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Spectroscopic study of drug–drug interactions: influence of two over-the-counter drugs on the albumin binding affinities of carbamazepine and its major metabolite

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Abstract

Background Multidrug regimens can increase the risk of drug–drug interactions at the level of albumin binding especially for drugs with narrow therapeutic windows such as carbamazepine (CBZ). This risk is particularly heightened for CBZ which is mainly metabolized to the active carbamazepine-10,11-epoxide (CBZE) that has been identified as contributory to both the therapeutic efficacy and severity of toxicity in CBZ-treated individuals. The objective of this study was to investigate the binding affinities of albumin with CBZ and CBZE, and to explore the influence of two competing over-the-counter medicines on the binding characteristics. CBZE was synthesized by epoxidation of CBZ and characterized using IR, NMR and mass spectrometry. The influence of paracetamol and ascorbic acid on the albumin complexes of CBZ and CBZE was investigated using absorption and IR spectrophotometry.

Results Protein–ligand complexation produced progressive hyperchromic changes in 278 nm band of bovine serum albumin (BSA) with formation constants of 10.28–10.44 and 12.66–13.02 M^{-1} for CBZ and CBZE, respectively. Thermodynamic considerations confirmed both binding processes as endothermic, spontaneous and driven by hydrophobic interactions. The presence of ascorbic acid increased the binding constants of both CBZ-BSA and CBZE-BSA complexes by non-competitive interference mechanism. Similarly, paracetamol increased the affinity of CBZ for albumin but then competitively interfered with the CBZE-BSA complex. The ratio of albumin binding affinities of CBZ–CBZE varied from 0.81 in the absence of competing drug to 1.29 and 1.0 with paracetamol and ascorbic acid, respectively. IR study confirmed that both CBZ and CBZE induced a reduction from the 67.34% *α*-helical content of free BSA to 42.56 and 56.43%, respectively. Competitive binding in the presence of either paracetamol or ascorbic acid induced further reduction in the *α*-helical content of BSA in the complexes. The most extensive perturbation in the secondary structure of BSA (22.78% *α*-helical content) which was observed with CBZE-BSA complex in the presence of paracetamol is probably due to the increased interaction of the protein for the analgesic.

Conclusion The study has revealed potential interference of paracetamol or ascorbic acid with the albumin binding of carbamazepine and its major metabolite.

Keywords Carbamazepine, Carbamazepine-10,11-epoxide, Drug–drug interactions, Albumin binding, Paracetamol, Ascorbic acid, UV–Visible spectroscopy, IR spectroscopy, Deconvolution

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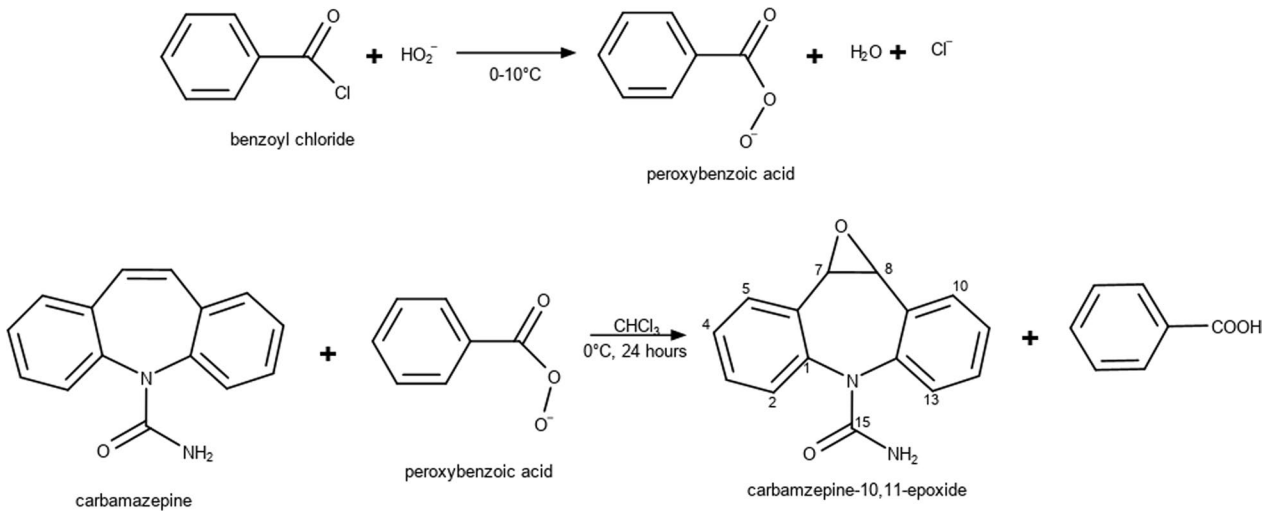

Scheme 1 Synthesis of CBZE

Background

Apart from its contributory roles in the control of pH and osmotic pressure of blood, serum albumin shows extensive capacity to bind to many endogenous and exogenous ligands [1, 2]. As a result, the protein plays important roles in the transportation, deposition and metabolism of many biologically active compounds including drugs. The in vitro study of the binding of molecules with human albumin or its homologue bovine albumin, therefore remains important in the safety assessment of drugs as binding dictates their pharmacokinetics and pharmacodynamics including bio distribution, bioaccumulation, efficacy and toxicity [3, 4]. This is particularly critical for drugs with a narrow therapeutic window such as carbamazepine (CBZ).

Carbamazepine, chemically known as 5H-dibenzo[b,f] azepine-5-carboxamide (Scheme 1), is a lipophilic tricyclic compound that is approved and widely used in the treatment of partial seizures, generalized tonic–clonic seizures and trigeminal neuralgia [2, 5]. It is also used in the management of bipolar disorder, schizophrenia and attention-deficit hyperactivity disorder [6, 7]. CBZ is metabolized to about 33 metabolites including its major metabolite, carbamazepine-10,11-epoxide (CBZE), which shows equivalent activity as the parent drug [8]. Plasma concentrations of CBZE that are up to 50% of the parent drug have been reported in patients [9]. However, CBZ because of its narrow target therapeutic range of 4–12 mg/L, can cause very severe and sometimes life-threatening adverse effects including cardiac dysrhythmias, hepatotoxicity, respiratory depression, hypotension, etc. [10]. Bioaccumulation of the main metabolite, CBZE, is known to contribute to CBZ intoxication and the severity of many adverse effects in CBZ-treated individuals [9, 10]. For example, the hepatotoxicity of CBZ is well characterized to be due to the accumulation of the toxic CBZE by cytochrome P450 isoenzymes [11]. From the foregoing, it is obvious the study of the safety of CBZ is incomplete without a profiling of its major metabolite as well. Metabolites, because they differ structurally from the intact drug, may exhibit altered pharmacokinetics especially with regards to bio accumulation, toxicity and potential drug–drug interactions.

The risk for drug–drug interactions increases with concomitant use of two or more drugs for the management of co-existing diseased conditions. This is particularly true for CBZ which is often included in multidrug regimens for the management of epilepsy [2]. This increases the likelihood of drug–drug interactions at the level of binding to albumin. The mechanisms of interactions may involve a competitive binding to the same binding site, non-competitive binding to different binding sites or by causing a change in the conformation of the protein [3]. In this regard, painkillers such as paracetamol, antibiotics and vitamin supplements which are some of the most commonly prescribed medicines pose an increased risk of interactions [12–15]. Although paracetamol is largely well tolerated and regarded as safe since it does not cause gastrointestinal damage, its concomitant use with CBZ has been reported to increase severity of paracetamol hepatotoxicity [16, 17]. Similarly, despite having been proffered as an adjunct to ameliorate some of the associated adverse effects of drug therapy with CBZ [18, 19], ascorbic acid has been previously implicated as one of 18 plasma-soluble drugs with a high potential of causing clinically significant interference with the quantification

of several drugs in biological matrices [20]. Furthermore, the potential interactions risks of paracetamol and ascorbic acid may be grossly underestimated as their over-the-counter availability increases the likelihood of being overlooked by health workers and patients during medication history [12].

The objective of this study was therefore to investigate the binding affinities of albumin with CBZ and CBZE as well as to explore the influence of competing paracetamol and ascorbic acid on the binding characteristics.

Methods

Materials and reagents

All reagents used were sourced from BDH UK except Bovine serum albumin (Glentham, UK), sodium hydroxide (Qualikems, India) and CBZ secondary reference. Stock concentrations of 1000 μM solutions of CBZ, CBZE, ascorbic acid, paracetamol as well as working concentration of 10 μM solution of albumin were prepared in 10 mM Tris–HCl buffer (pH 7.4).

Equipment used

UV–Visible spectrophotometer (Spectroquant Pharo 300) with 1 cm path length, Nanalysis 60PRO Multinuclear NMR spectrometer, HPLC (Agilent Technologies 1290 Infinity) hyphenated to mass spectrometer (Agilent Infinity G6125B), Perkin Elmer Spectrum Two IR spectrophotometer.

Synthesis of peroxybenzoic acid

Peroxybenzoic acid was synthesized using the method of Moyer and Manley (1964) with slight modifications [21]. Briefly, 5 g (0.04 mol) of 30% hydrogen peroxide solution was slowly added with continuous stirring into 12.5 mL aqueous solution of 5 g NaOH (0.12 mol) maintained in an ice-bath. The rate of addition and stirring was controlled to prevent the reaction temperature from exceeding 18 °C. The reaction mixture was filtered before the addition of 70 mL absolute ethanol and 0.2 g (0.81 mmol) $MgSO_4{\cdot}7H_2O$ dissolved in 6 mL water. The temperature of the reaction mixture was allowed to return to room temperature before the dropwise addition of 4.6 mL (0.04 mol) of benzoyl chloride over 10 min with continuous stirring. The solution was filtered, acidified with 30 mL of 20% aqueous sulfuric acid and extracted with 40 mL portions of carbon tetrachloride to obtain the product.

Synthesis of CBZE

0.002 mol of CBZ (0.47 g) and peroxybenzoic acid (0.28 g) dissolved in 50 mL chloroform was maintained at 0 °C for 24 h after which excess peracid was destroyed by the addition of an equal volume of 15% aqueous sodium sulfite solution. The organic layer was washed with 5% aqueous sodium bicarbonate solution, dried over anhydrous sodium sulfate and evaporated. The crude product was collected and recrystallized from methanol. The percent purity of the product was determined with an LC–MS method that involved gradient elution on a C-18 column using 0.1% formic acid in water: 0.1% acetic acid in methanol (70:30).

Photometric titrations of ligands with Bovine serum albumin

Absorption spectra of titration mixtures containing a fixed concentration of BSA and increasing concentrations of CBZ or CBZE ranging from $0.1 - 2.0 \times 10^{-5}$ M (r_i = [ligand]/[BSA] = 0.1, 0.2, 0.3, 0.4, 0.5, 0.75, 1.0, 1.5 and 2.0) were obtained after incubation for 30 min at 25 °C The procedure was repeated at incubation temperature of 37 °C.

The effect of the presence of paracetamol and ascorbic acid on the albumin binding of both CBZ and CBZE were also determined by photometric titrations. The concentrations of BSA and the competing drugs respectively were kept constant at 1×10^{-5} M while the concentrations of CBZ and CBZE ranged from $0.1 - 2.0 \times 10^{-5}$ M.

For each data acquisition, baseline correction of the spectrophotometer was carried out with solutions containing the appropriate ligand and competing drug concentrations.

FTIR study of ligand-induced conformational changes in albumin

Equal volumes of 0.3 mM BSA solution and CBZ or CBZE were mixed in Eppendorf tubes to give reaction mixtures containing albumin to ligand ratios of 1:1. The reaction mixtures were allowed to equilibrate at 25 °C for 2 h. Thereafter, the FTIR spectra of the protein–ligand complexes, buffer and BSA solutions were obtained at 4 cm^{-1} resolution using a 256-scan interferogram over the wavelength range of 4000–400 cm^{-1}. A difference spectrum was generated by digitally subtracting the spectrum of BSA from the protein–ligand complex while the quantitative estimation of the protein secondary structure was done using infra-red spectra deconvolution and curve fitting methods [22].

The effect of a competing drug on the structure of the protein–ligand complex was also investigated by the introduction of 0.3 mM final concentration of paracetamol or ascorbic acid and subsequent IR analysis of the resultant ternary mixtures.

Results

Synthesis of CBZE

CBZE was successfully synthesized by a two-step process. In summary, benzoyl chloride initially reacted with hydroperoxide ion to give peroxybenzoic acid which was then used to effect the epoxidation of the azepine residue of CBZ (Scheme 1).

Synthesis afforded CBZE in moderate yield of 50.1% and with a purity of 97.11%w/w when analyzed by LC–MS. The chromatographic conditions employed involved gradient elution on a C-18 column using 0.1% formic acid in water: 0.1% acetic acid in methanol (70:30) at a flow rate of 1 mL/min. The color and melting point of the product were consistent with those found in the literature [23]. Although the mass spectral analysis of the product did not yield a molecular ion, as is often the case with epoxides which are unstable to ionization energy, the mass spectrum revealed the most abundant peaks at m/z of 179.7, 191.7 and 207.8 [24]. This fragmentation pattern was consistent with some of the top peaks reported for CBZE in the literature [25].

Spectral data of CBZE

Color: white; mp: 190–193 °C Yield: 50.1%; ATR-FTIR cm^{-1}: 3431.0 (N–H), 1673.5 (C=O), 1096.1 (C–O); ^{1}H NMR (DMSO-d_6 60 MHz) δ : 7.31–7.47 (*m*, 8H Aromatic H), 6.92 (s, 2H CH–O–CH), 1.23 (*s*, 2H NH_2); ^{13}C NMR (DMSO-d_6 60 MHz) δ : 172.37 (C-15), 143.0 (C-1 and C-14), 130.87 (C-3 and C-12), 128.75 (C-5, C-10, C-6 and C-9), 126.62 (C-4 and C-11), 122.15 (C-2 and C-13), 70.69 (C-7 and C-8); negative ion ESI: *m/z* molecular peak not found, Calculated *M* 252.27.

Photometric titration of ligands with Bovine serum albumin

The changes in the 278 nm band of BSA with increasing concentrations of CBZ or CBZE are depicted in Fig. 1. Absorption measurements of the band showed progressive hyperchromic changes with increasing mole ratios of CBZ and CBZE. In addition, the band position of the CBZE-BSA complex showed a very small hypsochromic shift ($\Delta\lambda = -1$ nm).

The formation constants of complexes following the binding of small ligands to a protein molecule can be estimated from Eq. 1 [22].

$$\frac{1}{A - A_{\circ}} = \frac{1}{A_{\phi} - A_{\circ}} + \frac{1}{K\left(A_{\phi} - A_{\circ}\right)} \cdot \frac{1}{[\text{ligand}]} \qquad (1)$$

where $A_{\circ}$ and A are the absorbance values of BSA before and after the addition of increasing concentrations of the ligands, respectively. A_{ϕ} is the final absorbance of the ligand–protein complex.

The double reciprocal plots obtained are depicted in Figs. 2 and 3 for CBZ and CBZE, respectively. A linear relationship ($R^2 > 0.9$) between $1/A - A_{\circ}$ and reciprocal of ligand concentration can be observed in both instances. The formation constants of CBZ-BSA and CBZE-BSA complexes are depicted in Table 1. The values were determined from the ratio of the intercept to the slope of the respective plots. The thermodynamic parameters associated with the binding of the ligands with BSA are also shown in Table 1.

Effect of a competing over-the-counter medicine

The effects of the presence of paracetamol and ascorbic acid on the albumin binding affinities of CBZ and CBZE were also examined. The electronic absorption spectra of

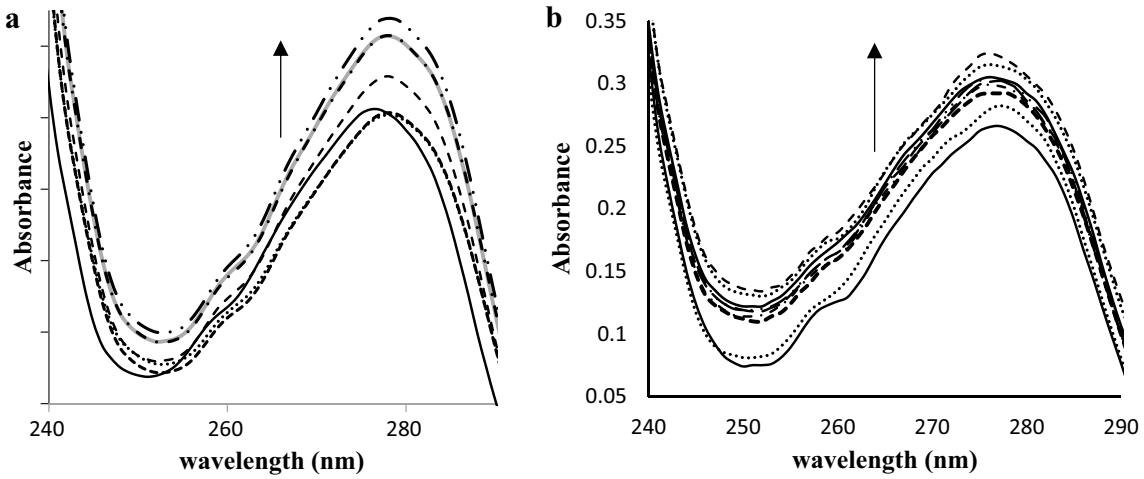

Fig. 1 Absorption spectra of BSA with increasing concentrations (0–20 μM) of (**a**) CBZ (**b**) CBZE

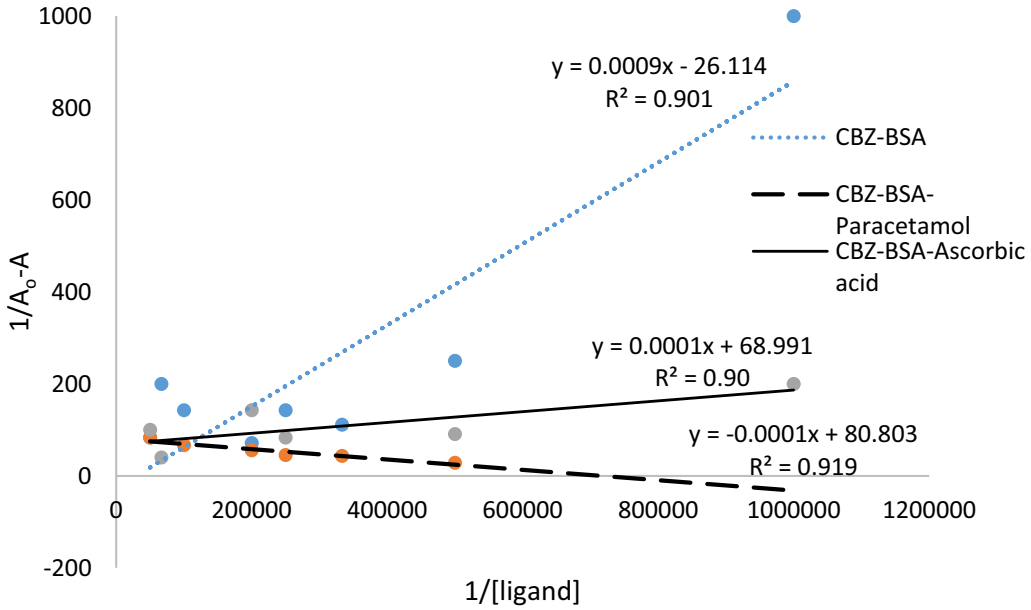

Fig. 2 Benesi–Hilderbrand plots for CBZ-BSA system in the absence and presence of competing drugs at 298 K

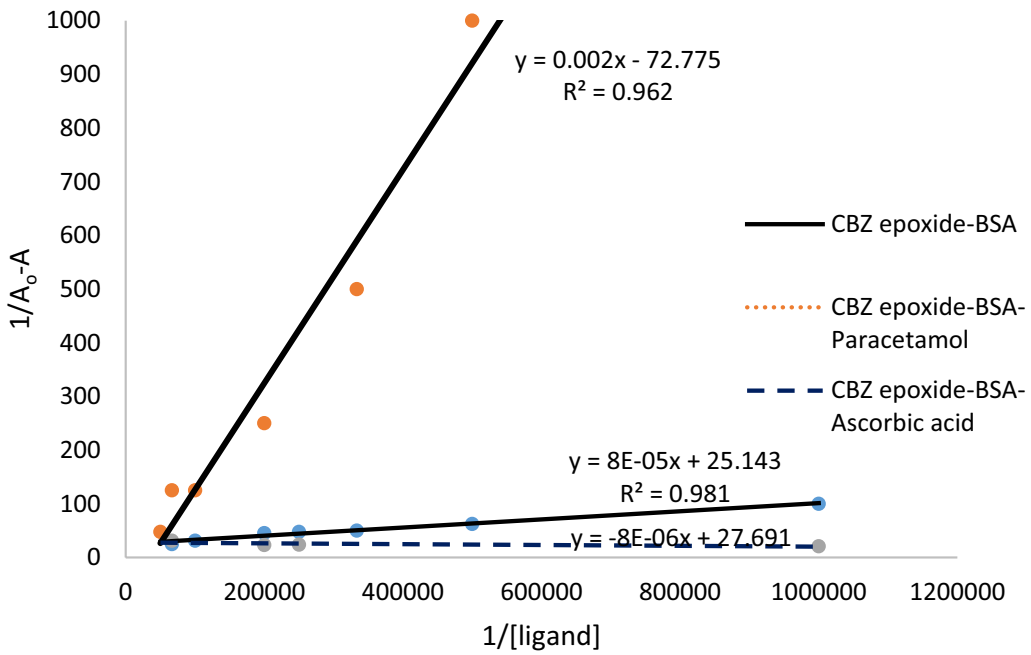

Fig. 3 Benesi–Hilderbrand plots for CBZE-BSA system in absence and presence of competing drugs at 298 K

Table 1 Binding and thermodynamic properties of the CBZ-BSA and CBZE-BSA systems

Interactions	25 °C		37 °C		ΔH (KJ/mol	ΔS (KJ/mol
	ln K_f	$-\Delta G$ (KJ/mol	ln K_f	$-\Delta G$ (KJ/mol		
CBZ	10.276	25.460	10.436	25.857	−8.445	0.002
CBZE	12.658	31.361	13.017	32.251	−8.550	0.001

a fixed concentration of BSA in the presence of increasing mole ratios of ligands and a fixed concentration of the competing drug showed progressive changes in the absorptivity of the band. As shown in the representative plots, incremental addition of CBZE in the presence of paracetamol and ascorbic acid respectively caused hyperchromic and hypochromic changes in the 278 nm band of BSA (Fig. 4).

The calculated binding constants and number of binding sites of the CBZ-BSA and CBZE-BSA systems in the presence of the competing drugs are depicted in Table 2. The number of binding sites of albumin involved in the formation and stabilization of the complexes were estimated using a modified Scatchard plot derived from Eq. 2 [3, 4]

$$\log \frac{A_{\circ} - A}{A} = \log K_a + n \log [\text{ligand}] \quad (2)$$

where A_o and A are the absorbance values of protein–ligand systems in the absence and presence of competing drug, Ka is binding constant, n is number of binding sites in BSA molecule and [ligand] is the concentration of the ligand.

Table 2 Ln Kf and *n* of ligand–protein systems in the presence and absence of competing drugs

Complex	ln K_f	*n*	*R*
CBZ-BSA	10.276	1.11	0.9
CBZ-BSA-paracetamol	13.602	1.94	0.92
CBZ-BSA-ascorbic acid	13.444	1.95	0.64
CBZE-BSA	12.658	1.27	0.98
CBZE-BSA-paracetamol	10.478	2.11	0.96
CBZE-BSA-ascorbic acid	13.442	0.56	0.91

**R* is correlation coefficient

IR study of albumin secondary structure

The difference spectrum of free albumin and CBZ-albumin complex are depicted in Fig. 5. The spectrum of free BSA showed the characteristic Amide I and II bands at

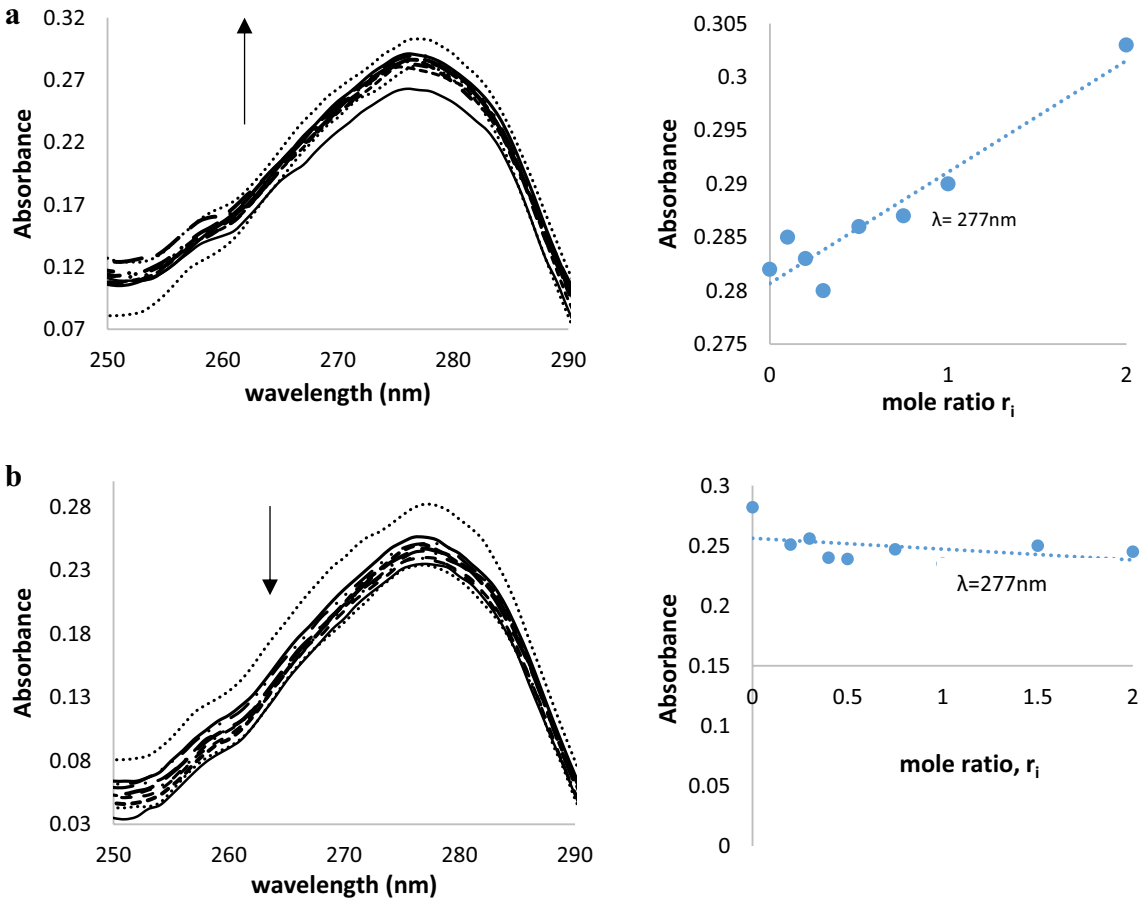

Fig. 4 Absorption of BSA with increasing concentrations of CBZE in presence of (**a**) paracetamol (**b**) ascorbic acid

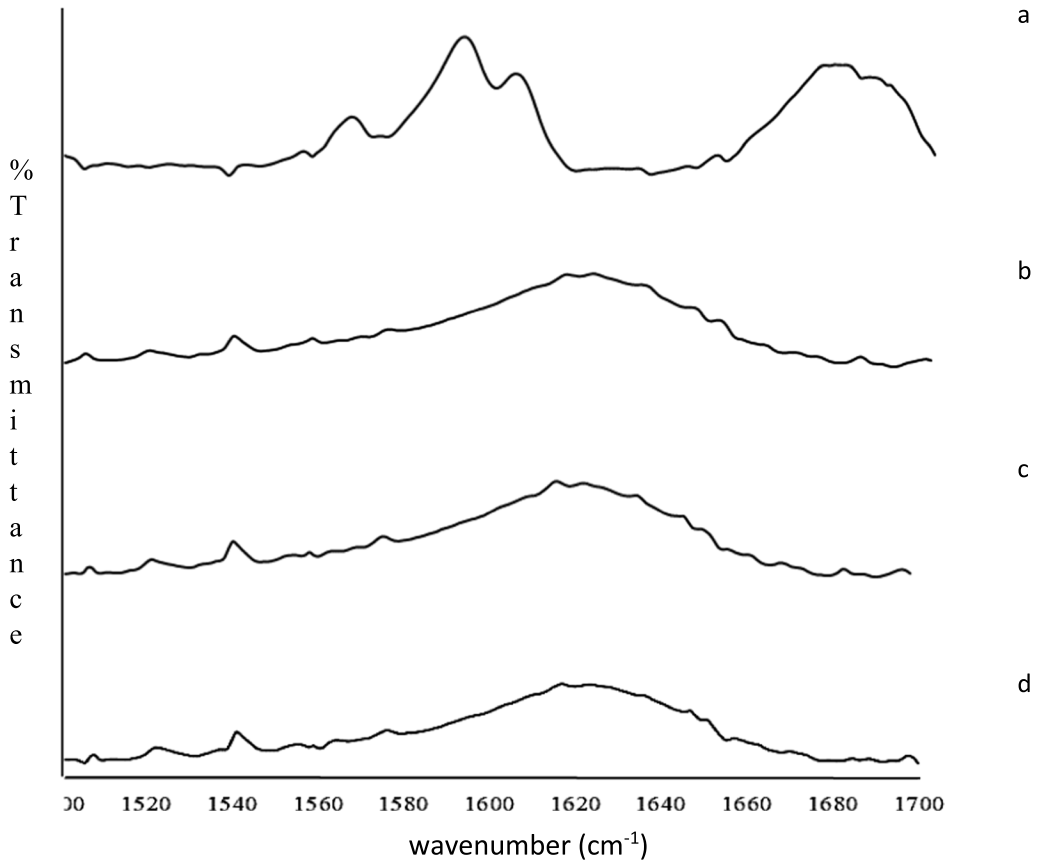

Fig. 5 Difference spectrum of (**a**) free BSA, (**b**) CBZ-BSA complex, (**c**) CBZ-BSA-paracetamol (**d**) CBZ-BSA-ascorbic acid

1677 and 1593 cm^{-1}, respectively. Following complexation with CBZ, a substantial decrease in intensity and position (from 1677 to 1623 cm^{-1}) of the Amide I band was observed. A similar decrease in band intensity of the CBZ-albumin complex was observed in the presence of interfering paracetamol or ascorbic acid. This was accompanied by a shift in Amide I band position to 1617 and 1624 cm^{-1}, respectively.

The difference spectrum of CBZE-albumin complex in the absence and presence of interfering drugs are also depicted in Fig. 6.

The reduction in the Amide I band was further confirmed by the quantitative estimation of the secondary structure of the protein using infra-red self-deconvolution and curve fitting methods. The α-helical content of albumin decreased from 67.34% in free BSA to 42.56 and less drastically, 56.43% upon interaction with CBZ and CBZE, respectively. In the presence of paracetamol and ascorbic acid interference, the CBZ-BSA complex showed a further decrease in the α-helical content of BSA to 37.76 and 34.87%, respectively. Similarly, the α-helical content of BSA in CBZE-BSA complex decreased to 46.74% in the presence of ascorbic acid while paracetamol induced more extensive perturbations in the secondary structure of BSA as the helical content was reduced to 22.78%.

Discussion

Binding constants and thermodynamic study

UV–Visible spectroscopy offers a simple but effective technique to study complex formation and structural changes following the interaction of ligands with proteins [26]. The UV–Visible spectrum of BSA revealed a high intensity band centered at 220 nm which can be attributed to the $\pi - \pi^*$ transition of C=O functional groups of the protein peptide backbone and a second less intense band centered at 278 nm due to the $\pi - \pi^*$ transition of its tryptophan, tyrosine and phenylalanine residues [4]. Following titrations with a ligand, changes in the 278 nm band arise from hydrophobic interactions involving the three amino acid residues and are often indicative of perturbations in the conformation of the protein. The very small hypsochromic shift ($\Delta\lambda = -1$ nm) in the band position of the CBZE-BSA complex is indicative of a change in the hydrophobicity of the protein arising from a change in polarity around the tryptophan residue and

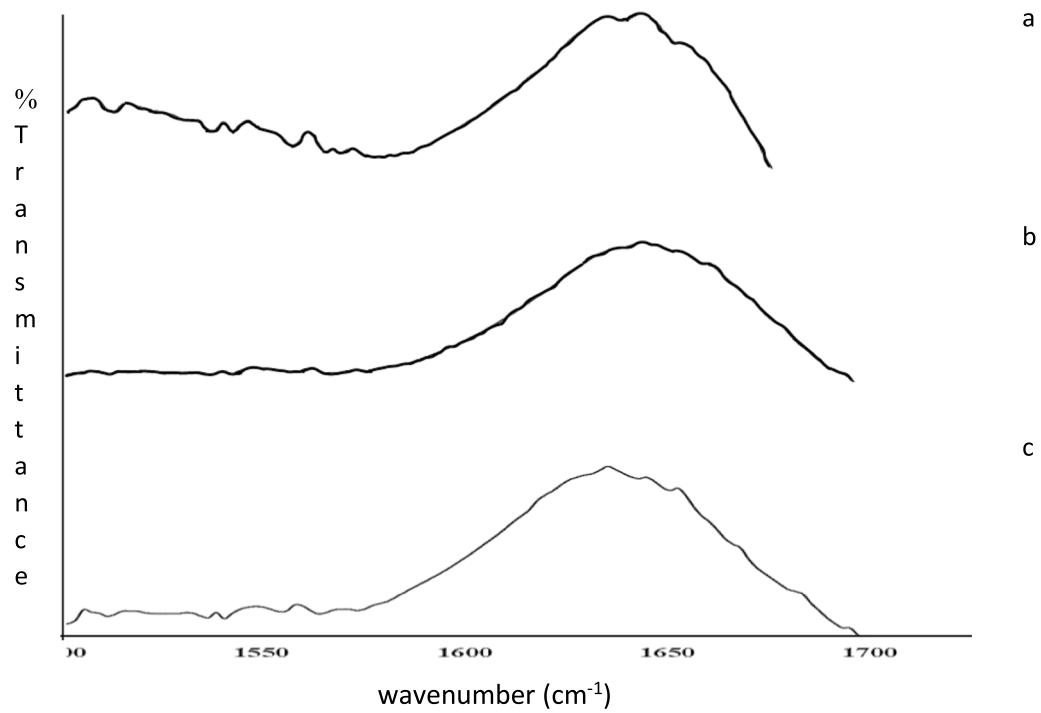

Fig. 6 Difference spectrum of CBZE-BSA complex (**a**) alone (**b**) with paracetamol (**c**) with ascorbic acid

peptide strand of BSA [22]. The perturbations observed in the absorptivity of BSA also identified ground state complex formation as the mode of interaction between the protein and the ligands. In contrast, absorption measurements do not change when the mode of interactions is driven by collisional (dynamic) quenching of the resultant complexes [3].

The formation constants of a ligand–protein complex are a direct measure of the binding affinity of a ligand for a protein. Binding affinity of a ligand is influenced by its structural chemistry and as such structural modifications by metabolism or degradation could lead to altered binding, pharmacokinetics and pharmacodynamics profiles. CBZE showed a higher affinity for BSA than the intact drug at the two temperatures investigated. Increased binding of the metabolite could lead to a more extensive distribution by circulating albumin, which often serves as an in vivo solubilizing agent for otherwise insoluble ligands [27]. This will result in the decreased clearance of the metabolite from the body and hence, a prolonged duration of action compared to the intact drug. This result is in agreement with clinical literature that puts the serum elimination half-lives of CBZ and CBZE in adults at 8–20 and 34 h, respectively [28].

The binding constants of both CBZ and CBZE increased with temperature indicating that the binding processes are endothermic while the negative free energy changes confirmed the spontaneity of the complex formation at the two temperatures investigated. The predominant intermolecular forces of interaction responsible for driving the formation and stabilization of the ligand–protein complexes can be deciphered from a consideration of the enthalpic and entropic contributions to the free energy changes associated with the binding process [4]. For drug–protein interactions, a positive ΔS often indicates hydrophobic interaction because of the increased disorderliness of water molecules that were initially arranged in an orderly fashion around the ligand and protein molecules prior to binding [29]. A combination of negative ΔH and positive ΔS values as obtained in the present study therefore confirmed that the binding of both CBZ and CBZE–BSA was driven and stabilized by hydrophobic interactions.

While our study represents, to the best of our knowledge, the first spectroscopic study of the in vitro albumin binding of CBZE, an earlier fluorimetric investigation of the binding of CBZ with albumin showed very similar results to those we obtained with the compound [29]. The comparable binding constants of 10.08 M^{-1} (ln *K*) and free energy changes of − 24.64 kJ/mol which were reported with fluorescence measurements as well as the correct identification of hydrophobic interactions as the predominant mode of interaction further validates our UV-spectroscopic approach. When properly designed, binding constants are independent of methods of estimation as large discrepancies in values arise not only from

limitations of instrumental methods but also critical experimental variables such as albumin concentration, pH of buffer, temperature control etc. [30].

Effect of the presence of a competing drug

The reference therapeutic windows for CBZ and CBZE are 4–12 µg/mL and up to 2.3 µg/mL, respectively [28]. The severity of CBZ toxicity increases with serum levels-nystagmus, tachycardia, dystonia are the prevalent symptoms at CBZ concentrations up to 40 µg/mL while higher serum levels may result in respiratory depression, coma and death [31]. Displacement of CBZ and CBZE (which are up to 75 and 60% bound to plasma proteins, respectively) from their binding sites can consequently potentiate their toxicity. This risk is expectedly heightened when the anticonvulsant is co-administered with competing drugs that are widely distributed in the body, exhibit extensive or inconsistent binding to albumin. A typical example is paracetamol which (despite is rapid absorption from the GIT and short elimination half-life of 1–3 h) exhibits widely varying binding to plasma proteins ranging from less than 20% at therapeutic doses to almost 50% with increasing doses [32]. Similarly, there exists substantial individual variability in dose-serum concentrations of ascorbic acid [33]. Ascorbic acid is also widely distributed into body tissues and shows dose-dependent increase in its absorption and elimination rates [34]. The effects of the two over-the-counter drugs on the albumin binding profiles of carbamazepine and its epoxide were therefore investigated.

As shown in Table 2, the presence of paracetamol or ascorbic acid increased the binding constants of the CBZ-BSA complex indicating a stronger binding of CBZ with albumin. An increase in the binding affinity of CBZE for albumin was also observed in the presence of ascorbic acid. On the contrary, the affinity of CBZE for albumin decreased in the presence of paracetamol indicating a decrease in the stability of the CBZE-BSA-paracetamol complex.

It has been established elsewhere that CBZ binds preferentially to site I of albumin [29]. It is more common for the albumin binding affinity of a drug to decrease in the presence of a second drug competing for the same binding site on the transporter protein (competitive interference) [3]. Furthermore, the two drugs may show independent binding in which they occupy different binding sites without causing a noticeable change in the binding affinities of each other. Alternatively, the simultaneous binding of two drugs may cause conformational changes in the transporter protein leading to an increase in the number of its binding sites or the spatial accessibility of existing binding sites [35]. This phenomenon known as non-competitive interference often results in an increase in binding affinity of a drug as was observed with CBZ-BSA-paracetamol, CBZ-BSA-ascorbic acid and CBZE-BSA-ascorbic acid ternary complexes. From the foregoing, it can be deduced that the binding constants of two co-administered drugs do not necessarily dictate their ability to displace each other from their complexes with albumin. Rather, the structure and binding dynamics of the drugs are more critical [3]. The results therefore showed that CBZ and CBZE when co-administered with ascorbic acid could be better transported by circulating albumin thereby increasing their access to storage sites. Increased binding may also reduce the free concentration of the antiepileptic agents in the plasma which is critical in producing both the desired therapeutic action and unwanted deleterious effects. The reduced plasma levels of CBZ and its active metabolite as a result of their increased albumin binding in the presence of ascorbic acid may be partly responsible for the reported ameliorative effects of the vitamin on the toxicity of CBZ [11, 18]. A similar reduction in the plasma levels of CBZE due to an increase in its biotransformation to stable dihydrodiols has been previously identified as the mechanism by which ascorbic acid ameliorates the hepatotoxicity of CBZ in rats [11]. In contrast, the presence of paracetamol led to a decrease in the albumin binding of CBZE and in the stability of the resultant ternary complex. This competitive interference by paracetamol will effectively increase the serum concentrations of the epoxide metabolite and the likelihood of side effects. It has been previously reported that increase in serum CBZE levels (unlike CBZ) readily results in a saturation of plasma proteins binding and increased availability of the metabolite for CNS toxicity [10]. A combination of these mechanisms might therefore partly explain the elevated serum concentrations of the anticonvulsant and sudden onset of adverse effects that have been reported following co-administration of paracetamol in CBZ-treated patients [17].

A cursory examination of the binding constants in Table 2 will also reveal large variations in the ratios of the binding affinities of CBZ to CBZE in the absence and presence of the interfering drugs. The ratio of the binding affinities of CBZ and CBZE varied from 0.81 in the absence of competing drug to 1.29 and 1.0 with paracetamol and ascorbic acid, respectively. It is noteworthy that in clinical medicine, there is considerable inter-individual variability in the serum levels of CBZ that is required to elicit optimal therapeutic response and/or toxicity [28, 31]. This in turn has been attributed to large inter-patient variations in serum CBZ/CBZE ratios with some previous studies having documented up to two- and three-folds inter-patient variations in the unbound fractions of CBZ and CBZE respectively [28, 36]. In a particular

case report which proved fatal, serum CBZE level that was 450% higher than the parent drug was reported [10]. While factors such as the differences in plasma protein concentrations as well as drug-concentration dependence in the binding of the ligands were shown to be responsible for the widely varying CBZ/CBZE ratios, this present study has confirmed the contributory role of competing interference, a factor that was also suggested in one of the earlier studies [36]. The tendency of ascorbic acid to even out the relative serum CBZ and CBZE levels will invariably contribute to its protective capacity in CBZ-toxicity.

Ligand-induced changes in secondary structure of albumin

Characteristic absorption bands in the FTIR spectrum of proteins can provide insightful information about their secondary structure [22]. These include Amide I and Amide II bands which arise from the interaction of infra-red with amide bonds linking the amino acids of a protein. The Amide I band which is characteristically found between 1600–1700 cm^{-1} can be attributed to the stretching vibrations of the C=O bonds while the Amide II band (1470–1570 cm^{-1}) are due to the bending vibrations of N–H bond [37]. Since both bands are involved in the intermolecular bonding stabilization of the secondary structure of proteins, a study of the bands position can be employed to quantify the secondary structure content of proteins [38]. The observed changes in the intensity and position of the Amide I band therefore indicate a ligand-induced change in the secondary structure of albumin. Such changes have been identified from previous studies to be due to hydrogen bond interactions between ligands and the C=O and/or N–H functional groups of the protein [22]. The reduction in the Amide I band was further confirmed by the quantitative estimation of the secondary structure of the protein using the Byler and Susi method [39]. The reduction in the α-helical content and the consequential conversion to other components of BSA secondary structure is indicative of the partial unfolding of the protein following ligand binding. The most extensive perturbation in the secondary structure of BSA (22.78% α-helical content) which was observed with CBZE-BSA complex in the presence of paracetamol is probably due to the increased interaction of the protein for the analgesic.

Conclusions

The interaction of CBZ and its major metabolite, CBZE with BSA in the absence and presence of two over-the-counter medicines were investigated using UV and IR spectrophotometry. The presence of ascorbic acid may increase the binding affinities of CBZ and CBZE for plasma proteins thereby reducing their free plasma concentrations and therapeutic/toxicity activity. Similarly, paracetamol increased the affinity of CBZ for albumin but caused a decrease in the binding constant of the CBZE-BSA system. IR study also revealed protein–ligand complex formation was associated with a change in the secondary structure of BSA as evidenced in marked reduction in the α-helical component of the unbound protein. The study has revealed potential interference of paracetamol or ascorbic acid with the albumin binding of CBZ and its major metabolite.

Abbreviations

BSA	Bovine serum albumin
CBZ	Carbamazepine
CBZE	Carbamazepine-10,11-epoxide
IR	Infra-red
UV	Ultraviolet
LCMS	Liquid chromatography hyphenated to mass spectrometry
NMR	Nuclear magnetic resonance

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Author contributions

OET was involved with study conceptualization, designed, supervised experiments, analyzed the data, drafted and reviewed the manuscript. AA, YSO and DD were involved in data generation and analysis. All authors read and approved the final manuscript.

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Availability of data and materials

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

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Studies involving plants

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Competing interests

The authors declare that they have no competing interests.

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