International Journal of Applied Research 2017; 3(4): 668-673


**ISSN Print:** 2394-7500
**ISSN Online:** 2394-5869
**Impact Factor:** 5.2
IJAR 2017; 3(4): 668-673
www.allresearchjournal.com
Received: 16-02-2017
Accepted: 17-03-2017

**Arifur Rahman**
Department of Pharmacy, BRAC University, 41 Pacific tower, Mohakhali, Dhaka, Bangladesh

**Md. Reazul Islam**
Department of Clinical Pharmacy and Pharmacology, Faculty of Pharmacy, University of Dhaka, Dhaka, Bangladesh

**Sabreena Chowdhury Raka**
Department of Pharmacy, Faculty of Allied Health Sciences, Daffodil International University, 4/2-Sobhanbagh, Dhanmondi, Dhaka, Bangladesh

**Abul Hasnat**
Department of Clinical Pharmacy and Pharmacology, Faculty of Pharmacy, University of Dhaka, Dhaka, Bangladesh

**Md. Saiful Islam**
Department of Clinical Pharmacy and Pharmacology, Faculty of Pharmacy, University of Dhaka, Dhaka, Bangladesh

**Correspondence**
**Arifur Rahman**
Department of Pharmacy, BRAC University, 41 Pacific tower, Mohakhali, Dhaka, Bangladesh

# Analysis the interaction of Imipramine hydrochloride with bovine serum albumin measured by fluorescence quenching for a predictive, preventive, safe and personalised medicine

**Arifur Rahman, Md. Reazul Islam, Sabreena Chowdhury Raka, Abul Hasnat and Md. Saiful Islam**

**Abstract**
The plasma protein binding profile of a drug molecule plays a vital role to comprehend the pharmacokinetics and pharmacodynamics properties of that molecule. It has arisen a great concern since it intensely effects drug distribution process and regulates the free fraction. The present study has been done to investigate the interaction of Imipramine hydrochloride (IPM) with bovine serum albumin (BSA) under physiological condition (pH 7.40) using UV absorption and fluorescence spectrophotometry at different temperatures (298K and 308K). To obtain a detail and insightful information of protein binding of Imipramine hydrochloride with BSA, UV-visible and fluorescence spectroscopy were used. Quenching of BSA was also observed in presence of IPM by the fluorescence method. Quenching constants were determined at different temperatures (298K and 308K) using the Stern-Volmer equation. The thermodynamic parameters namely, enthalpy change (ΔH), entropy change (ΔS), and Gibb's free energy change (ΔG) were analysed based on Van't Hoff equation. It was found that with the increase of temperature, the value of Stern-Volmer constant increases in case of IPM. From this quenching mechanism, it was found that quenching of BSA-IPM system is static. Based on the thermodynamic parameters, hydrophobic interaction and hydrogen bonding were found to be involved in the formulation of complexes in the BSA-IPM system. As Gibb's free energy change was negative, the interaction was a spontaneous process for the BSA-IPM system at both temperatures (298K and 308K). Binding constants (K) and the number of binding sites (n) were determined at different temperatures (298K and 308K). Based on the findings from the experiment regarding the interaction of BSA-IPM, the drug-protein binding mole ratio is 1:1 at temperatures 298K and 308K. The binding process is reversible and spontaneous.

**Keywords:** Bovine serum albumin, Fluorescence, Antidepressant, Pharmacokinetics, Personalised medicine, Fraction V

## 1. Introduction
To interpret the pharmacokinetics and pharmacodynamics properties of a drug molecule, plasma protein binding plays a substantial factor. It has arisen a great concern since it intensely effects drug distribution process and regulates the free fraction [1]. Most abundantly found proteins in blood are the serum albumins. They are engaged in various significant physiological functions. For example, they are mainly responsible for sustaining the blood pH and maintain the osmotic pressure [2]. However, the most important functions of serum albumins are the drug binding, transport and delivery of the drug through the bloodstream to their target organ [3]. Therefore, the investigation of such interaction of drug-protein complex has a great obligatory and inevitable significance [4].
Bovine serum albumins and human serum albumins illustrate approximately 76% sequence homology and the 3D structure of BSA is considered to be similar with HAS [5]. Since the presence of high-affinity binding sites for the formation of drug-protein complex and as the results obtained from the studies are consistent with HAS [5]. In this investigation, bovine serum albumin (BSA) is carefully chosen as the target protein model.
The drug- protein complex formed by the reversible and irreversible process [5]. In the case of reversible processes, the drug-protein complexes formed through weaker chemical bonds,

for example, hydrogen bonds or Van der Waals forces [7]. The reversible drug-protein complex formation has attained a great concern in pharmacokinetics. The formation of the irreversible drug-protein complex is due to the chemical activation of the drug candidates, which then tends to bind with the protein or macromolecule through stronger chemical bonds, for example, covalent bonds [9]. Due to the formation of irreversible drug-protein complex formation or reactive chemical intermediates, it is chiefly responsible for certain types of toxicities, for example, chemical carcinogenesis which may occur as a result of prolong use. If protein binding of a drug molecule is declined, the free concentration of that drug will increase eventually. Therefore, more drugs will be available to interact with the receptor site. This will produce an intense pharmacological effect [11]. The duration of action of a drug molecule is also influenced by the protein binding.

Although researchers have performed various experiments to elucidate the structure, the properties of serum albumin and their possible way of interaction with small molecules such as dyes, drugs and toxic chemicals using the fluorescence method [14-17]. But there has been no report on the interaction of Imipramine with protein albumin. In this regards, the present work has been performed to obtain a detail and insightful information of protein binding of Imipramine hydrochloride with BSA by considering the interactions using mainly UV-visible and fluorescence spectroscopy. The aim is to optimise the use of Imipramine as a predictive, preventive, safe and personalised medicine.

## Materials and method

For the study of the reactivity of chemical and biological systems, spectroscopic methods are the most influential tool. As it permits a nonintrusive measurement of substances at low concentrations, it is widely used for the measurement under physiological conditions [18-19]. Here fluorescence spectroscopy and UV spectroscopy were used to perform the study. 100 ml of a 0.1M solution of Imipramine hydrochloride was prepared as the stock solution by taking 2.9983g of Imipramine hydrochloride (Mol. Wt. 299.836) in a 100 ml volumetric flask and dissolving in nano-pure water. Bovine serum albumin (BSA) was used as the target protein model. Bovine serum albumin is a fatty acid-free, fraction V, and 96-98% pure protein which was purchased from the Sigma-Aldrich Chemical CO, USA. A solution of BSA was prepared at a concentration of $2.5\times10^{-6}$ M by measuring 41.875 mg of protein correctly and dissolving in a 250 ml volumetric flask with nano-pure water. The process was done carefully in order to ensure that there was no foam formation. The protein solution was preserved at 4° C until further use. Since BSA is a fluorescence active molecule in this system, the intensities at the excitation wavelength of 280 nm and 293 nm for BSA were taken. The fluorescence spectra were measured at different temperatures (298 K and 308 K).

## Results and discussion

### Analysis of fluorescence quenching mechanism

Fluorescence quenching is the reduction of the quantum yield of fluorescence from a fluorophore. When a fluorophore is substantially induced with quencher molecule by a variety of molecular interaction (i.e. excited state reactions, energy transfer, molecular rearrangements and ground-state complex formation) quenching of fluorescence is evident [23-24].

In order to ensure the quenching mechanism, the fluorescence data are usually evaluated by Stern-Volmer equation [25]:

$F_0/F = 1 + K_{SV}[Q]$

Here $F_0$ and F are the fluorescence intensities of BSA in the absence and presence of quencher, respectively. [Q] denotes the concentration of quencher which is IPM in this case, and $K_{SV}$ is the Stern-Volmer quenching constant, which signposts the strength of the interaction between the IPM and BSA. $K_{SV}$ is the slope of the plot of $F_0/F$ against [IPM] based on the fluorescence data at different temperatures.

### Effect of IPM on the fluorescence spectra of BSA at 298 K

The fluorescence spectra of BSA was measured in the absence and presence of various concentration of IPM at the excitation wavelength of 280 nm and 290 nm.

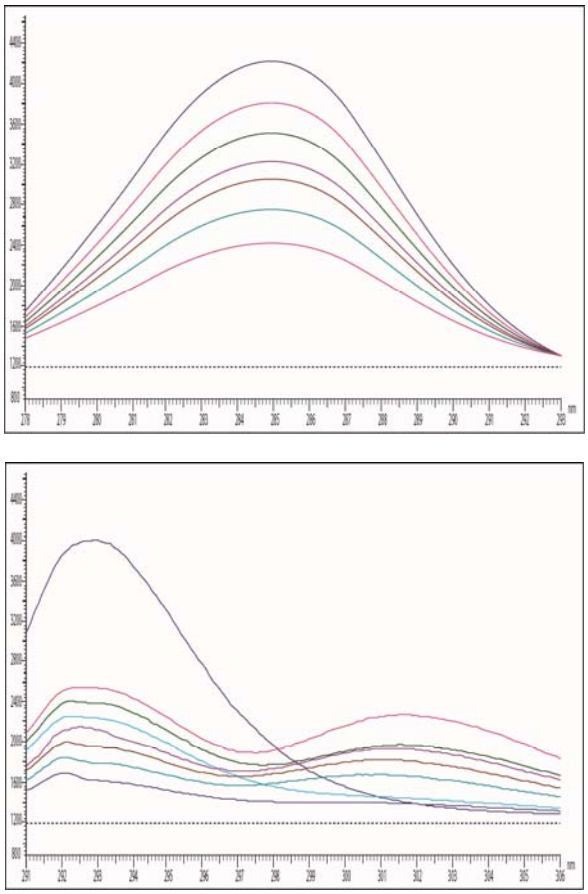

**Fig 1:** Fluorescence titration curve of BSA in the presence of IPM at the excitation wavelength of 280 nm and 293 nm at 298 K.

The fluorescence spectra of BSA demonstrate a broad band with the absorption maximum at 283.4 nm. Fluorescence quenching is observed for BSA in the presence of different IPM concentration. Quenching spectrum is a hint of a sturdy interaction and energy transfer occurred between IPM and BSA at 298 K. If a ligand is adequately close to tryptophan and tyrosine fluorescent residues, a decrease in intrinsic fluorescence intensity can be detected. To resolve whether both the tryptophan and tyrosine residues are engaged in the interaction with IPM, the fluorescence intensities of BSA at

the excitation wavelength of 280 nm and 293 nm was measured in the presence of IPM. When the excitation wavelength of 280 nm is used, the fluorescence of albumin comes from both tryptophan and tyrosine residues, whereas when the excitation wavelength of 293 nm is used, it only excites the tryptophan residues [21].

**Table 1:** Data for interaction of IPM with BSA at 298K

| | $F_0$ | F | $F/F_0$ | [IPM]/[BSA] | | $F_0$ | F | $F/F_0$ | [IPM]/[BSA] |
|---|---|---|---|---|---|---|---|---|---|
| 280 nm | 3653 | 3530 | 1.034 | 1 | 293 nm | 4177 | 4162 | 1.003 | 1 |
| | 3653 | 3485 | 1.048 | 2 | | 4177 | 4151 | 1.006 | 2 |
| | 3653 | 2998 | 1.218 | 4 | | 4177 | 3666 | 1.139 | 4 |
| | 3653 | 2736 | 1.335 | 6 | | 4177 | 3520 | 1.186 | 6 |
| | 3653 | 2401 | 1.521 | 8 | | 4177 | 3214 | 1.299 | 8 |
| | 3653 | 2310 | 1.581 | 12 | | 4177 | 3182 | 1.312 | 12 |
| | 3653 | 1601 | 2.281 | 16 | | 4177 | 2503 | 1.668 | 16 |

The plot $F/F_0$ against [IPM]/ [BSA] is presented in figure 2, which indicates that in the presence of IPM, the fluorescence of BSA at the excitation wavelength of 280 nm obviously diverges from that the excitation wavelength of 293 nm. This substantial differences between the fluorescence quenching of serum albumin illustrate that there are alterations in the participating fluorophores of BSA at different wavelengths. At 280 nm fluorescence of BSA comes from both tryptophan and tyrosine residues, and at 293 nm fluorescence of BSA only involves tryptophan residue [22].

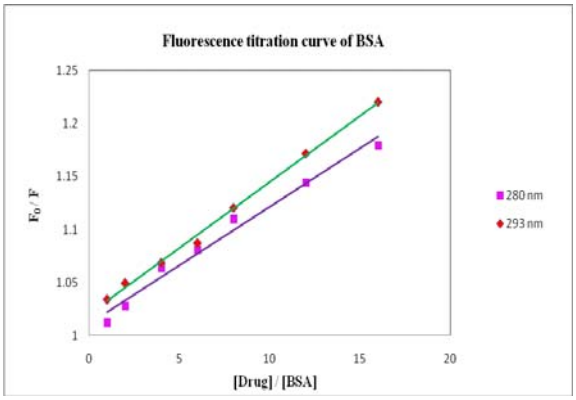


**Fig 2:** The plot F/F0 against [IPM]/ [BSA]

## Effect of IPM on the fluorescence spectra of BSA at 308 K

The fluorescence spectra of BSA with varying concentration of IPM at the excitation wavelength of 280 nm at 308K.

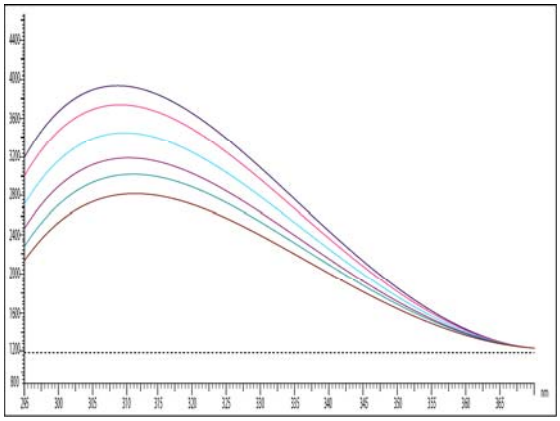

**Fig 3**: Fluorescence titration curve of BSA in the presence of IPM at the excitation wavelength of 280 nm at 308 K

The fluorescence spectra of BSA show a broadband with an absorption maximum at 312.5 nm. The quenching of fluorescence is observed for BSA in the presence of different IPM concentration. This quenching spectrum is a hint of a sturdy interaction and energy transfer between IPM and BSA at 308 K.

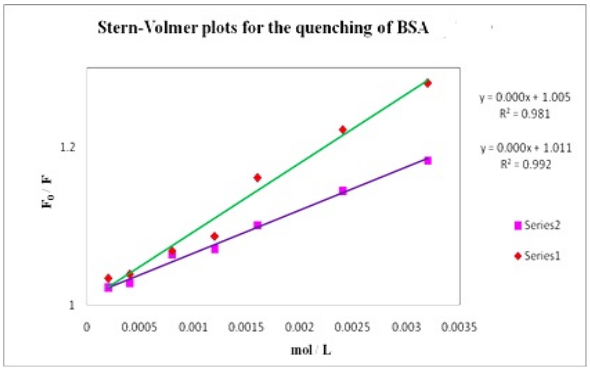


**Fig 4:** The Stern-Volmer plots for the quenching of BSA by IPM at 298K and 308K

The plot displays that in the above-mentioned concentrations, the results are in accordance with the Stern-Volmer equation. The plots are linear and Stern-Volmer quenching constants are obtained from the slopes at various temperatures, listed in table 2. The Stern-Volmer quenching constant decreases with increasing temperature for static quenching while for dynamic quenching the reverse effect [22]. From table 2, it is clear that the probable quenching mechanism of the IPM- BSA binding reaction is not initiated by dynamic quenching but by static quenching resulted from the complex formation.

**Table 2:** Stern-Volmer quenching constant $K_{SV}$ of the system of IPM- BSA at 280nm (R =Correlation co-efficient)

| T (K) | $K_{SV}$ (L $mol^{-1}$) | R |
|---|---|---|
| 298 | 0.0037 | 0.992 |
| 308 | 0.0019 | 0.989 |

## Thermodynamic parameters and nature of the binding forces

The forces involve the interaction between quencher and fluorescence active molecule may be included as the hydrophobic force, electrostatic interactions, Vander Waals interactions, hydrogen bonds, etc. [27]. Thermodynamic parameters namely the change of enthalpy, the entropy and Gibb's free energy are anticipated for revealing the interaction between the IPM and BSA. The main reasons for

carrying out the thermodynamic study of BSA-IPM interactions is to scrutinise the factors responsible for overall binding affinity and specificity of the drug molecule for the protein [28]. The thermodynamic parameters can be assessed from the Van't Hoff equation:

$\ln K_a = -(\Delta H/RT) + (\Delta S/R)$

Here $\Delta H$, $\Delta S$ denotes as the change of enthalpy and the entropy, correspondingly; constants $K_a$ are equivalent to the Stern-Volmer quenching constants $K_{SV}$ at the corresponding temperature [29], R is the gas constant. If the temperature does not differ so meaningfully, then the change of enthalpy ($\Delta H$) can be regarded as a constant [30]. The change of enthalpy ($\Delta H$) and the entropy ($\Delta S$) can be assessed from the slope and intercept of the fitted curve of $\ln K_{SV}$ against $1/T$, correspondingly.

From the subsequent equation Gibb's free energy ($\Delta G$) can be assessed:

$\Delta G = \Delta H - T\Delta S$

The temperature changes affect these binding forces as follows:

- When $\Delta H$ and $\Delta S$ are positive, it indicates a typical hydrophobic interaction [31].
- When $\Delta H$ and $\Delta S$ are negative, it is an indication of Vander Waals and hydrogen bonding in low dielectric media [31].
- Negative $\Delta H$ might have a role in electrostatic interactions, $\Delta H$ is assumed to be very little or almost zero [31].
- For the spontaneous binding of biomolecules and ligand. $\Delta G$ is always negative [32].

From the table 4, the change of free energy ($\Delta G$) is negative, the change of enthalpy ($\Delta H$) and the entropy ($\Delta S$) are positive. Different sign and magnitude of the thermodynamic parameters gives the impression of different interaction forces [33] such as the positive value of $\Delta S$ and $\Delta H$ is considered as the evidence of hydrophobic interaction from the point of view of $H_2O$ molecule structure while negative $\Delta H$ and $\Delta S$ values considered as the evidence of hydrogen bonds, Van der Waals interactions and protonation additional association. Electrostatic interactions are characterised by positive ΔS and when ΔH is almost zero [32]. The negative sign for $\Delta G$ reveals that the binding process is spontaneous [32]. Thus it can be settled that hydrogen bonding and Van der Waals interactions are present in the BSA-IPM binding.

**Table 3:** Data of Van't Hoff plot for BSA-IPM system at 280 nm at two different temperatures

| $K_{SV}$ ( L mol$^{-1}$) | ln $K_{SV}$ | T | 1/T |
|---|---|---|---|
| 0.0037 | -5.599 | 298 | 0.0033 |
| 0.0019 | -6.265 | 308 | 0.0032 |

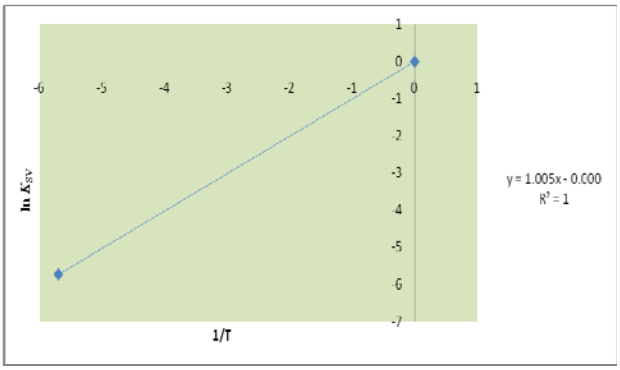


**Fig 5**: The Van't Hoff Plot for BSA-IPM system at 280 nm at two different temperatures

**Table 4:** Thermodynamic parameters of the system of BSA-IPM at 280nm

| *T(K)* | $\Delta H$ (KJ/mol ) | $\Delta S$ (J/mol) | $\Delta G$ (KJ/mol) |
|---|---|---|---|
| 298 | 0.0037 | 0.9259 | -275.91 |
| 308 | 0.0019 | 0.9626 | -296.47 |

**Binding constant and binding points**

Once small molecule resides independently to a set of equivalent sites on a macromolecule, the equilibrium constant between free and bound molecule can be attained from the subsequent equation [33]:

$\log [(F_0 - F)/F] = \log K + n \log [Q]$

Here, $K$ and $n$ denote the binding constant to a site and the number of binding per molecule, correspondingly. From the values of intercept and slope of the plot of log $[(F_0-F)/F]$ versus log [IPM], the values of $K$ and $n$ are intended, correspondingly.

**Table 5:** Data regarding binding constant and binding points for BSA-IPM system at 298 K and `308K

| | $F_0$ | F | log $[(F_0-F)/F]$ | [IPM] mol/L | log [IPM] | | $F_0$ | F | log $[(F_0-F)/F]$ | [IPM] mol/L | log [IPM] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 298K | 3653 | 3530 | -1.457 | $20\times10^{-6}$ | -4.699 | 308K | 4177 | 4162 | -2.443 | $20\times10^{-6}$ | -4.699 |
| | 3653 | 3485 | -1.316 | $40\times10^{-6}$ | -4.397 | | 4177 | 4151 | -2.203 | $40\times10^{-6}$ | -4.397 |
| | 3653 | 2998 | -0.660 | $80\times10^{-6}$ | -4.096 | | 4177 | 3666 | -0.855 | $80\times10^{-6}$ | -4.096 |
| | 3653 | 2736 | -0.474 | $120\times10^{-6}$ | -3.920 | | 4177 | 3520 | -0.729 | $120\times10^{-6}$ | -3.920 |
| | 3653 | 2401 | -0.282 | $160\times10^{-6}$ | -3.795 | | 4177 | 3214 | -0.523 | $160\times10^{-6}$ | -3.795 |
| | 3653 | 2310 | -0.235 | $240\times10^{-6}$ | -3.619 | | 4177 | 3182 | -0.504 | $240\times10^{-6}$ | -3.619 |
| | 3653 | 1601 | 0.107 | $320\times10^{-6}$ | -3.494 | | 4177 | 2503 | -0.174 | $320\times10^{-6}$ | -3.494 |

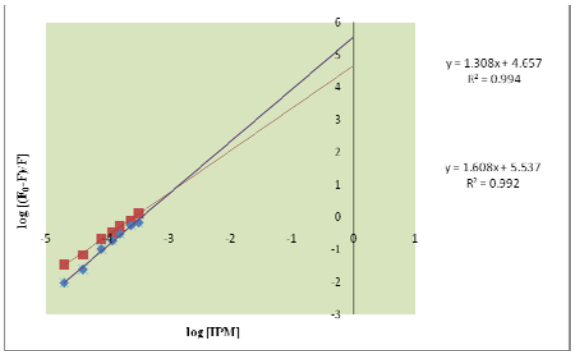


**Fig 6**: Plot for the determination of binding constant and binding points of BSA-IPM system at 298K and 308 K

**Table 6:** Binding constant and binding points of the system of IPM- BSA at 280nm

| T (K) | *K* (L/mol) | *n* |
|---|---|---|
| 298 | 4.6578 | 1.3083 |
| 308 | 5.5372 | 1.6084 |

From the table 6, it is evident that the values of *K* and *n*, at 280 nm are different at different temperatures (298K and 308K), which was obtained from the intercept and slope, separately. It was detected that the binding constant decreases with the increase in temperature, resulting in the reduction of the stability of the BSA-IPM complex, which is a further proof that the fluorescence quenching mechanism of BSA-IPM system is static. The values of *n* remained constant at different temperatures. From the experimental data, it is evident that the binding mole ratio for the BSA-IPM system is 1: 1.

## Conclusion

Analyses of the interactions of Imipramine hydrochloride (IPM) with BSA under the physiological buffer (pH 7.4) were carried out by fluorescence spectroscopic technique. The fluorescence quenching of BSA was also observed in presence of IPM. The experimental results indicated that tyrosine residues also participate with tryptophan residues in the interactions of IPM with BSA at 280 nm excitation wavelength.

From Stern-Volmer constant value at different temperature (298 and 308K), the quenching of fluorescence of BSA initiated by IPM was found to be static. In order to determine the factors responsible for the overall binding affinity and specificity of the drugs, thermodynamic parameters namely enthalpy change (ΔH), entropy change (ΔS), and Gibb's free energy (ΔG) for BSA-IPM system was measured on the basis of Van't Hoff equation. The observation demonstrates the existence of hydrogen bonding and hydrophobic interaction in BSA-IPM complex formation which indicates reversible binding. The interaction process was spontaneous and the mole ratio of BSA-IPM is 1:1 (which indicated that 1 mole of IPM binds with 1 mole of BSA). The binding process is reversible and spontaneous.

The experimental consequences bear a great impact in pharmacy, pharmacology and biochemistry, and are expected to provide important insight into the interaction of the drug molecule with protein albumin. From the protein binding study, accurate calculation of different pharmacokinetic parameters of Imipramine is possible. Thus, the study will be helpful in designing actual dosage regimen and finding out new drugs with lower side effects. So from the interaction study of BSA-IPM, future study can be done besides having an analgesic effect. This study will help to discover some new kind of pharmacological properties of Imipramine hydrochloride and to design a predictive, preventive, safe and personalised medicine.

## Acknowledgement

The authors express earnest thanks and gratitude to Novartis (Bangladesh) Ltd. for giving the Imipramine as a gift sample. We are grateful to Center for Advance Research and Sciences (CARS) and Department of Clinical Pharmacy and Pharmacology, University of Dhaka for their logistic support and motivation.

## References

1. Lazaro E, Lowe PJ, Briand X, Faller B. Medicinal chemistry. 2008; 51:2009-20017.
2. Hu YJ, Liu Y, Sun TQ, Bai AM, Lü JQ, Pi ZB. Binding of anti-inflammatory drug cromolyn sodium to bovine serum albumin. Int J Biol Macromol. 2006; 39(4-5):280-5.
3. Tian J, Liu J, He W, Hu Z, Yao X, Chen X. Probing the binding of scutellarin to human serum albumin by circular dichroism, fluorescence spectroscopy, FTIR, and molecular modelling method. Biomacromolecules. 2004; 5(5):1956-61.
4. He XM, Carter DC. Atomic structure and chemistry of human serum albumin. Nature. 1992; 358(6383):209-15.
5. Shargel L, Andrew Y. Applied Biopharmaceutics and Pharmacokinetics (5th ed.). 2005; 10:253-02.
6. Wise SA, Watters RL. Bovine Serum Albumin (7 % Solution). Certificate of Analysis. United States National Institute of Standards & Technology. 2011.
7. Skoog. Principles of Instrumental Analysis (6th ed.). Thomson Brooks/Cole. 2007, 169-173.
8. Stormo GD. DNA binding sites: representation and discovery. Bioinformatics. 2000; 16(1):16-23.
9. Sultana S, Sayeed Bin MS, Islam MS, Bahar A, Sultan MZ, Hasnat A. Interaction of nalbuphine hydrochloride with deoxyribonucleic acid measured by fluorescence quenching. Drug Res. 2013; 63:224-227.
10. Yun-Kai LV, Pan LI, Miao-Lun JIAO, Bao-Sheng LIU, Chao YANG. Fluorescence quenching study of moxifloxacin interaction with calf thymus DNA. Turkish Journal of Chemistry. 2014, 1-8.
11. Zhang G, Keita B, Craescu CT, Miron S, de Oliveira P, Nadjo L. Molecular interactions between Wells-Dawson type polyoxometalates and human serum albumin. Biomacromolecules. 2008; 9(3):812-7.
12. Gillen C, Haurand M, Kobelt DJ, Wnendt S: Affinity, potency and efficacy of Imipramine and its metabolites at the cloned human mu-opioid receptor. Naunyn Schmiedebergs Arch Pharmacol. 2000 Aug; 362(2):116-21.
13. Raffa RB, Friderichs E, Reimann W, Shank RP, Codd EE, Vaught JL: Opioid and nonopioid components independently contribute to the mechanism of action of Imipramine, an 'atypical' opioid analgesic. J Pharmacol Exp Ther. 1992 Jan; 260(1):275-85.
14. Ran D, Wu X, Zheng J, Yang J, Zhou H, Zhang M *et al*. Study on the interaction between florasulam and bovine serum albumin. J Fluoresc. 2007; 17(6):721-6.

15. Wang YQ, Zhang HM, Zhang GC, Tao WH, Tang SH. Binding of brucine to human serum albumin. J Mol Struct. 2007; 830:40-5.
16. Yuan T, Weljie AM, Vogel HJ. Tryptophan fluorescence quenching by methionine and selenomethionine residues of calmodulin: orientation of peptide and protein binding. Biochemistry. 1998; 37(9):3187-95.
17. Zhang X, Li L, Xu Z, Liang Z, Su J *et al*. Investigation of the interaction of naringin palmitate with bovine serum albumin: Spectroscopic analysis and molecular docking. 2013; 8(3):e59106.
18. Grond S, Sablotzki A. Clinical pharmacology of Imipramine. Clin Pharmacokinet. 2004; 43(13):879-923.
19. Li Y, He WY, Liu H, Yao X, Hu Z. Daidzein interaction with human serum albumin studied using optical spectroscopy and molecular modelling methods. J. Mol. Struct. 2007; 831(1-3):144-150.
20. Monti S, Manet I, Manoli F, Capobianco ML, Marconi G. Gaining an insight into the photoreactivity of a drug in a protein environment: a case study on nalidixic acid and serum albumin. J Phys Chem B. 2008; 112(18):5742-54.
21. Parisa Dorraji S, Fahimeh Jalali. Investigation of the interaction of sertraline with calf thymus DNA by spectroscopic methods. J. Braz. Chem. Soc. 2013; 24(6).
22. Steinhardt J, Krijn J, Leidy JG. Differences between bovine and human serum albumins: binding isotherms, optical rotatory dispersion, viscosity, hydrogen ion titration, and fluorescence effects. Biochemistry. 1971; 10(22):4005-15.
23. Silva D, Cortez CM, Cunha-Bastos J, Louro SR. Methyl parathion interaction with human and bovine serum albumin. Toxicol Lett. 2004; 147(1):53-61.
24. Cui F, Wang J, Cui Y, Li J, Lu Y, Fan J *et al*. Anal. Sci. 2007; 23:719-725.
25. Bhattacharyya M, Chaudhuri U, Poddar RK. Evidence for cooperative binding of chlorpromazine with haemoglobin: equilibrium dialysis, fluorescence quenching and oxygen release study. Biochem Biophys Res Commun. 1990; 167(3):1146-53.
26. Lakowicz JR. Principles of Fluorescence Spectroscopy (2nd ed.). New York, Plenum Press. 1999, 237-265.
27. Liu Xuyang, Shi Zhihong, Sun Hanwen. Study on the interaction characteristics of lomefloxacin and/or cefazolin with bovine serum albumin by spectroscopic technique. 2007.
28. Papadopoulou A, Green RJ, Frazier RA. Interaction of flavonoids with bovine serum albumin: a fluorescence quenching study. J Agric Food Chem. 2005; 53(1):158-63.
29. Haq I. Thermodynamics of drug-DNA interactions. Archives of Biochemistry and Biophysics. 2002; 403:1-15.
30. Sun SF, Zhou B, Hou HN, Liu Y, Xiang GY. Studies on the interaction between Oxaprozin-E and bovine serum albumin by spectroscopic methods. Int J Biol Macromol. 2006; 39(4-5):197-200.
31. He LL, Wang X, Liu B, Wang J, Sun YG. The interaction between ranitidine hydrochloride and bovine serum albumin in aqueous solution. J Solution Chem. 2010; 39: 654–664.
32. Aki H, Yamamoto M. Thermodynamics of the binding of phenothiazines to human plasma, human serum albumin and alpha 1-acid glycoprotein: a calorimetric study. J Pharm Pharmacol. 1989; 41(10):674-9.
33. Ross PD, Subramanian S. Thermodynamics of protein association reactions: forces contributing to stability. Biochemistry. 1981; 20(11):3096-102.
34. Ghosh A, Bansal M. A glossary of DNA structures from A to Z. Acta Crystallogr D Biol Crystallogr. 2003; 59(4):620-6.
35. Hu YJ, Liu Y, Hou AX, Zhao RM, Qu XS, Qu SS. Study of caffeine binding to human serum albumin using optical spectroscopic methods. Acta Chim. Sinica. 2004; 62:1519-1523.