSA. The relationship between the proportion of unbound medication and serum albumin has been widely

acknowledged as a determinant of pharmacological response (51). It is not recommended for patients undergoing LP administration to concomitantly consume CAF from any source, including drugs, food, or beverages. This precaution is necessary due to the potential for mutual nullification of therapeutic effects or the occurrence of unfavorable pharmacological responses.

The findings of our study indicate that CAF presence can affect the LP binding constant with BSA, potentially affecting the therapeutic efficacy of the medicine. Consequently, healthcare professionals should caution patients against the concurrent use of CAF and LP during therapy.

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There is nothing to declare. The contributing author supported the work.

Conflicts of Interest

The authors declare that they have no conflicts of interest in this work.

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