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ORIGINAL ARTICLE

Studying Kinetic and Thermodynamic Parameters of the Interaction of Ceftriaxone with Albumin Extracted from Healthy and Covid-19 Plasma

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ABSTRACT

Because it has been well defined, bovine serum albumin (BSA) is highly suited to pharmacological effects, biotransformation, and bio-distribution of medicines initial research. Drug–protein interactions in the blood stream are known to have a significant impact on drug distribution, free concentration, and metabolism. Coronavirus disease 2019 (COVID-19) has spread globally as a severe pandemic. It is a serious threat to healthcare systems, economies, and is devastating to some populations, such as the elderly and those with comorbidities. Unfortunately, there is still no effective cure for COVID-19, especially the critically ill patients. The link between the mortality risk of patients hospitalized for COVID-19 and the function of blood albumin levels has been investigated. Because of albumin biological significance, the study goal conducted to apply spectroscopic methods to explore and comparing the kinetic and thermodynamic aspects of ceftriaxone's interaction with BSA, albumin isolated from healthy and covid-19 plasma.

A pooled plasma from healthy and hospitalized covid-19 individuals (Karbala Province / Iraq) was used to purify albumin using HPLC technique. UV-vis spectrophotometric measurements of albumin–ceftriaxone complex formation recorded at different pH (7, 7.2, 7.4, 7.6 & 7.8) in phosphate buffer solution, and at six different temperatures (298, 301, 304, 307, 310 & 313) K. The equilibrium constant and the thermodynamic parameter such as ΔG, ΔH and ΔS were calculated.

The drug-albumin (BSA, healthy and Covid-19 albumin) complexes are stable in 60 to 300 minutes, as evidenced by the steady absorbance studies. The reaction is from the first false order for drug-albumin (BSA, healthy and covid-19 plasma) complexes. Our finding suggesting the reaction is from the first false order. In the case of plasma albumin from individuals infected with - Covid-19, the values of (R2) are closer together. This is because the medication dominates the formation of a more stable complex with albumin. The stoichiometric ratio (coordination number) of complex between ceftriaxone and albumin at 298 k and pH=7.4 is 1:1.

The Gibbs free energy for albumin– ceftriaxone is negative, indicating that the reaction is spontaneous. The positive enthalpy of contact indicates that the process is endothermic, requiring energy input. Positive enthalpy and entropy change also refer to the hydrophobic association and electrostatic contact that occurs between albumin molecules and ceftriaxone. It is worth noting that the complex formed between bovine albumin and the medication has less absorbency at pH = 7.4. That is, the complex is more stable, and it prefers natural helical shapes. Additionally, as the pH value shifts away from physiological (> 7.4 <), the intensity of complex absorption increases.

Keywords: Ceftriaxone, Bovine serum albumin, Covid-19 albumin, purification of albumin

INTRODUCTION

In life sciences, chemistry, and clinical medicine, the interaction of biomacromolecules, particularly plasma proteins and medicines, has been a fascinating research topic (Lu et al., 2010). Drug-albumin complexes can be used as models to learn about drug-protein interactions at a fundamental level(Yue, Shen, Wang, Gao, & Liu, 2012). Because it has been well defined, bovine serum albumin (BSA) is highly suited to pharmacological effects, biotransformation, and bio-distribution of medicines initial research (Guo et al., 2004).

The largest extracellular protein in human plasma, serum albumin, accounts for 60% of total plasma protein content and has a concentration of 3.4-5.0 g/100ml (Pan & Han-wen, 2009). Extravascular fluid also contains it. In terms of drug binding characteristics, human serum albumin (HSA) is of special importance. Because of its ability to bind a wide variety of ligands, albumin plays a crucial role in the transportation, distribution, and metabolic of both endogenous and exogenous substances (Vuignier et al., 2010). It also plays a role in the transport of several of these substances across organ/circulatory interfaces like the liver, gut, kidney, and brain (Q. Wang, Liu, Su, Shi, & Sun, 2014). The human serum albumin protein is primarily made up of -helices, with an overall structure that resembles a heart. Sites I and II are two key binding sites on human serum albumin. Bulky heterocyclic drugs like azapropazone, phentylbutazone, and warfarin have a preferential binding affinity for Site I. Aromatic chemicals, such as ibuprofen, appear to bind preferentially to Site II (Otagiri & pharmacokinetics, 2005; Sudlow, Birkett, & Wade, 1975).

Despite the fact that worldwide vaccination in the COVID-19 pandemic has been accelerated, much currently unexplained about the patterns and factors associated as the pandemic spreads internationally (Challen et al., 2021). The function of blood albumin levels has been studied in relation to the mortality risk of COVID-19 patients admitted to the hospital. Hypoalbuminemia, or a significant drop in albumin levels, has been identified as a predictive marker of patient with severe illness. (Huang et al., 2020; Li et al., 2020). Drug–protein interactions in the blood stream are known to have a significant impact on drug distribution, free concentration, and metabolism (Hu, Liu, Shen, Fang, & Qu, 2005; Kamat & analysis, 2005; Naik, Chimatadar, Nandibewoor, & Spectroscopy, 2009). Furthermore, the drug–albumin combination can be used as a model for learning more about drug–protein binding in general (Naik, Chimatadar, Nandibewoor, & Biology, 2010).

Cephalosporin antibiotics are used to treat a wide range of bacterial infections, including serious or life-threatening illnesses. Cephalosporin derivatives include ceftazidime, cephradine, cefazolin, cephalexin, cefuroxime, cefamandole, cefuroxime axetil, cefotaxime, ceftriaxone, cefoperazone, cefoselis, cefepime, ceftobiprole, and ceftaroline, which all have identical core structures but different side chains. Ceftazidime has been found as a possible medication to prevent SARS-CoV-2 infection. It is one of the first COVID-19 therapy medications established (Lin et al., 2021). These antibiotics have been discovered to bind to serum albumin with a variety of affinities (Nerli, Farruggia, & Pico, 1996; Nerli, Pico, & international, 1994). Ceftriaxone is a 3rd cephalosporin antibiotic manufactured by the fungus Cephalosporium. Similar to penicillins, these are beta-lactam antibiotics having broad-spectrum activity against Gram-positive and Gram-negative bacteria. The usage of ceftriaxone in Covid-19 infected patients were reported in many studies (D. Wang et al., 2020), (Borba et al., 2020). Ceftriaxone comprises the functional groups –NH2, –COOH, –CO, and N–C, which are all electron

donors (figure 1).

Drug binding to plasma proteins is important because it affects their pharmacokinetic and pharmacodynamic properties, and it can also interfere with the binding of other endogenous and/or exogenous ligands due to binding site overlap and/or conformational changes. As a result, a thorough examination of drug–protein interactions are essential for a thorough knowledge of a drug's pharmacokinetic behavior and the development of analogues with useful pharmacological features [15].

In the investigation of drug-albumin binding in physiological conditions, spectroscopic techniques have been widely used. Because of its biological significance, we believe it is worthwhile to apply spectroscopic methods to explore the binding and interaction mechanism of ceftriaxone with BSA, human albumin isolated from plasma of healthy (Hum-PC) and covid-19 (Hum-PP) individuals. This study could aid researchers in determining and comparing the kinetic and thermodynamic aspects of ceftriaxone's interaction with BSA, albumin isolated from healthy and covid-19 plasma.

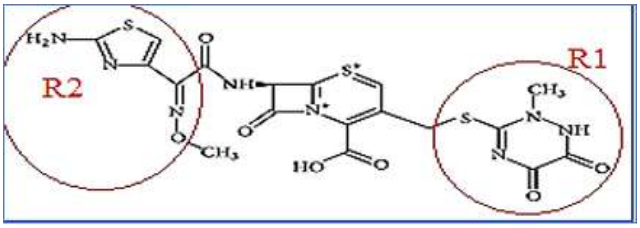

Figure 1: Structure of ceftriaxone

MATERIALS AND METHODS

Instruments: The UV–Visible spectro-Photometer Double beam were obtained (Shimadzu UV-1800) coupled with a 1.0 cm quartz cell. All pH readings were taken using a digital pH meter (Shanghai Leici Device Works, China). Proteins (BSA, Hum-PC & Hum-PP) and ceftriaxone solutions were stored in volumetric flasks of 5.0 ml volume and introduced into water bath KARL KOLB (D-6072) at the required temperature of 25, 28, 31, 34, 37 and 40 ◦C, and were maintained within a particular range (±0.1◦C) all through experiment.

Materials: All of the reagents employed in this study were analytical-reagent grade. In a Milli-Q system, double-distilled deionized water was purified (Millipore, Bedford, MA, USA). BSA (Sigma) was supplied and used to make a stock solution (50 mM), that was maintained at 4°C in a brown flask. A 1.0 mM stock solution of ceftriaxone was prepared. Phosphate buffere saline (PBS) was made with monosodium phosphate and disodium phosphate to maintain a pH of 7.4.

The solutions of ceftriaxone and BSA were prepared in 0.1 M phosphate buffer of pH 7.4 containing 0.15 M NaCl. Ceftriaxone was first dissolved in a slight fraction of methanol (approximately 5 mL) and then diluted to 100mL in a volumetric flask with double distilled water. The required concentrations of the drug or albumin were diluted with phosphate saline buffer pH 7.4.

Purification of albumin: After collection of appropriate amounts (about 135 ml) of pooled plasma from (for one healthy person) and (about 75 ml) from 25 hospitalized covid-19 individuals (from Al-Hussein Teaching Hospital / Karbala Province). HPLC technique was used to purify albumin from the plasma of healthy and covid-19 participants. The study protocol was approved by the Ethics Committee of the College of Sciences/University of Baghdad.

For stander solution, 0.5 mg of the high purity standard (BSA) was taken and placed in a volumetric flask of 10 ml capacity, and was prepared in phosphate buffer pH (7.2) solution was added and the volume was completed to the mark where the initial concentration (50 ppm) and using the dilution law, the concentration injected into the device was prepared. The HPLC condition were: Mobile phase = (acetonitrile: D.W: Triflouroacetic acid) (80: 10: 10 V/V); Column = C 18 – ODS (25 cm * 4.6 mm); Flow Rate = 1 ml / min and Detector = UV – 220 nm (Heidary, Imani, & Mostafavi, 2017).

Absorption measurements: The absorption spectra of BSA and HAS (purified from control and patient plasma) in the presence and absence of ceftriaxone were measured at 200–350 nm (n = 3 repetitions), at 298 K. Protein concentration was set at 5×10-5M, whereas ceftriaxone concentration was set at 5×10-4M. Albumin and ceftriaxone binding investigations are conducted in two approaches. First, the concentration of BSA was set to 0.5 M, and then a series of ceftriaxone standard solutions were added. Second, purified albumin from control and covid-19 subjects and ceftriaxone, the concentrations was maintained as in BSA.

RESULTS

Absorption spectroscopy (UV-Vis Absorption Studies): To determine the complexion of albumin (BSA, purified – control, purified – patient) with ceftriaxone, UV–VIS absorption experiments were conducted. Structure alterations and complex development are studied using UV-Vi's absorption measurements (Borba et al., 2020). Figure 2 shows the UV absorption spectra of BSA–ceftriaxone, Hum-PP–ceftriaxone, and Hum-PC–ceftriaxone. BSA has a strong band with a maximum at 208 nm and a weak band with a maximum at 279 nm in its UV absorption spectra. The intensity of the ceftriaxone-BSA system's absorbance (279nm) rose with increasing ceftriaxone concentration, and the peak displayed a minor blue shift (from 275nm to 278nm). The absorption at 204nm to 206nm, on the other hand, did not alter appreciably. Changes in the conformation of the peptide backbone associated with helix-coil transition have been found to cause differences in the spectral peak of 204nm (Baler et al., 2014; Rondeau & Bourdon, 2011). Furthermore, the polarity of the microenvironment around BSA tryptophan and tyrosine residues is linked to the peak at 279nm.The interaction between ceftriaxone and BSA changes the polarity of the microenvironment around BSA's tryptophan and tyrosine residues but does not modify BSA's conformation.

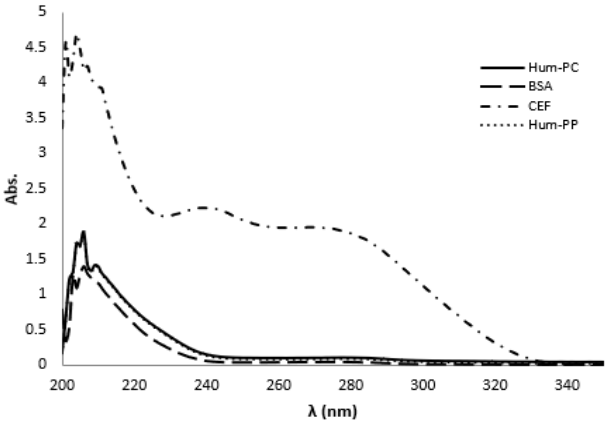

Figure 2: Absorption spectra of albumin (5x10-4M in a phosphate buffer of pH7.4) for BSA, Hum-PC, Hum-PP and Ceftriaxone (CEF) (5x10-5M) in a phosphate buffer of pH7.4.

The absorption spectrum of ceftriaxone was recorded at 298 K in the range 300–500 nm (n = 3 replicates). The emission spectrum of BSA with different concentrations (10-3, 10-4 & 10-5 M) was represented in figure 3(A). The absorbance spectra for various concentrations of ceftriaxone were shown in figure 3(B), while the spectra of the different concentrations of the complexes between constant concentration of albumin (10-3M) and (10-3, 10-4 & 10-5 M) of ceftriaxone were also recorded at 298 K, as shown in figure 3(C). The energy transfer was then calculated using the overlap of ceftriaxone's UV absorption spectra with that of BSA. As a result, the best drug concentration is 10 μM, and 1 mM of albumin will lower the absorption curve due to the formation of a complex between the drug and albumin.

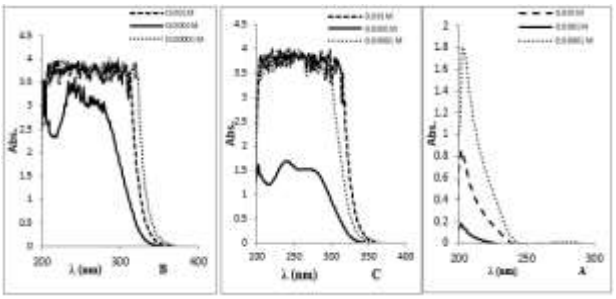

Figure 3: Absorption spectra for different concentration of Albumin (A), different concentration of ceftriaxone (B) and for the complex of albumin (0.001M) with different concentration of ceftriaxone (C).

Stoichiometric Analysis: The stoichiometry of interaction of the drug (ceftriaxone) with albumin were calculated by the method of continuous variation, these methods sometimes known as Job's method (Lin et al., 2021). The stoichiometry of the complex between ceftriaxone and albumin were obtained by preparing a series of ten solutions of ceftriaxone and albumin with a total concentration of (1×10-5M) to (10×10-5M) in phosphate buffer of pH =7.4, at the maximum wavelength of ceftriaxone. The plot of absorbance against the mole fraction of medication could be used to compute the coordination number (n). The Job's plot indicates that the stoichiometric ratio n of albumin-drug at 298 k and pH7.4 is 1:1, as shown in figure 4.

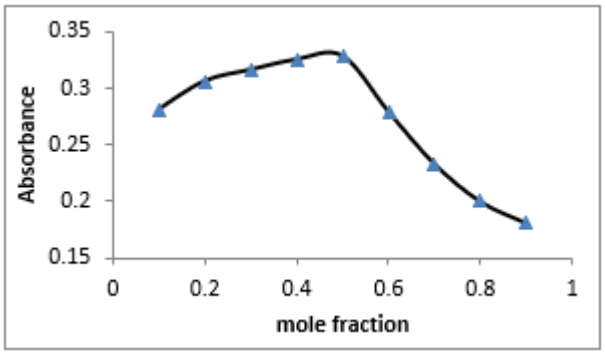

Figure 4: Job's method of continuous variation plot for BSA-ceftriaxone complexes (5×10-4M in a phosphate buffer of pH7.4) for BSA and ceftriaxone (5x10-5M in a phosphate buffer of pH7.4). Extrapolation of linear portion can be used to locate the position of break point.

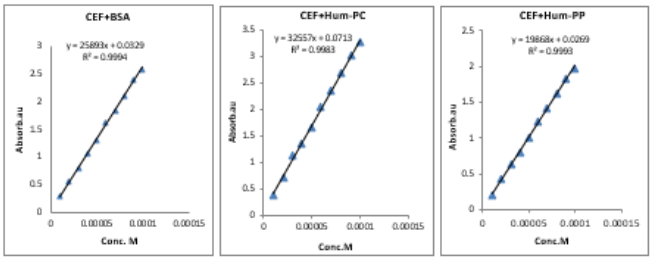

Figure 5: Absorbance vs concentration of ceftriaxone with albumin (BSA–CEF), (Hum-PC–CEF) and (Hum-PP–CEF) complexes in phosphate buffer pH 7.4.

The equilibrium constant was calculated using the continuous variation method (Borba et al., 2020; Nerli et al., 1996; Nerli et al., 1994; D. Wang et al., 2020), as flowing:

Drug + Albumin ↔ [Albumin–complex] (1)

Keq = [BSA–complex] / [Drug] eq × [BSA] eq (2)

Knowing the formula of the complex between albumin and the drug which was (1:1) it is possible to determine the equilibrium constant of this complex. The concentration of the complex formed at equilibrium was calculated as (Heidary et al., 2017; Ross & Subramanian, 1981)

[Albumin–drug] eq = Absorbance (max) / ☐ l … (3)

Where; ☐= molar absorptivity and l = length of light path

Plotting of the absorbance of the complex against concentration given a straight line with the slope equal to ☐ complex (figure 5), the obtained data were listed in table (1).

Table 1: Absorption characteristics of the studied drugs in a phosphate buffer of pH 7.4. Albumin with 5×10-4M concentration and ceftriaxone with 5×10-5M.

Albumin-Drug complex	Molar absorptivity ε_{max} ($cm^{-1}.L.mol^{-1}$)	R^2 correlation coefficient
BSA + ceftriaxone	25893	0.9994
Hum-PC+ ceftriaxone	32557	0.9983
Hum-PP+ ceftriaxone	23517	0.9992

The equilibrium constant for the interaction of albumin (BSA, Hum-PP and Hum-PC) with the drug was then calculated at six different temperature (298, 301, 304, 307, 310 and 313) K; the results were shown in table (2).

Thermodynamic Parameters: The intermolecular interacting forces between a small molecule and a macromolecule include hydrogen bonding, van der Waals force, electrostatic, and hydrophobic interactions. For confirming the binding mode, the thermodynamic parameters of the reaction, enthalpy change (ΔH) and entropy change (ΔS), are crucial. As shown in figure 6, temperature-dependent thermodynamic parameters for the ceftriaxone-BSA system are used to characterize the intermolecular interactions (ΔH◦ and ΔS◦) between ceftriaxone and BSA can be calculated by using the Van'tHoff equation:

$$\ln k = \frac{-\Delta H^\circ}{RT} + \frac{\Delta S^\circ}{R}, \quad (4)$$

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

Table (3a, 3b & 3c), shows the results of K, ΔH◦, ΔS◦, and the associated values of Gibbs free energy (ΔG◦) for the systems: BSA-ceftriaxone, Hum-PP–ceftriaxone and Hum-PC–ceftriaxone. The sign and amplitude of the thermodynamic parameters were linked to a variety of interactions that could occur during protein interaction. The negative values of ΔG, as well as the positive values of ΔH and ΔS, are obtained for the albumin with ceftriaxone interaction. ΔG < 0 indicates that the binding is spontaneous, while the ΔS > 0 indicates that there are hydrophobic interactions (Ross & Subramanian, 1981). Furthermore, ΔH > 0 and ΔS > 0 are refrered to the electrostatic interaction features in aqueous solution. As a result, ceftriaxone's interactions with albumin were primarily hydrophobic and electrostatic. According to table 3, increasing the temperature increases the values of the equilibrium constant between ceftriaxone and albumin (bovine, human healthy, and patient). Because the reactions are endothermic according to Le Chatelier's rule, the increase in absorbance is due to a decrease in the interaction between the drug and albumin.

Table 3: Thermodynamic parameters for albumin–drug complexes in phosphate buffer pH 7.4

(BSA–CEF)			
T(K)	ΔG°($kJ.mol^{-1}$)	ΔH°($kJ.mol^{-1}$)	ΔS°($kJ.K^{-1}.mol^{-1}$) *10^{-3}
298	-23.952	43.158	225.201
301	-24.508	43.158	224.804
304	-25.237	43.158	224.984
307	-25.816	43.158	224.671
310	-26.515	43.158	224.752
313	-27.362	43.158	225.304
(Hum-PP–CEF)			
298	-21.152	11.029	107.991
301	-21.498	11.029	108.064
304	-21.725	11.029	107.745
307	-22.119	11.029	107.975
310	-22.431	11.029	107.937
313	-22.783	11.029	108.027
(Hum-PC–CEF)			

298	-25.055	41.953	224.860
301	-25.373	41.953	223.682
304	-26.332	41.953	224.623
307	-26.718	41.953	223.685
310	-27.473	41.953	223.956
313	-28.438	41.953	224.892

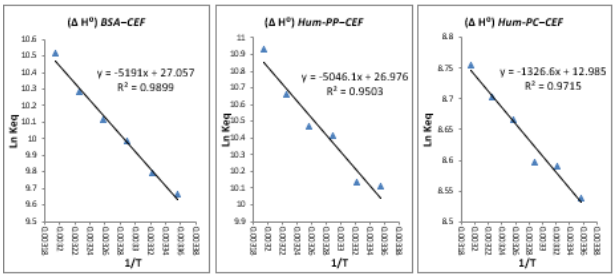

Figure 6: Van't Hoff plot for (BSA–CEF), (Hum-PP–CEF) and (Hum-PC–CEF) complexes in pH 7.4.

Interaction Kinetics: The absorbance of the albumin-ceftriaxone complex over time at a defined wavelength was used to assess the interaction kinetics of the examined medication (ceftriaxone). The first order rate equation (7) was used, as well as the second order rate equation (8).

lnA-lnA =-kt first order equation (7)

k: rate constant for the reaction, which is independent of the concentration but depends on the temperature.

1/ [A]-1/ [A] =kt Second order equation … (8)

The compound will be stable in 60 to 300 minutes, as evidenced by the steady absorbance. Figure 7 (A and B) depicts the application of the first and second orders of the reaction for albumin with drug.

As shown in figure 7, the reaction is from the first false order because the correction factor (R2) is bigger than the correction factor for the second order, but in the case of those infected with -Covid-19, the values of (R2) are closer to each other. This is owing to the drug's effect in dominating the creation of a more stable complex with albumin.

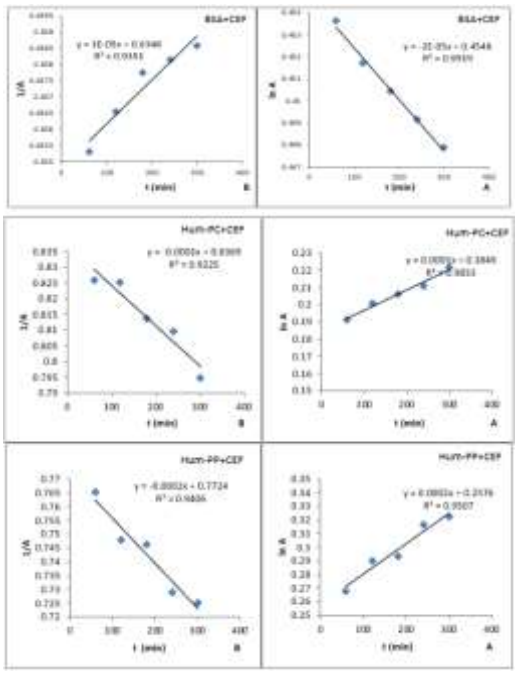

Figure 7: The application of the first (A) and second order (B) reaction equation for complex of (BSA–CEF), (Hum-PC–CEF) and (Hum-PP–CEF) complexes

It is worth noting that the complex formed between bovine albumin and the medication has less absorbency at PH = 7.4. That is, the complex is more stable, and it prefers natural helical shapes. As seen in figure 8, absorption rises as pH drops, indicating that the complex is dissociating, particularly at pH = 7. So, in addition to the natural shape of protein (native), bovine albumin may take other multiple forms (folding and unfolding). Because of the drug's affinity for albumin, the intensity of absorption of ceftriaxone-bovine albumin complex decreases compared to the drug alone, as illustrated in figure 9. Additionally, as the pH value shifts away from physiological (> 7.4 <), the intensity of complex absorption increases.

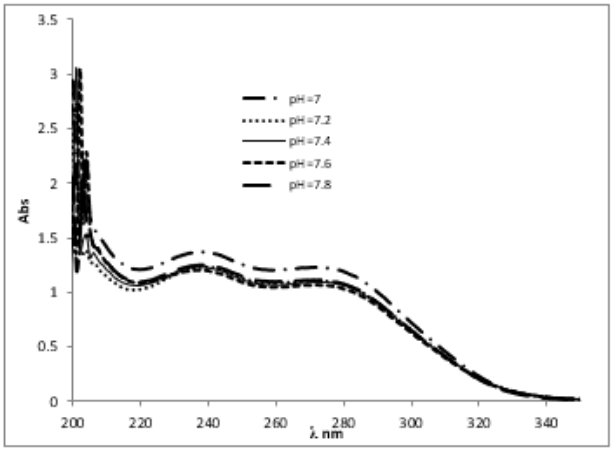

Figure 8: The absorption spectrum of BSA–ceftriaxone complex in phosphate buffer at various pH.

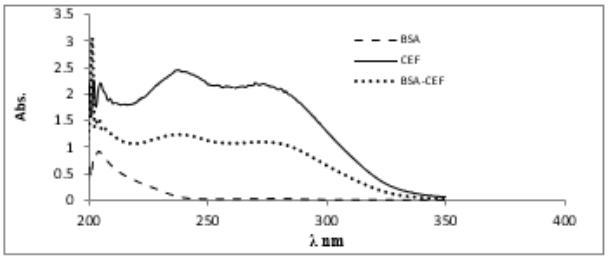

Figure 9: The absorption spectrum of BSA (1X10-3), Ceftriaxone (1x10-4) and BSA–ceftriaxone in phosphate buffer at pH=7.4

Solution stability: The complex solution (albumin-ceftriaxone) was kept at room temperature for 24 hours to test its stability. The difference between the initial absorbance and the absorbance after 24 hours was noticed. The following formula was used to compute the similarity factor [23]:

Similarity factor = absorbance of initial solution / absorbance of solution after 24 h

For the studied complex (albumin-ceftriaxone):

Similarity factor = 1.585 / 1.561= 1.015

The solution's stability was assessed using the similarity factor (0.995), which was judged to be within the acceptable range of 0.98-1.02.

DISSCUSION

A wide spectrum of medicines are delivered to their target organs/tissues by binding with human serum albumin (HAS) (Yamasaki, Chuang, Maruyama, & Otagiri, 2013). As a result, HSA not only protects bound medicines from oxidation and regulates drug distribution in vivo, but it also changes drug pharmacokinetics and pharmacodynamics (Tayyab, Feroz, & biology, 2021). Furthermore, medications that bind to HSA at the same time may interact, altering HSA binding behavior and potentially affecting the

therapeutic efficacy of the pharmaceuticals (Tesseromatis, Alevizou, & pharmacokinetics, 2008). Binding of a drug to plasma and tissue proteins is usually reversible and happens as a result of weak non-covalent interactions such as hydrophobic interactions, van der Waals forces, and hydrogen bonding (L. M. J. E. o. o. d. m. Berezhkovskiy & toxicology, 2008).

Multiple binding sites with varying affinities characterize the protein in general. For albumin, the unbound drug fraction is commonly calculated using two groups of binding sites on the protein with equal affinity in each group (low and high affinity groups) (L. M. J. J. o. p. s. Berezhkovskiy, 2007; Borgå, Borgå, & biopharmaceutics, 1997). Because albumin's tertiary and quaternary structures are flexible and can be influenced by a variety of circumstances (such as pH, Cl-, and albumin and drug concentrations), it has been dubbed a "breathing" molecule . (Dröge, Wilting, & Janssen, 1982). The N → B (native to binding) transition is one such phenomenon that is strongly impacted by pH. Drugs bind more strongly to binding sites (Site I) and (Site II) in the B conformation of albumin than in the N form (Wanwimolruk, Birkett, & Enzymology, 1982).

Our result suggests that ceftriaxone forms strong connections with bovine protein (i.e. a complex with poor dissociation) at pathological conditions pH (>7.4<) & temperature >37°<C) as depicted in figure 8 and table 2. Plasma albumin concentration was reduced significantly (p<0.5) in covid-19 patients compared to control individuals (data not shown), this reduces the number of drug-binding sites available, increasing the risk of undesirable displacement events and higher-than-normal free drug concentrations. Because only unbound pharmaceuticals can attach to protein drug receptors on cells and exert therapeutic actions (Baler et al., 2014), a negative impact on drug control was proposed, especially since the drug has a half-life of 5 to 9 hours Drug binding to human serum albumin (HSA) is typically altered due to either drug-drug interactions, in which other ligands (typically other drugs) bind to HSA and cause the first drug to be displaced from the protein, or abnormal physiology, in which HSA binding capacity varies significantly from the normal range. Under specific conditions, these occurrences can alter the concentrations of a medication's free/bound fractions in the plasma, and hence its pharmacodynamic and pharmacokinetic properties.

Structural changes in HSA are common in several diseases, as reported in diabetes (Rondeau & Bourdon, 2011), where crystallographic data have established that site I can accommodate two molecules of glucose, with one of them covalently bonded to Lys-195 at the opening of the binding site and partially blocking it (Y. Wang et al., 2013) as a result reduction in drug binding occurs (Anguizola et al., 2013). Albumin structural changes also documented in hemodialysis patients, where higher levels of oxidized albumin have reported in these patients. This is clinically significant as this form of albumin has an altered conformation with lower drug binding capacity, especially toward site II ligands (Mera et al., 2005).

The chemical origin of the binding and the structural binding interactions of -lactam antibiotics to serum albumin have been studied, however the results are mixed. However, it has been demonstrated that the interactions between HSA and several -lactam antibiotics were mostly electrostatic, and that an electron-rich heterocyclic group was involved. It has been found a skewed relationship between lipophilicity and CEF-HSA binding affinity [18].

The structural features of the R2 substituents (as illustrated in figure 1) have a major impact on albumin affinity [18]. The fluorescence intensity of both Trp and Tyr decreases when ceftriaxone is introduced, and there is a notable red shift at maximum emission, indicating that the protein's structure has changed. It's most likely because the hydrophobic amino acid structure surrounding tryptophan and tyrosine residues in BSA collapses, exposing them to the aqueous phase. The interaction of ceftriaxone with BSA modifies the polarity surrounding Trp and Tyr residue micro regions, according to Miller, J. (1979), (Miller, 1979), which is consistent with the results obtained using the UV-Vis spectroscopic approach previously discussed. The study of binding phenomena is critical for gaining a foundational understanding of pharmacological effects, biotransformation, and biodistribution (Yue et al., 2012).

CONCLUSIONS

Our findings indicate that ceftriaxone establishes strong connections with bovine protein (i.e., a complex with poor dissociation) under pathogenic conditions (pH >7.4< & temperature >37<°C). Plasma albumin concentrations were considerably lower (p<0.5) in covid-19 patients compared to controls (data not shown). This limits the availability of sites for drug binding, increasing the chance of undesirable displacement events and higher than usual free drug concentrations. A negative impact on drug control was hypothesized, especially given the medication's half-life of 5 to 9 hours. Our finding suggesting the reaction is from the first false order. In the case of individuals infected with - Covid-19, the values of (R2) are closer together. This is because the medication dominates the formation of a more stable complex with albumin.

Ceftriaxone may attach to bovine serum albumin via hydrogen bonding and van der Waals forces, according to the findings. Ceftriaxone caused certain microenvironmental and structural alterations in bovine serum albumin molecules, according to the findings. The presence of ceftriaxone could affect the conformation of bovine serum albumin throughout the binding process. The findings are relevant in pharmacy, pharmacology, and biochemistry, and are expected to shed light on how medications interact with the physiologically vital protein bovine serum albumin.

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