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Spectroscopic Investigations of Conformational Change in Bovine Serum Albumin (BSA) with Rising Concentration of a Mood Stabilizing Drug: Lamotrigine

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Abstract

The current study investigates lamotrigine's binding to bovine serum albumin (BSA) using UV visible, fluorescence, synchronous fluorescence (SFS), CD spectroscopy, and molecular docking. The addition of lamotrigine at increasing concentrations suppressed the intensity of the BSA's fluorescence. Synchronous, and UV-vis fluorescence experiments demonstrated the effects of lamotrigine on the structure of BSA. CD spectroscopy was used to determine how the native BSA's stability changed when lamotrigine was included. To assess the binding sites and affinity of lamotrigine to BSA with simultaneous interactions, molecular docking studies were done using AutoDock. This work contributes to our perception of the mechanism underlying the drug-binding effect at higher concentrations by elucidating the molecular interactions and modifications between lamotrigine and BSA.

Keywords: Lamotrigine, BSA, Interaction study, Spectroscopic techniques

Introduction

Lamotrigine was initially sold in Ireland in 1991 (Weisler et al., 2008) (Alizadeh et al., 2008) (Beck et al., 2006) and it was authorized for usage there in 1994 (Figure 1). According to the WHO model list of essential medicines lamotrigine is enlisted. Lamotrigine was added to the 20th list, which was published in 2017. Lamotrigine is added to NLEM (National List of Essential Medicines) India. The addition of these drugs to the above-mentioned lists, itself indicates the importance of drugs (OMS, 2019). Lamotrigine is offered in generic form under numerous brand names all over the world and is marketed under the brand name Lamictal (original name). Lamotrigine, often known as Lamictal, is a mood stabiliser that is prescribed to treat epilepsy and bipolar disorder. This covers tonic-clonic seizures, focal seizures, and LGS (Lennox-Gastaut Syndrome) seizures in the context of epilepsy. Lamotrigine appears to be helpful in reducing the likelihood of future depressive episodes in people without symptoms, but it is not a dependable treatment for acute depression in bipolar illness. Lamotrigine differs chemically from other anticonvulsants since it is a phenyltriazine. Though its exact mode of action is unknown, it seems to prevent the release of excitatory neurotransmitters in neurons by blocking voltage-sensitive sodium and voltage-gated calcium channels (Bendtsen et al., 2019),(V. S. Patil1, 2, S. N. Labade1 et al., 2023).

The study of the interaction of lamotrigine with bovine serum albumin (BSA) needs of intense research (Figure 2). The importance of this research work is to create and produce medications as well as find new ones, it is essential to comprehend serum albumin and drug interaction research. The binding interaction between BSA and lamotrigine brings about an accountable conformation shift in BSA. Our study's main objective was to find the binding interaction between lamotrigine and BSA using UV - Visible spectroscopy (UV), fluorescence quenching spectroscopy, synchronous fluorescence spectroscopy (SFS), circular dichroism spectroscopy (CD) and molecular docking (Patil et al., 2022). This allowed us to collect important information about BSA's fluorescence quenching, lamotrigine’s binding site and modes, and the conformational change that occurred when lamotrigine was bound to BSA. The goal of this work is to provide a comprehensive understanding of lamotrigine dynamics and to illustrate the molecular binding processes of lamotrigine with BSA (Vidya Patil, Sandeep labade, Ranjana Jadhav, 2023).

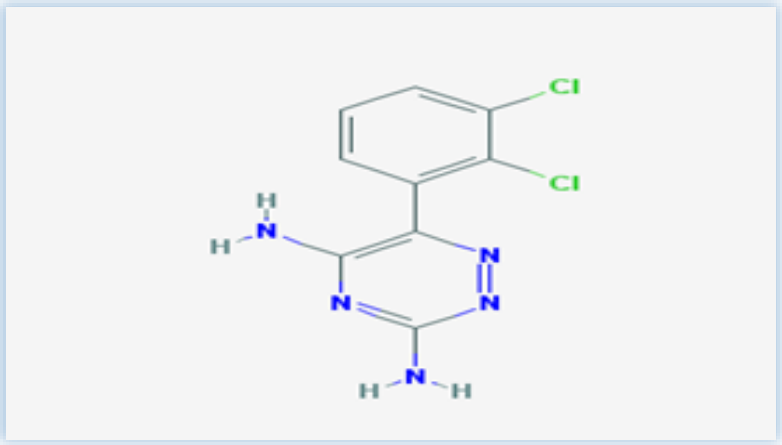

Figure 1: Structure of Lamotrigine

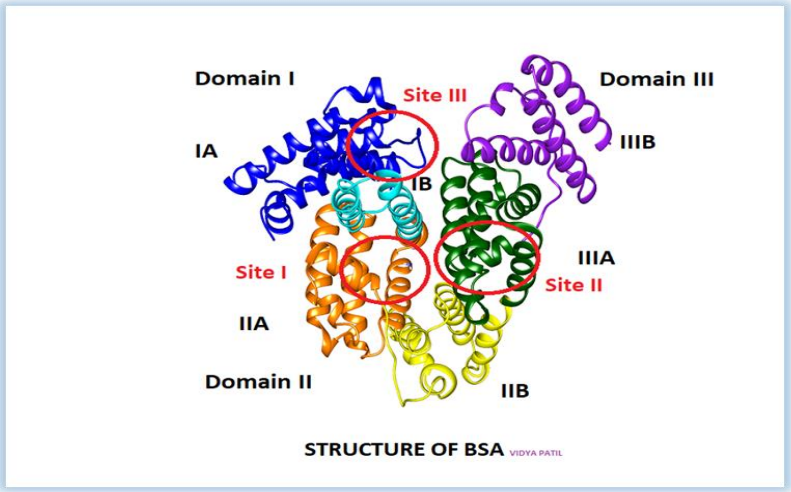

Figure 2: Bovine Serum Albumin (BSA) Structure

Materials and Methods

2.1 Reagents and Solutions

Bovine Serum Albumin (M.W. ~66 KDa, ≥99% essential fatty acid, endotoxin, protease, DNase, RNase free) and Phosphate Buffered Saline (PBS) 10X (pH = 7.40) were purchased from SRL India. Lamotrigine (≥98%) was purchased from TCI-America (98 %). Stock solutions of BSA (200 μMdm^{-3}) and lamotrigine (200 μMdm^{-3}) were prepared by using 1X PBS buffer solution (pH = 7.40).

2.2 UV-visible Spectra Measurements

Using 10 mm quartz rectangular cells ranging in wavelength from 200 to 400 nm, the UV-visible absorption spectra of albumin, lamotrigine, and albumin were recorded at room temperature using a UV-1600 spectrophotometer (Shimadzu, Kyoto, Japan). The reference solution was 1X PBS solution (pH=7.40) (Ma & Allen, 2004).

2.3 Fluorescence Quenching Spectra Measurements

Using a 10 mm quartz rectangular cell with a scan range of 270 to 500 nm, the steady-state fluorescence spectra of BSA solutions with and without lamotrigine at different doses were measured at pH 7.40 on an FP-8300 Spectrofluorometer (Jasco, Japan) with λex set at 278 nm. Both slit widths were maintained at 5 nm, and the scan speed was set at 500 nm/min.

2.4 Synchronous Fluorescence Spectra Measurements

On an FP-8300 Spectrofluorometer with a 10 mm quartz rectangular cell (Jasco, Japan) at room temperature, the synchronous fluorescence spectra of BSA solution and BSA in the presence of different concentrations of lamotrigine were measured at different scanning intervals of Δλ (Δλ= λem – λex, where λem and λex are the emission and excitation wavelengths, respectively). The Tyr residue's characteristics are described by the values of Δλ, which were set at 60 nm and 15 nm, respectively. With λex set at 278 nm, the scan range was set between 270 and 500 nm. Both slit widths were maintained at 5 nm, and the scan speed was maintained at 500 nm/min (Lakowicz, 2006).

2.5 Circular dichroism (CD) Spectroscopy:

Spectral analysis is done by using a CD spectrophotometer with stop-flow and a Jasco CD Spectrometer Model J - 1500 with quartz cells with a 10 mm path length. With a data interval of 1 nm and a scanning speed of 100 nm min^{-1}, spectra in the 190 – 300 nm wavelength range were obtained. The spectra are the three-scan averages. Due to the impact of oxygen, the measurements were conducted in a nitrogen environment. Spectra Manager II software and Protein Analysis Programme Package were used for the analysis of the evidence (Ma & Allen, 2004).

JASCO spectra manager (CD Multivariate SSE) program: The [CD Multivariate SSE] program estimates the secondary structure of a protein sample from a spectrum obtained by using the multivariate analysis technique. It includes the CD spectra of protein sample measured by JASCO and the calibration models created from these data. A unified platform for the entire line of JASCO analytical instruments, including as Visible, UV - Visible, near-infrared, infrared, Raman, fluorescence, polarimetry, and circular dichroism (CD) spectrometers, is provided by JASCO Spectra Manager TM, an integrated software suite.

2.6 Molecular Docking:

Molecular docking studies were used to investigate ligand binding modalities to bovine serum albumin (BSA) and their molecular interactions. Docking studies were carried out using the Autodock Tool (ADT). (Vidya Patil, Sandeep labade, Ranjana Jadhav, 2023)The rigid-receptor/flexible-ligand model and Lamarckian genetic algorithm are the two docking techniques that are most frequently used to represent the interaction between lamotrigine and BSA protein. On BSA, three ligand-binding sites are simple to locate with MD software. All research used the 3D crystal structure of BSA (PDB ID: 4OR0) from the Protein Data Bank (PDB), in our study it was produced in UCSF Chimera to eliminate crystal water and heteroatoms. Chain A of BSA is considered for docking study with Lamarckian Genetic Algorithm (LGA).
[(https://pubs.acs.org/doi/10.1021/acs.jchemed.5b00404#:~:text=The%20Research%20Collaboratory%20for%20Structural,associated%20small%20molecules%20)].
The 3D structures of Lamotrigine (LMZ; PubChem ID: 3878) were retrieved in SDF format from PubChem Database [(https://pubchem.ncbi.nlm.nih.gov/)]. Then 3D structures of ligand were prepared by optimization and preparing using MMFF94 force field in Chem3D 15.0 with a maximum number of iteration 500 and minimum 0.1 RMS gradient. The ligand structure was saved in PDBQT file format instead of pdb. extension and used for docking studies with BSA. The grid box was set at the dimension of 122 Å × 100 Å × 94 Å with 0.375 Å grid spacing encompassing the binding site (Sudlow's site I and II) of BSA, and the grid center at x = 1.077, y = 21.684, z = 111.471 for all ligand molecules. During analysis, the receptor (BSA) was kept rigid and ligand flexible. Further, a 0.02 rate of mutation and 0.8 crossing-over rate with a population size of 150 to generate 20 docked conformations for 27000 generations and 250000 evaluations was used during docking simulation. The generated conformations clustered based on binding energy and default RMSD 2.0, further lowest binding energy (highest binding affinity) conformations were selected for analysis of molecular interactions by utilizing software like UCSF Chimera and Discovery Studio (Patil et al., 2022).

Result and Discussion

3.1 UV spectra measurements

This is a very useful method for evaluating protein conformational changes and identifying the existence of a protein-ligand interaction in UV-visible spectroscopy. By examining the UV spectra of BSA and Lamotrigine-BSA the complex's formation was

verified (Figure 3). UV-visible absorption spectroscopy data shows that the peak BSA intensity has increased in tandem with the growth in lamotrigine concentration. When lamotrigine bound to BSA, a ground state complex called Lamotrigine-BSA was formed, which resulted in a **noticeable blue shift of 2 nm at 278 nm**. Absorption between 260 to 300 nm is due to the polarity of the microenvironment (Trp, Phe, and Tyr). **200-400 nm** can additionally provide detailed information about **secondary and tertiary conformation structural changes.** BSA binds to lamotrigine, resulting in conformational changes around the **Trp and Tyr.**

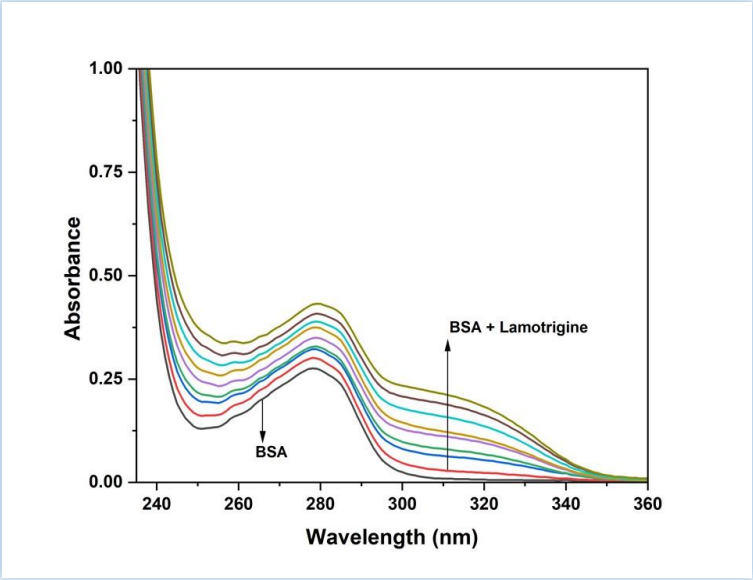

Figure 3: UV absorption spectra of BSA (2 µMdm^{-3}) with various concentrations of lamotrigine from 0, 20, 40, 60, 80, and 100 µMdm^{-3} respectively at pH 7.4.

3.2 Fluorescence Spectroscopy

An extensive examination of the relationship between BSA and lamotrigine was conducted by a fluorescence quenching experiment. Because BSA contains traces of Tyr, Phe, and Trp, it glows naturally. The fluorescence characteristic of BSA is mostly caused by the Trp residue, as evidenced by the ratio of Trp to Tyr to Phe fluorescence intensity of 100:9:0.5 (Vidya Patil, Sandeep labade, Ranjana Jadhav, 2023). The structure of BSA contains two sensitive Trp residues (Trp 134 and 213). The intrinsic fluorescence of the BSA macromolecule is mostly caused by these aromatic moieties. The binding interactions between BSA and lamotrigine were demonstrated by the dimming of BSA's fluorescence

spectra. Energy is also transferred between the quencher, Lamotrigine, and the fluorophore, which in this case is the Trp and Tyr residues in BSA, during the quenching process. Lamotrigine efficiently quenched the intrinsic fluorescence of BSA, forming the Lamotrigine-BSA combination. The discrepancy in the absorption spectra of the fluorophore (BSA) and the Lamotrigine-BSA complex indicates a substantial possibility of static quenching. The results (Figure 4) show that Trp residues are mainly responsible for the high fluorescence emission peak that BSA displays at **341 nm** at **λex = 280 nm**. **The blue shift** of the maximum emission wavelength (λem) (~11 nm); indicates a considerable microenvironment change around fluorescent Trp residues. **Modification in tryptophan** residues microenvironment. Static quenching and strong remarkable binding interactions existed between lamotrigine and BSA. It suggests that BSA changes into a non-native form when lamotrigine is present.

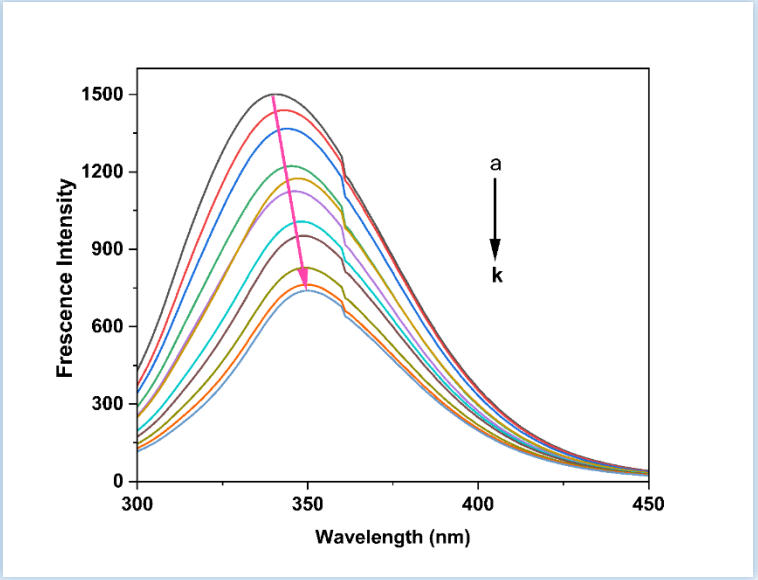

Figure 4: Fluorescence spectra of BSA (20 µMdm^{-3}) with various concentrations of lamotrigine from 0,10, 20, up to 150 µMdm^{-3} increasing order concentrations (pH 7.4)

3.3 Binding Site and Constant Study

The Stern - Volmer equation was utilized to compute the quenching characteristics of lamotrigine that were inserted in the BSA. The binding site (n) and binding constants (K_b) of the Lamotrigine-BSA complex can be found using a double logarithmic plot. Figures 5 and 6 express the two graphs.

$$\frac{F_0}{F} = 1 + K_q \tau_0 [Q] = 1 + K_{sv}[Q] \quad \text{Eq. 1}$$

$$\log \frac{(F_0 - F)}{F} = \log K_b + n \log[Q] \quad \text{Eq. 2.}$$

The BSA fluorescence intensities with and without lamotrigine are represented by F_0 and F, respectively, while the concentration of lamotrigine is shown by [Q]. A quenching constant is K_{sv}. Plotting log [(F_0 – F)/F] vs. log [Q] is an analytical technique for determining protein quenching rate constants (K_q), binding constants (K_b), and binding site numbers (n). At the temperature under investigation, the (n) value of the combination Lamotrigine-BSA is one, indicating that BSA has one lamotrigine binding site. The four forces responsible for the drug-protein binding response are hydrogen bonds, electrostatic forces, van der Waals forces, and hydrophobic interaction forces. These forces are frequently indicated by the thermodynamic parameter signs and magnitudes of ΔH^0 (enthalpy change) and ΔS^0 (entropy change). ΔH^0 and ΔS^0 can be considered constants in this case. The calculation method is the Van 't Hoff equation.

$$\ln K_b = \frac{-\Delta H^0}{RT} - \frac{\Delta S^0}{R} \quad \text{Eq. 3}$$

Where R is the gas constant, T is the experimental temperature, and K_b is a binding constant of corresponding T. Then ΔG^0 can be raised from the equation:

$$\Delta G^0 = -RT \ln K_b = \Delta H^0 - T\Delta S^0 \quad \text{Eq. 4}$$

The binding between lamotrigine and BSA is significantly influenced by van der Waals forces and H-bonds, as indicated by the negative values of ΔS^0 and ΔH^0. On the other hand, abrupt lamotrigine binding with BSA is indicated by $\Delta G^0<0$, $\Delta H^0<0$, and $|\Delta H^0|>|T\Delta S^0|$; this reaction is exothermic and driven by enthalpy.

Table 1: Different constants, binding sites, and energy obtained from fluorescence data

	K_{sv} / M^{-1}	K_q / $M^{-1}s^{-1}$	K_b / M^{-1}	n	ΔG^0 / JM^{-1}
Value	6.8149×10^3	3.4075×10^{12}	3.2017×10^5	1.4594	-1.3640×10^4

The data may indicate a significant binding between BSA and lamotrigine. Data generated from the theoretical parameter under study, based on fluorescence measurements, are

displayed in Table 1. 6.8149 x10^3 is the value of the Stern - Volmer dynamic quenching constant (K_{sv}). It was discovered that the bimolecular quenching rate constant (K_q) was 3.4075 x 10^{12}. The values of K_b and n for lamotrigine with BSA binding were determined to be 3.2017 x 10^5 and one, respectively. Considering the (n) value, it makes sense that lamotrigine would attach to the BSA and have one binding site. Binding affinity is indicated by a binding constant (K_b = 3.2017 x 10^5). The fluorescence study calculations indicate that there is a quick binding of lamotrigine with BSA, with an exothermic and enthalpy-driven binding process, as shown by the negative values of ΔG^0 = -1.3640×10^4 / JM-1 (ΔG^0<0). Van der Waals and H-bonds forces are the main mechanisms by which BSA and lamotrigine bind. The binding and occupation of lamotrigine with BSA are significantly

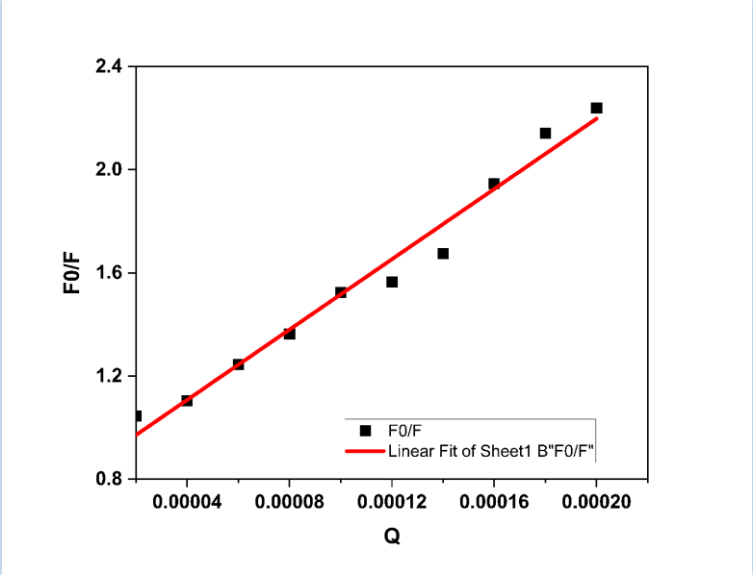

Figure 5: Stern - Volmer plot for the quenching of BSA (20 µMdm^{-3}) induced by lamotrigine

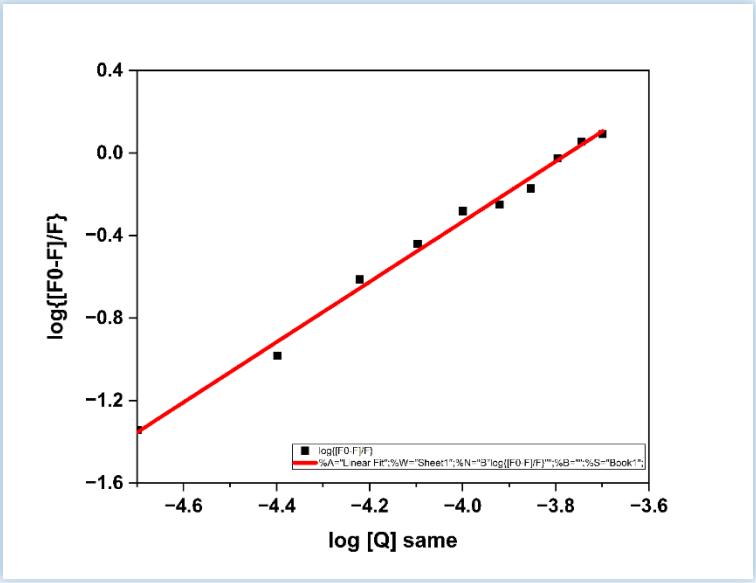

Figure 6: Modified Stern - Volmer plot for the quenching of BSA (20 µMdm^{-3}) induced by lamotrigine

3.4 Synchronous Fluorescence Measurement

SFS measures the polarity variations surrounding the fluorophore by computing the maximum emission wavelength. Consistent fluorescence emission wavelength shift measurements are employed in spectroscopy to investigate the microenvironment change information of Tyr and Trp residues. The Trp and Tyr residues' microenvironments alter at $\Delta\lambda$ = 15 and 60 nm, respectively. A λem shift is frequently employed to describe the shift in the polarity of the surrounding environment in the vicinity of Tyr and Trp residues. In Figure 7, the synchronous fluorescence spectra of Tyr residue with $\Delta\lambda$ = 15 nm in 20 µMdm^{-3} BSA are shown with different concentrations of lamotrigine (10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 µMdm^{-3}). The concurrent fluorescence spectra of the Trp residue with $\Delta\lambda$ = 60 nm in 20 µMdm^{-3} BSA at different dosages of lamotrigine (10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 µMdm^{-3}), are shown in Figure 8. When lamotrigine was present, **no significant shift was observed.** It concludes that there is no change around the microenvironment polarity of Tyr residues. The **1 nm blue shift** is observed at 338 nm for 60 nm SFS, a **significant observation.** It concludes that there is a remarkable change around the microenvironment polarity of Trp residues. Consequently, the hydrophobicity

increases and the polarity surrounding the Trp residues slightly alters when BSA binds to lamotrigine. It is possible to conclude that lamotrigine binds to BSA significantly in the Trp region with the folding state being impacted.

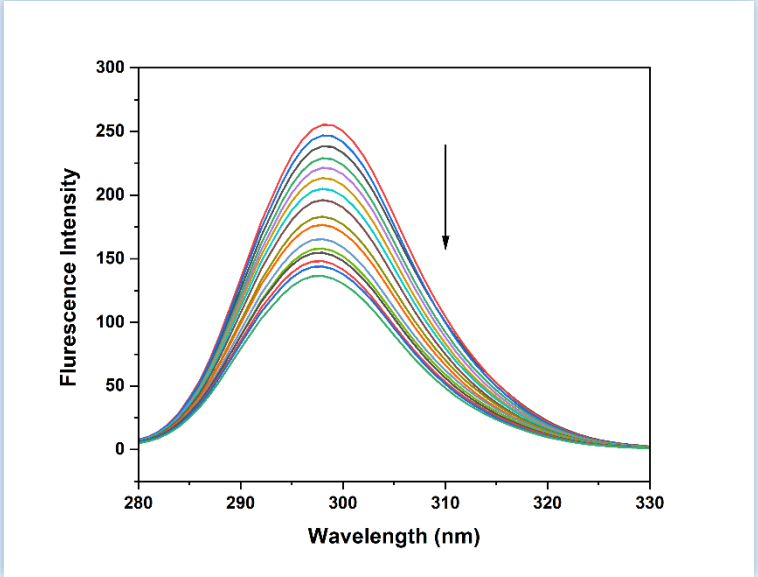

Figure 7: The synchronous fluorescence spectra of Tyr residue with $\Delta\lambda = 15$ nm in BSA of 20 µMdm^{-3} with various increasing concentrations of lamotrigine 0, 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 µMdm^{-3}

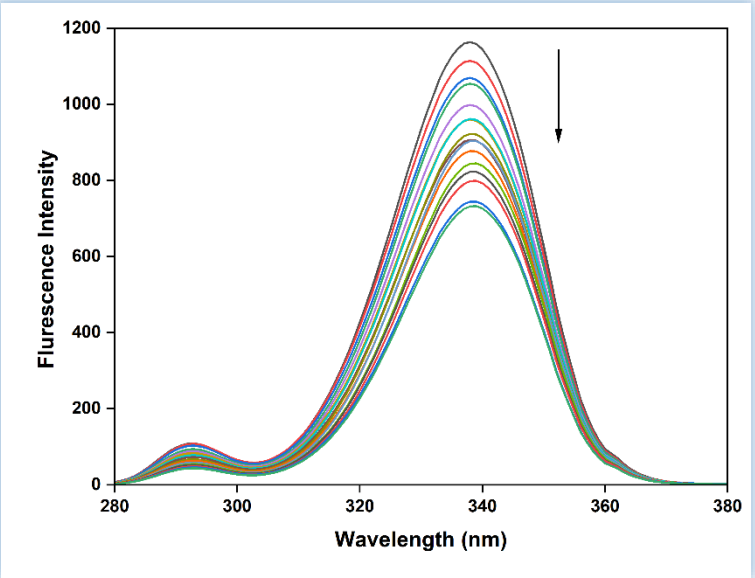

Figure 8: The synchronous fluorescence spectra of Trp residue with $\Delta\lambda = 60$ nm in BSA of 20 µMdm^{-3} with various increasing concentrations of lamotrigine 0, 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 µMdm^{-3}

3.5 Circular dichroism spectroscopy

CD spectroscopy is used to examine the protein structure of complex systems comprising BSA and Lamotrigine-BSA at a pH of 7.4. The study examined complex systems including BSA and lamotrigine, where lamotrigine is present in 1:0, 1:1, and 1:2 ratios concerning BSA, as well as BSA in a solution containing 20 µMdm^{-3}, using CD spectra. The P20-L00, P20-L20, and P20-L40 complex systems were employed during the investigation; the corresponding effective doses of lamotrigine were 0, 20, and 40 µMdm^{-3} (Figure 9). The findings indicated that the $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ electron transfers in the α helix peptide bond take place at 222 and 208 nm, which are associated with negative bands. Lamotrigine added to these bands made them less strong. The secondary structure of BSA and Lamotrigine-BSA complexes was investigated using a quantitative multivariate analysis tool called Jasco Spectra Manager TM software and a Jasco J-1500 CD spectrometer. The percentage change in the BSA structure with various conformations is displayed in the data. The findings indicated that **α helix decreased (41.3% to 36%) and β sheet decreased (24.3% to 19.2%).** However, when Lamotrigine-BSA complex systems are in a 1:1, 1.2, or higher ratio, there is an enhanced content of β-Turn, and random coiling with 8.20%, and 2.10% respectively, in the analyzed BSA. The secondary structure of BSA

has remarkably broken down, indicating that lamotrigine has bound to BSA. Through intercalation and helicity disruption, it modifies the structure of the protein. Table 2 shows the results of the secondary structure analysis of the BSA and Lamotrigine-BSA complex systems performed in the YANG reference study. The conformational shift affects the hydrophobicity. β-pleated sheet, β-Turn, and random coil structures make the surrounding of tryptophan residues in BSA more hydrophobic. Thus, it appears that hydrophobic contact is necessary to maintain the stability of the Lamotrigine-BSA complex.

Table 2: Secondary structure analysis of BSA and Lamotrigine-BSA complex systems by YANG reference study

Secondary structure analysis of BSA and Lamotrigine-BSA complex systems by YANG reference study					
JASCO Spectra Manager TM software is used which is a quantitative multivariate analysis program					
System Name →	**P20-L00**	**P20-L20**	**P20-L40**		
Conformation ↓	% Ratio	% Ratio	% Ratio	% Change	Observation
Helix	41.3	40.6	36	5.30%	Decreased
Beta	24.3	21	19.2	5.10%	Decreased
Turn	7.2	9.3	15.4	8.20%	Increased
Random	27.2	29.1	29.3	2.10%	Increased
Total	100	100	100	0%	Same

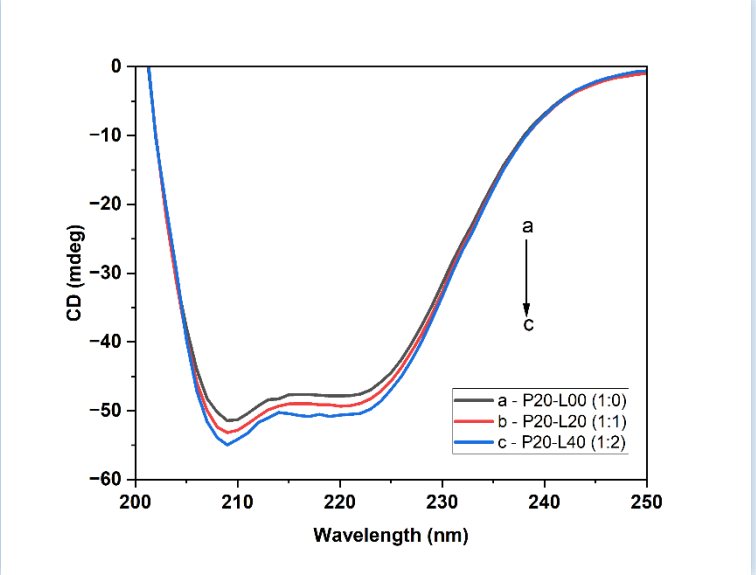

Figure 9: Circular dichroism spectra of BSA solutions (20 µMdm^{-3}) in the presence of lamotrigine at 298K with the concentrations of lamotrigine 0, 20, and 40 µMdm^{-3} respectively

3.6 Molecular docking

To assess the binding sites and affinity of lamotrigine to BSA with simultaneous interactions, molecular docking studies were done using AutoDock. Discovery Studio tps://discover.3ds.com/discovery-studio-visualizer-download] (*Discovery Studio Visualizer (BIOVIA, 2021)*, n.d.) was used to create 2D and 3D interaction graphs (Figure 10). The binding energies analysis shows that lamotrigine has a strong affinity for docked BSA, binding with the lowest value of **-7.09 kcal/mol**. The lowest binding energy conformation was chosen for molecular interaction analysis utilizing UCSF Chimaera and Discovery Studio to comprehend the molecular interactions involved in lamotrigine's binding to the BSA (Das et al., 2020). Lamotrigine has a significant affinity for binding BSA, which indicates the intensity of the binding, according to molecular docking investigations.

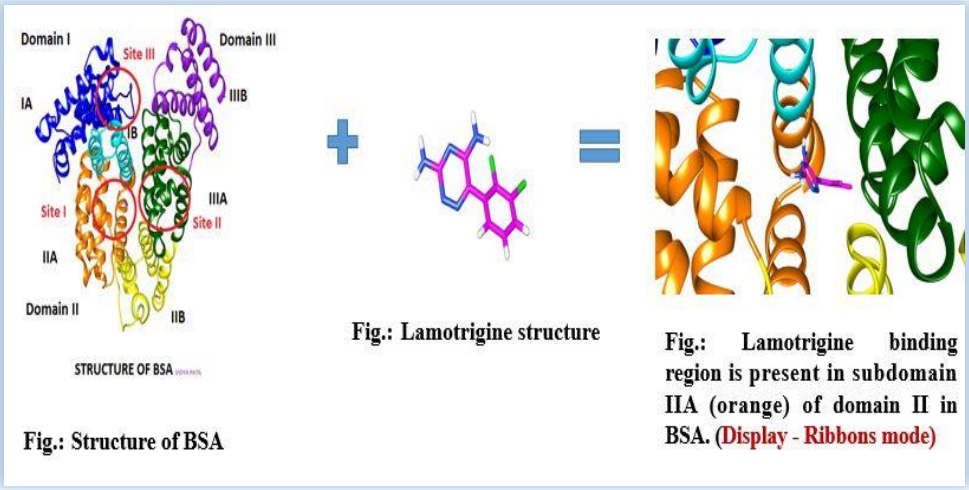

Figure 10: Lamotrigine binding region is present in subdomain IIA of domain II of BSA

Two conventional hydrogen bonds between Arg194 and Arg198 of BSA are entangled in the binding of lamotrigine with BSA and numerous hydrophobic contacts stabilize the connection, as seen in 2D and 3D interaction images (Table 3). Lamotrigine is also involved in non-covalent interactions such as interaction between Trp213 and lamotrigine. Residue Trp213 in the hydrophobic site (Domain IIA) is responsible for intrinsic fluorescence (Figure 11). A comprehensive examination of the residues implicated in the interactions between BSA and lamotrigine is found in Table 2. Hydrophobic pi-sigma interaction found at Leu197 in binding of lamotrigine with BSA. Pi-alkyl interactions were observed between Val342 and Val481 of BSA. Van der Waals interactions play a crucial role in the binding of lamotrigine to BSA by using Gln195, Ser201, Leu480, Ser343, Ser453, Asp450, Arg217, and Leu454 residues. **Arg198 and Trp213 are present in subdomain IIA of domain II which is a drug binding site I (Sudlow Site I).** As can be observed in the 2D interaction docked image (Figure 11), each residue participates in a variety of interactions, including alkyl, pi-alkyl, van der Waals, conventional hydrogen bonds, and carbon-hydrogen bonds.

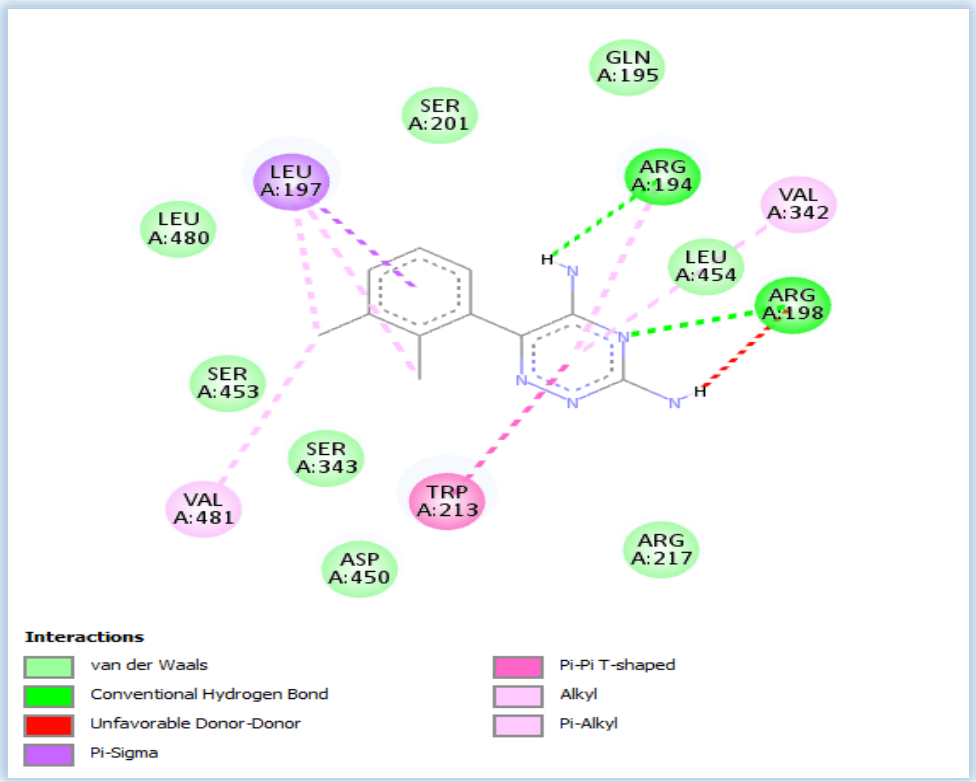

Figure 11: 2D Interaction plot showing the residues involved in various interactions

Table 3: Comprehensive analysis of residues involved in interactions of Lamotrigine-BSA

Lamotrigine		
Sr. No.	**Name of Bond/ Interaction**	**Residues of BSA Involved in Interactions**
1	Conventional hydrogen bond - 2	Arg194, Arg198
2	Pi-Pi interaction	Trp213
3	Pi-Sigma interaction	Leu197
4	Pi-Alkyl interaction	Val342, Val481
5	Van der Waals interactions	Gln195, Ser201, Leu480, Ser343, Ser453, Asp450, Arg217, Leu454

Conclusion

This work describes the interaction between lamotrigine and BSA by using multi-spectroscopic techniques. The Stern - Volmer quenching constant (K_{sv}) and quenching

constant (K_q) indicate that chlorpromazine BSA binding reaction occurs by static quenching mechanism. The value of (n) is onereveals that one binding site on BSA for lamotrigine. K_b value suggests the maximum affinity of lamotrigine towards BSA. The results of synchronous fluorescence spectra are indicative of conformational changes of BSA upon binding with lamotrigine. The hydrophobic interaction plays an important role during the fluorescence quenching of BSA by lamotrigine. The CD spectra measurements indicate a change in the secondary structures of BSA. Molecular docking analysis showed that hydrophobic interaction along with various interactive forces of lamotrigine with BSA. The binding of drugs to serum protein is a key factor in determining their pharmacokinetics as well as pharmacological effects. Hence such a study of interaction between BSA and tranquilizer drugs would be useful in clinical medicine, pharmaceutical industry, and life sciences studies.

Future scope of research study

The spectroscopy-based interaction analysis of lamotrigine and BSA provides accurate information about how these medications attach to blood proteins. It offers important details on binding sites, affinities, structural changes, and interaction thermodynamics. These discoveries have significant implications for pharmaceutical design, delivery, and overall therapeutic efficacy, potentially improving safer and more effective drugs. The use of spectroscopic methods to analyze the interaction of tranquilizers and bovine serum albumin (BSA) opens up several new avenues for future research. Here are some possible areas for additional exploration.

Acknowledgment

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