Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Spl Issue [1] January 2023: 274-281.

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD

ORIGINAL ARTICLE **OPEN ACCESS**

Spectroscopic Study of Conformational Change in Bovine Serum Albumin with Increasing High Concentration of Chlorpromazine

Vidya Patil[1,2], Sandip Labade[1,3], Ranjana Jadhav[1] and Shakuntala Sawant[1,4*]
[1]Research Centre in Chemistry, S. M. Joshi College, Pune - 411028, Maharashtra, India
[2]Yashavantrao Chavan Institute of science, Satara - 415001, Maharashtra, India.
[3]Dr. B. N. Purandare Arts, Smt. S. G. Gupta Commerce and Science College, Lonavala - 410403, Maharashtra, India.
[4]R. S. B. mahavidyalaya Aundh-Khatav - 415510, Maharashtra, India.
Contact email ID – vidya08.patil@gmail.com

ABSTRACT
The present work explores the binding of chlorpromazine (CPZ) with the bovine serum albumin (BSA) by UV-visible, CD, and fluorescence spectroscopic studies. Fluorescence intensity of BSA was quenched on increasing concentration addition of chlorpromazine. BSA structure alteration by chlorpromazine was shown by UV-vis, synchronous and 3D fluorescence studies. Change in stability of native BSA in presence of chlorpromazine was given by CD spectroscopy. This study explains interaction and modification at molecular level between chlorpromazine and BSA and helps to understand the mechanism of drug binding effect in higher concentrations.
Keywords: *Chlorpromazine, BSA, Fluorescence, Spectroscopy*

Received 10.10.2022 Revised 23.11.2022 Accepted 20.12.2022

INTRODUCTION

Drug binding with plasma proteins is an important pharmacological checkpoint as it affects drug distribution, elimination, duration and pharmacological effect. Psychotropic drug association to plasma proteins is an important factor in determination of its clinical response.
Small modification in BSA leads to a significant change in its biochemical response as on interaction with drug molecules. Thus, formation of a drug–BSA complex may be useful as a model to gain fundamental insights into drug-protein interaction and elucidate its applications.
Serum protein plays major role in transport of small hydrophobic or sparingly soluble molecules such as steroids, bilirubin, fatty acids, hormones, drugs and their metabolites [1] and also transfers metal ions. [2]. BSA [Fig. 1] [3] is a single chain serum protein obtained from blood serum of bovine. It is a globular heart shaped protein with approximate dimensions of 80 × 80 × 30 Å and a molecular weight of ~66.4 kDa. Albumin contains 3 domains (I, II, III), which are separated into 9 loops (L1–L9) by 17 disulfide bridges. Each domain carries two subdomains A and B. It contains 583 amino acids with two tryptophan residues Trp-134 and Trp-213 in subdomain IB and IIA respectively and 20 tyrosine residues dispersed over 3 domains [4]–[8].
Binding of ligands with carrier proteins has great interest in the medicinal chemistry field. BSA is generally used as model protein to study binding of several small molecules including drugs with serum protein since its similarity with human serum albumin, less cost and no ethical issue.

Chlorpromazine with trade names thorazine and largactil is an antidepressant phenothiazine drug [Fig. 2] [9] widely used in psychiatric treatment and possesses a tricyclic ring bend at the NS axis [10], [11]. Chlorpromazine is an effective antagonist of dopamine D_1 and D_2, serotonin 5-HT_{1A}, muscarine M_1, and adrenergic alpha 2A receptors. CPZ binds to blood components like RBC membranes, albumin and lipoproteins. Interaction of CPZ with lipid bilayer and proteins of biomembranes, influencing their permeability [12], [13]. Binding of CPZ with lipids changes the lateral organization and functions of biomembranes. Such bindings may influence the bioavailability of drugs [14].

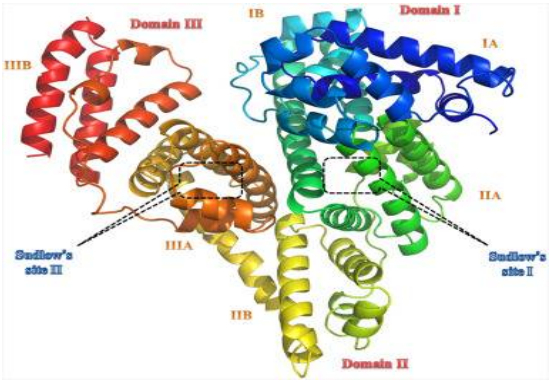

Fig. 1 BSA Structure

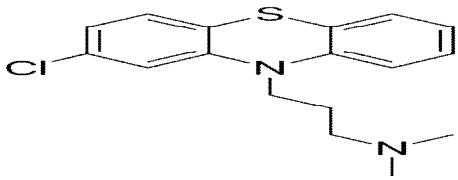

Fig. 2 CPZ Structure

CPZ with BSA studies explains that concentration of drug and protein influences the absolute and relative values of the CPZ free fraction [15]. Drug binding to plasma proteins affects its distribution and elimination as well as the duration and intensity of its physiological effects [16]. It is well known that the interaction of CPZ with blood components influences bioavailability.

In recent years few reports are available on interactions of tranquilizer drugs with BSA, such as alprazolam [17], clonazepam, [18], Lorazepam, oxazepam, bromazepam [19]. Few reports are available on fluorescence quenching by chlorpromazine [20] [21], spectroscopic and calorimetric studies of chlorpromazine binding with BSA [22].

Like other drugs, its chronic use may cause side effects such a cornea opacity, parkinsonism and respiratory troubles, and is suspected to be related to iron sediments in nervous cells and blood vessels [23]. On the other hand CPZ, high concentration toxicity effects mentioned in Drug Bank data are agitation, coma, convulsions, difficulty breathing, difficulty swallowing, dry mouth, extreme sleepiness, fever, intestinal blockage, irregular heart rate, low blood pressure, restlessness [24].

Chlorpromazine involve in cytotoxic and genotoxic effects by interacting with macromolecules since photoactivated and radical cation forms of chlorpromazine binds with DNA, RNA and proteins it is considerably and irreversibly respectively [25] [26].

Based on these facts, we studied the high concentration CPZ interaction with albumin. In the present work, interactions were studied, using UV-visible, fluorescence and circular dichroism (CD) spectroscopy. Further docking and MD simulations studies were carried out to investigate CPZ binding effect on BSA structure. Hence studies on the interaction of chlorpromazine and bovine serum albumin will be significant for elucidating its toxicity action studies.

MATERIAL AND METHODS

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) was purchased from SRL India. Chlorpromazine (≥98%) was purchased from Sigma Aldrich. Stock solutions of BSA (200 µM) and chlorpromazine (200 µM) were prepared by using 1X PBS buffer solution (pH = 7.40).

UV-visible Spectra Measurements

UV-visible absorption spectra of albumin, CPZ, and albumin in presence of varying concentrations of chlorpromazine were recorded at room temperature on UV-1600 spectrophotometer (Shimadzu, Kyoto, Japan) with 10 mm quartz rectangular cells from 200 to 400 nm. The 1X PBS solution (pH=7.40) was used as the reference solution.

Fluorescence Quenching Spectra Measurements

The steady-state fluorescence spectra of BSA solutions in absence and presence of chlorpromazine with varying concentrations were measured at pH 7.40 on an FP-8300 Spectrofluorometer (Jasco, Japan) with 10 mm quartz rectangular cell from scan range 270 to 500 nm while λ_{ex} was set at 278 nm. Scan speed was maintained as 500 nm/min and both slit widths were kept at 5 nm.

Synchronous Fluorescence Spectra Measurements

The synchronous fluorescence spectra of BSA solution and BSA in presence of varying concentrations of chlorpromazine were measured on FP-8300 Spectrofluorometer with 10 mm quartz rectangular cell (Jasco, Japan) instrument at different scanning intervals of Δλ (Δλ= λ_{em} – λex, λ_{em} and λ_{ex} are the emission and excitation wavelengths, respectively) at room temperature. The Δλ values were set at 15 nm and 60 nm, respectively, which describe the properties of Tyr and Trp residues, respectively. The scan range was

set at 270 to 500 nm with λ_{ex} as 278 nm. Scan speed was maintained as 500nm/min and both slit widths were kept at 5 nm.

Fluorescence 3D Spectra Measurements

The 3D fluorescence spectra measurement of BSA and BSA with chlorpromazine solutions were performed on FP-8300 Spectrofluorometer with 10 mm quartz rectangular cell (Jasco, Japan) with 1 nm slit widths at room temperature. The λ_{ex} and λ_{em} were set from 220 to 380 nm and from 230 to 500 nm respectively.

RESULTS AND DISCUSSION

UV spectra measurements

UV absorption measurement is frequently used to measure the change in the conformation of protein binding interaction between protein and drug and to know the complex formation. The UV absorption spectra of BSA and chlorpromazine were shown in Fig. 3. In the results weak band near 280 nm belongs to the $\pi \rightarrow \pi^*$ transition of the aromatic amino acids [27][28].

The absorption intensity of these bands near 280 nm increased with increasing the concentration of chlorpromazine. In addition, the slight blue shift of the band near 280 nm was also observed. This indicates that chlorpromazine can bind to BSA, resulting in forming Chlorpromazine–BSA complex and a change in the conformation of BSA. Strong absorption peaks around 250 nm are observed; these are characteristic peaks of chlorpromazine that do not affect the protein's absorption peak at 280 nm.

A) B)

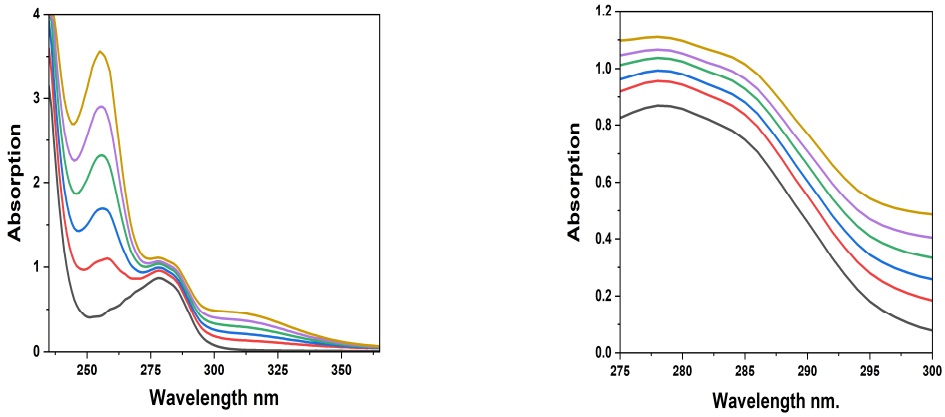

Fig. 3. A) UV absorption spectra of BSA (20 µM) with various concentrations of chlorpromazine from 0, 20, 40, 60, 80, 100 µM respectively at pH 7.4. B) Zoom view at 280nm.

Fluorescence Quenching of BSA

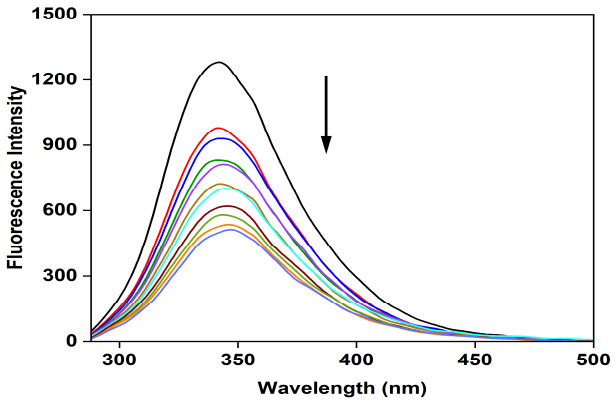

Fig. 4. Fluorescence spectra of BSA (20 µM) with various concentrations of chlorpromazine) 0, 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 µM respectively (λ_{ex} = 278 nm, pH 7.4).

Trp, Tyr and Phe residues can make the protein generate endogenous fluorescence. The most sensitive to the changes in the surrounding micro-environment is Trp which has strongest fluorescence intensity [29] and is used as an endogenous fluorescent probe to understand the binding interaction between protein and drugs. Steady-state fluorescence spectra of BSA solutions in absence and presence of chlorpromazine were shown in Fig. 4.

Strong fluorescence emission peak is obtained at 342 nm at λ ex =285 nm, which mainly comes from Trp residues. Fluorescence intensity of BSA decreased after addition of chlorpromazine from 0 to 120 µM along with the blue shift of the maximum emission wavelength (λ em) (~ 5 nm). This shows that there is an alteration in the microenvironment surrounding Trp residues due to binding of chlorpromazine to BSA.

Binding Site and Constant Study

To clarify the quenching mechanism of BSA induced by chlorpromazine, fluorescence quenching experiments were performed and the quenching parameters of BSA induced by chlorpromazine were calculated according to the Stern–Volmer equation. Binding constants (K_b) and binding site (n) for the chlorpromazine-BSA complex can be determined with double logarithmic plots [30].

$$\frac{F_0}{F} = 1 + K_q\tau_0[Q] = 1 + K_{sv}[Q] \qquad (1)$$

$$log\frac{(F_0-F)}{F} = logK_b + nlog[Q] \qquad (2)$$

F_0 and F are the BSA's fluorescence intensities in the chlorpromazine absence and presence, and [Q] denotes the chlorpromazine concentration. K_{SV} is a quenching constant. The k_q is quenching rate constant of protein, K_b is binding constant, n is binding sites number, it could be mathematically determined from the plot of log[$(F0 - F)/F$] vs. log [Q] and τ_0 is the protein's fluorescence lifetime with a quencher, about two ns for BSA [31].

The 'n' value for complex chlorpromazine BSA at the studied temperature is one, representing the existence of chlorpromazine binding sites on BSA.

The K_b value in order of 10^4 at studied temperature indicates a robust chlorpromazine's binding interaction with BSA.

A) B)

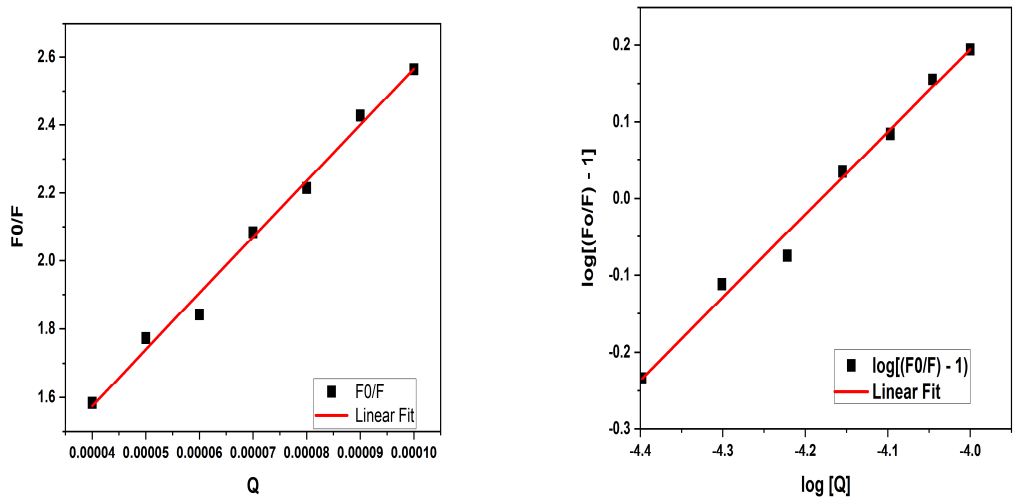

Fig. 5. A) Stern-Volmer and B) Modified Stern-Volmer graph for BSA with varying concentration of chlorpromazine.

Thermodynamic Study

The dominant binding forces between drugs and proteins are hydrogen bonds, Van der Waals forces, electrostatic forces, hydrophobic interactive forces usually thermodynamic parameter signs and magnitudes of the ΔH^0; enthalpy change and ΔS^0; entropy change, the main forces can be accounted in the binding reaction is encountered. Experiments were done out at 298K. Here ΔH^0 and ΔS^0 can be assumed

constants. The Van't Hoff equation is applied for calculation.

$$lnK_b = \frac{-\Delta H^0}{RT} - \frac{\Delta S^0}{R} \qquad (3)$$

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

$$\Delta G^0 = -RTlnK_b = \Delta H^0 - T\Delta S^0 \qquad (4)$$

Table.1 specifies fluorescence data results. The negative ΔS^0 and ΔH^0 values suggest that H-bonds and Van der Waal's forces are significantly involved in chlorpromazine binding to BSA. However, $\Delta G^0<0$, $\Delta H^0<0$, and $|\Delta H^0|>|T\Delta S^0|$ indicate sudden chlorpromazine binding with BSA; reaction is an exothermic and enthalpy driven process.

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	$\Delta G^0/J\ M^{-1}$
1.6514×10^3	8.277×10^{12}	5.084×10^3	0.88442	-1.1026×10^4

Synchronous fluorescence measurements

The synchronous fluorescence spectrum is used to obtain molecular environment information in a chromosphere vicinity. When the wavelength interval (Δλ=λ em -λ ex) between λ em and λ ex is stabilized at 15 nm or 60 nm, the synchronous fluorescence spectra is used to distinguish the characteristics of Tyr residues and Trp residues of BSA, respectively. Usually, shift of the λ em represents the change in Tyr or Trp residues surrounding microenvironment polarity [32].

Blue shift of λ em suggests the Trp or Tyr residues surrounding polarity decreases and increases the hydrophobicity with the increase of the macromolecules folding state[33]. The synchronous fluorescence spectra of BSA along with chlorpromazine were shown in Fig. 6 A) & B).

It is observed that gradually decrease in fluorescence intensities of Tyr and Trp residues together with a slight blue shift in the max emission wavelength with chlorpromazine from 0 to 120 µM addition. It is determined by decreasing the surrounding polarity of Tyr or Trp residue and increasing hydrophobicity with an increase of folding state of macromolecule due to binding of chlorpromazine to BSA.

A) **B)**

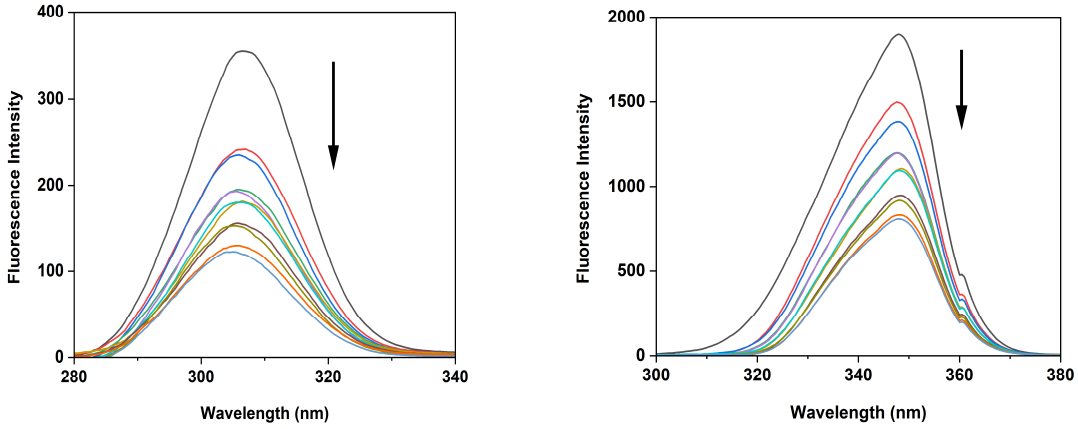

Fig. 6 The synchronous fluorescence spectra of Tyr residue with Δλ = 15 nm (A) and Trp residue with Δλ = 60 nm (B) in BSA of 20 µM with various concentrations of chlorpromazine 0,10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 µM respectively.

Fluorescence 3D measurements

3D fluorescence spectra are plotted with emission wavelength, excitation wavelength and fluorescence intensity as X, Y, and Z-axis. It reflects the fluorescence intensity of excitation wavelength and emission wavelength changed simultaneously and gives more information about the change in conformational of proteins. To further investigate the effect of binding chlorpromazine on the conformation and microenvironment of BSA, the 3D fluorescence spectra of BSA in the presence and absence of chlorpromazine were measured. As is shown in Fig. 7 (A), the Peak 2 area represents the band of Trp and

Tyr residues fluorescence emission.
Where intensity and maximum emission wavelength are closely related to the polarity of the microenvironment surrounding Tyr and Trp residues [34].
As noted in Fig 7 (B), the fluorescence intensity of Peak 2 decreased after the addition of chlorpromazine, indicating that there was interaction between BSA and chlorpromazine. As the intensity of peaks decreased after the addition of chlorpromazine. The reason for the phenomenon may be the formation of the chlorpromazine-BSA complex after the addition of chlorpromazine, leading to the increase in the macromolecule diameter.
Additionally, blue shifts of peak 2 at λ ex were observed, suggesting that the Trp and Tyr residues environment polarity decreased after adding chlorpromazine.

A) **B)**

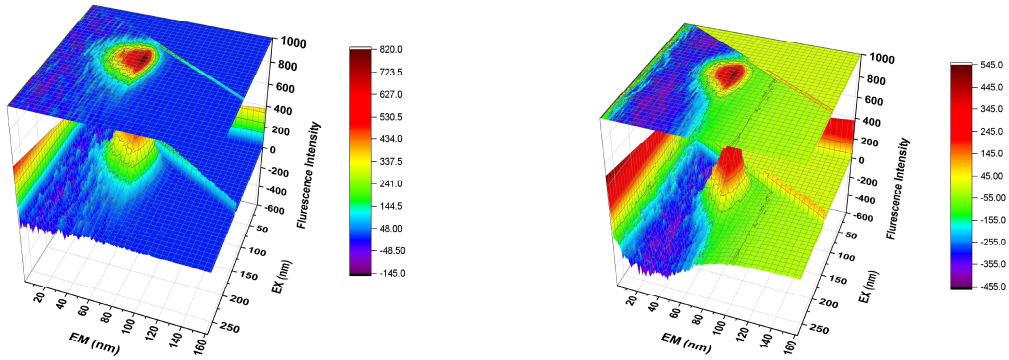

Fig.7 Three-dimensional (3D) fluorescence spectra of A) BSA and B) BSA- chlorpromazine1:1.

CONCLUSION

This work describes the interaction between chlorpromazine 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 dynamic quenching mechanism. The value of n is one revealed that one binding site on BSA for chlorpromazine. The results of synchronous fluorescence spectra and three dimensional fluorescence spectra are indicative of conformational changes of BSA upon binding with chlorpromazine. The hydrophobic interaction plays an important role during the fluorescence quenching of BSA by chlorpromazine according to positive values of ΔH^0 and ΔS^0.
The binding of drugs to serum protein is an important factor in determining their pharmacokinetics and pharmacological effect. Hence such study of interaction between BSA and chlorpromazine would be useful in clinical medicine, medicinal chemistry, pharmaceutical industry, and life sciences studies.

ACKNOWLEDGEMENT

The authors are grateful to the research advisory committee of Yashavavntrao Chavan Institute of Science, Satara, for giving seed money to work.

REFERENCES

1. C. M. M. D.E. Metzler, (2001). Biochemistry: The Chemical Reaction of Living Cells, Second ed. USA: Academic Press.
2. L. Jattinagoudar, M. Meti, S. Nandibewoor, and S. Chimatadar, (2016). “Evaluation of the binding interaction between bovine serum albumin and dimethyl fumarate, an anti-inflammatory drug by multispectroscopic methods,” Spectrochim. Acta Part A Mol. Biomol. Spectrosc., vol. 156, pp. 164–171, doi: https://doi.org/10.1016/j.saa.2015.11.026.
3. “BSA,image.” https://www.google.com/search?q=Bovin+Serum+Albumin+image&tbm=i sch&ved= 2ah UKEwir5KD
4. A. Jahanban-Esfahlan, V. Panahi-Azar, and S. Sajedi, (2016). “Interaction of glutathione with bovine serum albumin: Spectroscopy and molecular docking,” *Food Chem.*, vol. 202,doi: 10.1016/j.foodchem.2016.02.026.
5. S. K. Chaturvedi, E. Ahmad, J. M. Khan, P. Alam, M. Ishtikhar, and R. H. Khan, (2015). “Elucidating the

interaction of limonene with bovine serum albumin: a multi-technique approach," *Mol. Biosyst.*, vol. 11, no. 1, pp. 307–316, doi: 10.1039/C4MB00548A.
6. Z. K. D. C. Carter, B. Chang, J. X. Ho, K. Keeling, "(1994). *Eur. J. Biochem*, vol. 226, pp. 1049–1052.
7. S. Chatterjee and T. K. Mukherjee, "Spectroscopic investigation of interaction between bovine serum albumin and amine-functionalized silicon quantum dots," *Phys. Chem. Chem. Phys.*, vol. 16, no. 18, pp. 8400–8408, 2014, doi: 10.1039/C4CP00372A.
8. Q. Wang *et al.*, (2016). "Binding interaction of atorvastatin with bovine serum albumin: Spectroscopic methods and molecular docking," *Spectrochim. Acta Part A Mol. Biomol. Spectrosc.*, vol. 156, pp. 155–163, doi: https://doi.org/10.1016/j.saa.2015.12.003.
9. CPZ,Image,CPZimage." https://www.google.com/search?q=chlorpromazine&sxsrf=AOaemvJXJCnfyQHqH0jlHnCmKi5VWHAuUw:1632674025389&source=lnms&tbm=isch&sa=X&ved=2ahUKEwjtrsTaiJ3zAhVEdCsKHTAoDkoQ_AUoAXoECAEQAw&biw=1297&bih=657&dpr=0.9#imgrc=K0fBwIAxRjIthM.
10. M. Bhattacharyya, U. Chaudhuri, and R. K. Poddar, (1990). "Evidence for cooperative binding of chlorpromazine with hemoglobin: Equilibrium dialysis, fluorescence quenching and oxygen release study," *Biochem. Biophys. Res. Commun.*, vol. 167, no. 3, pp. 1146–1153.oi: https://doi.org /10.1016/0006-291X(90)90643-2.
11. J. Bhattacharyya and K. P. Das, (2001). "Aggregation of insulin by chlorpromazine11Abbreviations: CPZ, Chlorpromazine; and DTT, Dithiothreitol.," *Biochem. Pharmacol.*, vol. 62, no. 9, pp. 1293–1297, doi: https://doi.org/10.1016/S0006-2952(01)00786-9.
12. T. A. Ban, (2007). "Fifty years chlorpromazine: a historical perspective," *Neuropsychiatr. Dis. Treat.*, vol. 3, no. 4, pp. 495–500,, [Online]. Available: https://pubmed.ncbi. nlm.nih.gov/19300578.
13. W.Drugbank,"DRUG,INFORMATION,CPZ." https://www.sigmaaldrich.co /deepweb/assets /sigmaaldrich /product /documents/966/223/c8138pis.pdf.
14. A. Jutila, T. Söderlund, A. L. Pakkanen, M. Huttunen, and P. K. J. Kinnunen,(2001). "Comparison of the effects of clozapine, chlorpromazine, and haloperidol on membrane lateral heterogeneity," *Chem. Phys. Lipids*, vol. 112, no. 2, pp. 151–163, doi: https://doi.org/10.1016/S0009-3084(01)00175-X.
15. M. D. Rukhadze, G. S. Bezarashvili, N. S. Sidamonidze, and S. K. Tsagareli, (2001). "Investigation of binding process of chlorpromazine to bovine serum albumin by means of passive and active experiments," *Biomed. Chromatogr.*, vol. 15, no. 6, pp. 365–373, doi: https://doi.org/10.1002/bmc.82.
16. R. K. Verbeeck, J.-A. Cardinal, A. G. Hill, and K. K. Midha,(1983). "Binding of phenothiazine neuroleptics to plasma proteins," *Biochem. Pharmacol.*, vol. 32, no. 17, pp. 2565–2570. doi: https://doi.org/10.1016/0006-2952(83)90019-9.
17. M. Sarkar, S. S. Paul, and K. K. Mukherjea,(2013). "Interaction of bovine serum albumin with a psychotropic drug alprazolam: Physicochemical, photophysical and molecular docking studies," *J. Lumin.*, vol. 142, pp. 220–230. doi: https://doi.org/10.1016/j.jlumin.2013.03.026.
18. Y.-Y. Lou, K.-L. Zhou, D.-Q. Pan, J.-L. Shen, and J.-H. Shi,(2017). "Spectroscopic and molecular docking approaches for investigating conformation and binding characteristics of clonazepam with bovine serum albumin (BSA)," *J. Photochem. Photobiol. B Biol.*, vol. 167, pp. 158–167, doi: https://doi.org/10.1016/j.jphotobiol.2016.12.029.
19. R. G. Machicote, M. E. Pacheco, and L. Bruzzone, (2010). "Binding of several benzodiazepines to bovine serum albumin: Fluorescence study," *Spectrochim. Acta Part A Mol. Biomol. Spectrosc.*, vol. 77, no. 2, pp. 466–472, doi: https://doi.org/10.1016/j.saa.2010.06.020.
20. D. Silva, C. M. Cortez, and S. R. W. Louro, "Quenching of the intrinsic fluorescence of bovine serum albumin by chlorpromazine and hemin," *Brazilian J. Med. Biol. Res.*, vol. 37, no. 7, pp. 963–968, Jul. 2004, doi: 10.1590/S0100-879X2004000700004.
21. D. Silva, C. M. Cortez, and S. R. W. Louro, (2004). "Chlorpromazine interactions to sera albumins: A study by the quenching of fluorescence," *Spectrochim. Acta Part A Mol. Biomol. Spectrosc.*, vol. 60, no. 5, pp. 1215–1223, doi: https://doi.org/10.1016/j.saa.2003.08.003.
22. S. Khatun, Riyazuddeen, and F. A. Qais, (2020). "In-vitro binding analysis of bovine serum albumin with sulindac/chlorpromazine: Spectroscopic, calorimetric and computational approaches," *J. Mol. Liq.*, vol. 299, p. 112124 doi: https://doi.org/10.1016/j.molliq.2019.112124.
23. M. B. H. Youdim, D. Ben-Shachar, and P. Riederer, (1993). "The possible role of iron in the etiopathology of Parkinson's disease," *Mov. Disord.*, vol. 8, no. 1, pp. 1–12, doi: https://doi.org/10.1002/mds.870080102.
24. "CPZ Drugbank." https://go.drugbank.com/drugs/DB00477.
25. N. J. De Mol and R. W. Busker, (1984). "Irreversible binding of the chlorpromazine radical cation and of photoactivated chlorpromazine to biological macromolecules," *Chem. Biol. Interact.*, vol. 52, no. 1, pp. 79–92,doi: https://doi.org/10.1016/0009-2797(84)90084-X.

26. J. Piette, J. Decuyper, M.-P. Merville-Louis, and A. Van De Vorst, (1986). "Biomolecular photoalterations mediated by phenothiazine derivatives," *Biochimie*, vol. 68, no. 6, pp. 835–842 doi: https://doi.org/10.1016/S0300-9084(86)80098-0.
27. J.-H. Shi, J. Chen, J. Wang, Y.-Y. Zhu, and Q. Wang, (2015). "Binding interaction of sorafenib with bovine serum albumin: Spectroscopic methodologies and molecular docking," *Spectrochim. Acta Part A Mol. Biomol. Spectrosc.*, vol. 149, pp. 630–637 doi: https://doi.org/10.1016/j.saa.2015.04.034.
28. J. R. Lakowicz, (2007). Springer Science & Media,
29. X. Liu, Z. Ling, X. Zhou, F. Ahmad, and Y. Zhou, (2016). "Comprehensive spectroscopic probing the interaction and conformation impairment of bovine serum albumin (BSA) by herbicide butachlor," *J.Photochem.Photobiol.BBiol.*,vol.162,pp.332–339,2016,https://doi.org/10.1016/j.jphotobiol. 2016. 07.005.
30. J. Shi, J. Wang, Y. Zhu, and J. Chen, (2014). "Characterization of intermolecular interaction between cyanidin-3-glucoside and bovine serum albumin: Spectroscopic and molecular docking methods," *Luminescence*, vol. 29, no. 5, pp. 522–530,doi: https://doi.org/10.1002/bio.2579.
31. X. Guo *et al.*, (2014). "A spectroscopic study on the interaction between p-nitrophenol and bovine serum albumin,"*J.Lumin.*,vol. 149, pp. 353–360 https://doi.org/10.1016/j.jlumin.2014.01.036.
32. B. Hemmateenejad, M. Shamsipur, F. Samari, T. Khayamian, M. Ebrahimi, and Z. Rezaei, (2012). "Combined fluorescence spectroscopy and molecular modeling studies on the interaction between harmalol and human serum albumin," *J. Pharm. Biomed. Anal.*, vol. 67–68, pp. 201–208.doi: https://doi.org/10.1016/j.jpba.2012.04.012.
33. . Shi, Y. Zhu, J. Wang, and J. Chen, (2015). "A combined spectroscopic and molecular docking approach to characterize binding interaction of megestrol acetate with bovine serum albumin," *Luminescence*, vol. 30, no. 1, pp. 44–52, doi: https://doi.org/10.1002/bio.2688.
34. F.-F. Tian *et al.*, (2010). "Synthesis of a Novel Hydrazone Derivative and Biophysical Studies of Its Interactions with Bovine Serum Albumin by Spectroscopic, Electrochemical, and Molecular Docking Methods," *J. Phys. Chem. B*, vol. 114, no. 46, pp. 14842–14853, doi: 10.1021/jp105766n.

CITATION OF THIS ARTICLE

V. Patil, S. Labade and R. Jadhav, Shakuntala Sawant Spectroscopic Study of Conformational Change in Bovine Serum Albumin with Increasing High Concentration of Chlorpromazine., Bull. Env. Pharmacol. Life Sci., Spl Issue [1]: 2023:274-281.