Scatchard analysis of analgin with BSA at various pH and molecular modeling study.

Pisudde Ajay, Tekade Pradip[*] and Thakare Shrikant

Department of Chemistry, Jankidevi Bajaj College of Science, Jamnalal Bajaj marg, civil lines, Wardha (India).
Email: pradiptekade@gmail.com

Manuscript Details

Cite this article as:

ABSTRACT

Study of the binding of drug with plasma protein by the acoustical properties shows simple and effective method. Analgin is a pain reliever and antipyretic drug. We studied binding of analgin with plasma protein by ultrasonic, FT-IR and molecular modeling techniques. In the present study, we used ultrasonic method for the study of the binding of analgin with BSA which is the novel method for study of binding of analgin with BSA. Study of interaction of analgin with BSA shows successful binding with BSA. Binding of BSA with analgin further confirmed using FT-IR spectroscopy and molecular modeling study. Effect of pH on the binding of analgin with BSA was also studied. The values of the association constant calculated from the Scatchard plot at varying pH 3, 4 and 5 are 0.5012, 0.4994 and 0.5014 respectively. Study of interaction by FT-IR spectroscopy gives the changes in peak positions of amide bands. The amide I changes from 1635 to 1642 cm^{-1} and amide II 1538 to1556 cm^{-1}. It shows the secondary structure of BSA changes on binding with analgin. binding interaction of analgin with BSA was further confirmed by using molecular modelling study. The energy value obtained (-213.34) shows that the analgin efficiently binds with BSA.

Keywords Ultrasonic study, FT-IR spctroscopy, Scatchard analysis, association constant, molecular modeling study.

INTRODUCTION

Transportation of drug is one of the important characteristic of blood and binding of drug is an efficiency feature of protein which binds the drug in plasma.Chiral drugs have various applications in day-to-day life as well as in commercial areas like clinical, medicinal, pharmaceutical industries and some metabolic activities of humans. Proteins are macromolecules; consist of one or more long chain of amino acid residues. Proteins do a vast array of functions within living organisms, including catalyzing metabolic reactions, replicating DNA, responding to stimuli and transporting molecules from one place to another. Basically there are three types of protein in the blood which have the ability to bind the drug, out of which alpha-1-acid glycoprotein (AGP) have ability to bind the basic drugs; Human Serum Albumin (HSA) shows the affinity for acidic drugs and lipoprotein which binds drugs on saturation of these proteins. In drug transportation HSA plays a vital role. It is difficult to get an HSA for experimental purposes. So in this study, we used bovine serum albumin (BSA) in place of HSA. BSA show similar bonding chemistry as that of HSA due to the high percentage of sequence identities. The vast studies were performed by researchers to see protein-drug interaction, such as ^{1}H NMR [1-2], FT-IR spectroscopy [3], fluorescence quenching & CD spectroscopy [4], U. V. Spectrophotometry [5] Fluorescence spectroscopy [6], thin layer chromatography [7], capillary electrophoresis [8-9], Mass spectrometry [10], spectroflurometry [11], HPLC method [12], isothermal titration calorimetry (ITC) [13], gel filtration [14], flow ultra- filtration technique [15], chromatography [16] and micro liquid-liquid interface [17]. Molecular modeling also shows some important aspects about protein-drug interaction [18-20].

Literature survey reveals that, drug-protein binding by ultrasonic interferometer was not done so far. Therefore, it is a topic of interest to study BSA-analgin interaction using acoustical factor. Ultrasonic interferometer is a simple and direct device to determine the ultrasonic velocity in liquids with a high degree of accuracy. Change in ultrasonic velocities for BSA and analgin are a measure of their interaction. Analgin is biologically active drug shows analgesic and antipyretic activity. Binding interaction of analgin with BSA was also confirmed by FT-IR spectroscopy and molecular modeling study.

METHODOLOGY

Acoustical parameters determined by Vi Microsystems Pvt. Ltd. India ultrasonic interferometer. Bruker's FT-IR spectrophotometer (Alpha model, Germany) equipped with Zn-Se attenuated total reflection (ATR) accessory at room temperature used for spectral analysis. All spectra analyzed via the ATR method with 4 cm^{-1} resolution and 60 scans in the region 1800-1300 cm^{-1}. For molecular modeling study software HEX 8.0 were used. BSA (M_r = 66,500, essentially fatty acid free) purchased from Chemsworth Chemicals Ltd (India) and used without further purification. analgin prepared by known method. Acidic buffer solutions of 3, 4 and 5 pH were used. All other chemicals used in the experiments were of commercial grade.

Measurement of binding affinity:
Ultrasonic study of analgin was performed at 1MHz. 0.15 µM BSA solution was prepared in acetate buffer of pH 3, 4 & 5 and ultrasonic velocity of these solutions were measured. Secondly 0.0005, 0.001, 0.0015, 0.0020, 0.0025 M analgin solutions were prepared in same buffer at pH 3, 4 and 5. Then the solutions of BSA and analgin of different concentrations have been mixed at pH 3 and allowed to stand for half an hour for maximum binding interaction. The ultrasonic velocities of these complex solutions were recorded. Graph of ultrasonic velocity versus concentrations of analgin have been plotted. Also the graph of specific binding versus concentration of analgin were plotted which gives the values of association constants of analgin with BSA (scatchard plot). Similarly ultrasonic velocities of BSA-analgin complexes at pH 4 and 5 were recorded. Scatchards plot was used to measure the specific binding of analgin with BSA.
Specific binding for the complex calculated by using equation:

$$V = \frac{\text{ultrasonic velocity of complex}}{\text{ultrasonic velocity of complex} + \text{ultrasonic velocity of BSA}}$$

Where, V = specific binding for complex solution.

Determination of 'saturation value' (n) and 'formation constant' (K_f) for protein-ligand interaction using Scatchard plot are in following graphs.

Measurement of binding affinity using FT-IR:

Binding interaction of analgin with BSA was confirmed by FT-IR Spectroscopy. Initially Spectrum of BSA recorded and it shows two amide bands, amide-I (C=O stretching) and amide-II (C-N stretching coupled with N-H bending). Then the complex of BSA and analgin prepared by mixing them in 1:1 ratio and allowed it to stand for half an hour for maximum binding. Spectrum for analgin-BSA complex recorded and changes in secondary structure of BSA were observed. Shift in peak positions of amide bands, changes the secondary structure of BSA. It confirms the binding of analgin with BSA.

Molecular modeling study:

Molecular modeling study of plasma protein with analgin was carried out by using HEX 8.0 software. Molecular modeling study gives the efficient energy value of binding. Crystal structure of BSA was obtained from RCSB protein data bank and 3D file for analgin was developed on Chem Draw Ultra 8.0. and run on HEX 8.0. It gives negative energy value for the newly formed complex showing its stability.

RESULTS AND DISCUSSION

Ultrasonic study:

The ultrasonic velocities of BSA Solution at pH 3, 4 and 5 are 1484.590, 1490.078 and 1495.607 respectively. Similarly, ultrasonic velocities at various pH for BSA-analgin complexes at different concentrations were also measured. Figures 1, 3 & 5 shows change in ultrasonic velocity of complexes at pH 3, 4 & 5 respectively. Figures 2, 4 & 6 shows Scatchard plot of BSA-analgin complexes respectively. From scatchard plot association constants have been calculated. The association constants (K_f) for BSA-analgin complex are 0.5014, 0.4994 & 0.5012 at pH 3, 4 & 5 respectively. The association constant is more at pH 3, which shows the binding is more at pH 3.
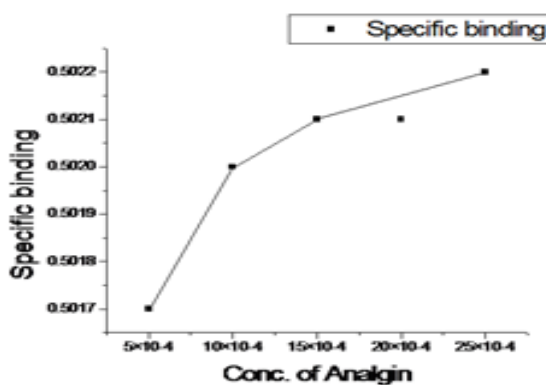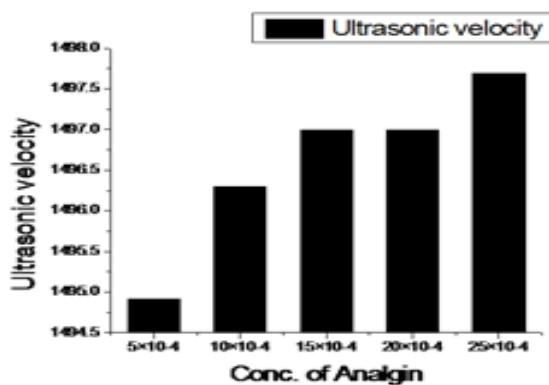

Figure 1: Ultrasonic velocity Vs conc.   analgin at pH 3

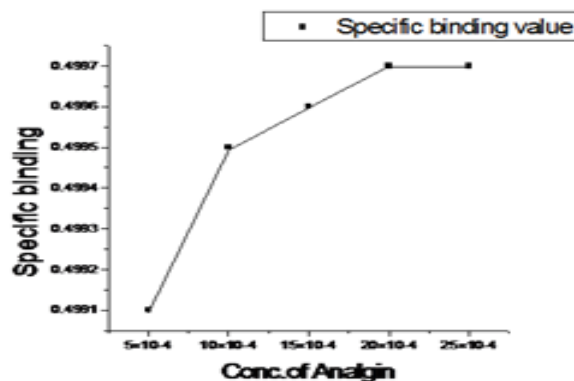**Figure 2**: Specific binding Vs conc. of analgin at pH 3
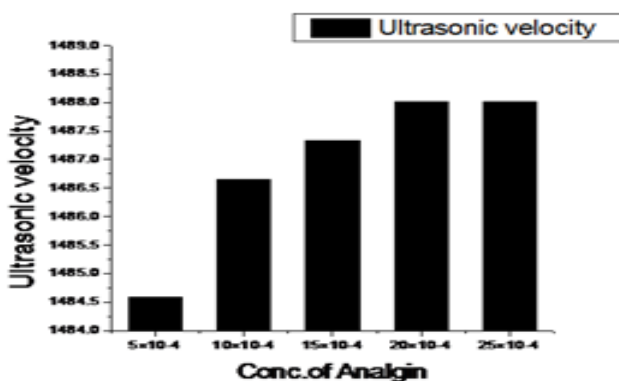

Figure 3: Ultrasonic velocity Vs conc. analgin at pH 4

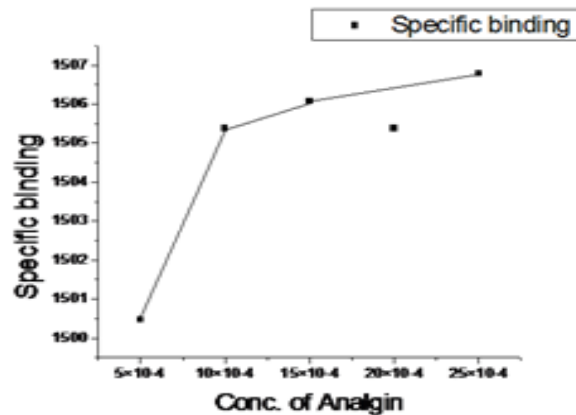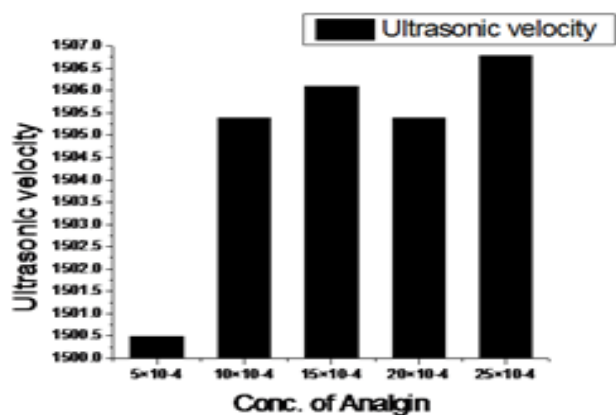**Figure 4**: Specific binding Vs conc. of analgin at pH 4

Figure 5: Ultrasonic velocity Vs conc. analgin at pH5

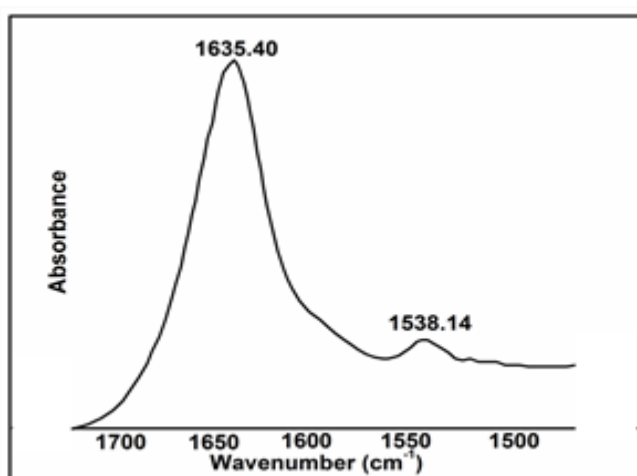**Figure 6**: Specific binding Vs conc. of analginpH

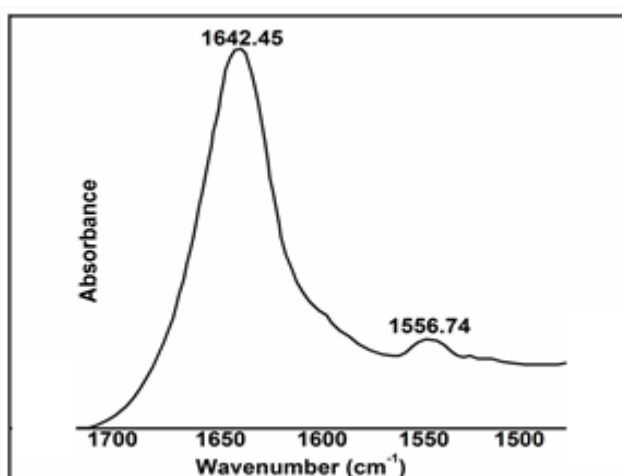**Figure 7**: FT-IR spectrum of BSA

Figure 8: FT-IR spectrum of BSA-analgin complex
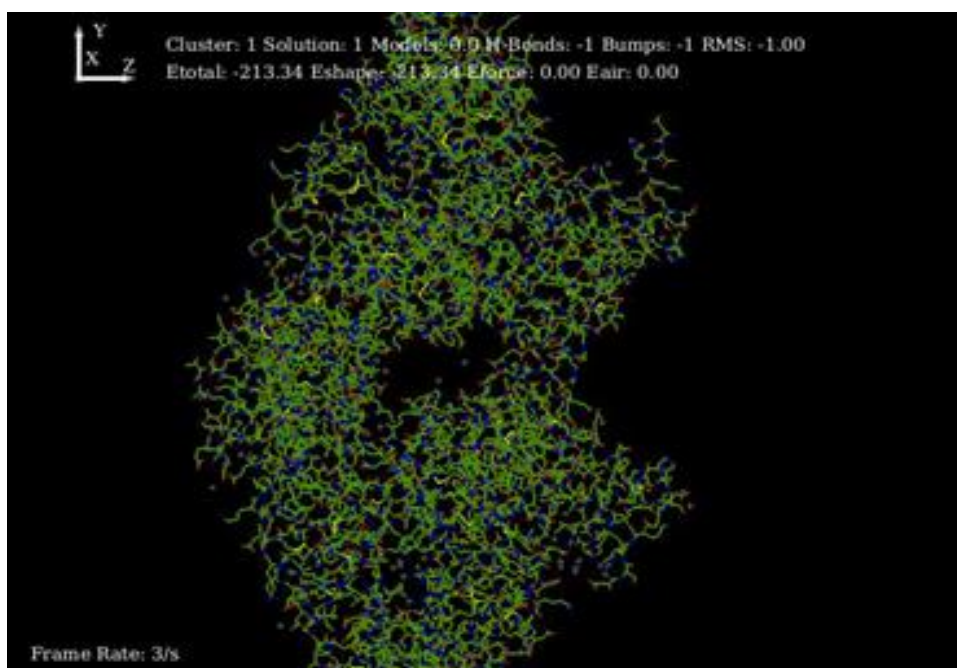

Figure 9: Molecular Modeling interaction between BSA and analgin

FT-IR study:

Binding analysis of analgin with BSA was further studied by FT-IR spectroscopy. BSA consists of mainly two bands. Amide I at 1635.40 cm^{-1} due to C=O stretching and amide II at 1538.14 cm^{-1} is due to C-N stretching coupled with N-H bending mode (figure 7). On binding interaction of analgin with BSA, the changes in peak positions of these amide bands were observed. Amide I shift to 1642.45 from 1635.40 cm^{-1} and amide II shifted to 1556.74 from 1538.14 cm^{-1} respectively (figure 8). Shifting in peak positions of amide bands of BSA changes the secondary structure of BSA. No significant binding observed at pH 4 & 5.This confirms the successful binding of analgin with BSA at pH 3.

Molecular modeling study:

Molecular modeling is an efficient method for measurement of interaction between protein and drug. The energy value obtained from the study is the measure of binding of drug to the serum protein. Binding interaction between BSA and analgin was by using software HEX 8.0. The energy value obtained (-213.34) shows that the analgin efficiently binds with BSA. Diagrammatic representation of interaction between BSA and analgin (figure 9).

CONCLUSION

Acoustical Study of binding interaction of analgin with BSA is the simple and effective method. Analgin is a pain reliever as well as antipyretic. In this study, we have used multifrequency ultrasonic interferometer for determination of binding affinity of analgin with BSA, which is the novel method. It is concluded that, analgin binds successfully with BSA at lower pH. Scatchard plot gives significant values of the association constants (K_f) at pH 3, 4 and 5 viz. 0.5014, 0.4994 and 0.5012. The value of association constant (K_f) is more at pH 3 than pH 4 and 5. The order of binding is 3>4>5. It means that binding of analgin with BSA is more efficient at a more acidic pH.

FT-IR spectroscopic study also gives positive statistics for binding affinity of analgin with BSA. Changes in secondary structure of BSA (change in peak positions of amide I and II) confirm the binding of analgin with BSA. Molecular modeling study was also used to confirm binding of analgin with BSA. This gives the efficient energy value (-213.34) for BSA-analgin complex formation. This energy value shows that the complex formed is stable. It concludes that the analgin successfully binds with BSA.

Conflicts of interest: The authors stated that no conflicts of interest.

REFERENCES

1. Fielding L, Rutherford S, I-bruprofen, salicylic acid & warfarin, binding with protein. Magnetic res. Chemistry, 2005; 43: 463-472.

2. Yuangyuan, et al, NMR Spectroscopic approach of human blood plasma associated with protein drug interaction. Analytical chemistry, 2013; 85: 8601-8608.

3. Bakkialakshmi S, Barani V, FT-IR study on the interaction of quercetin and amantadin with egg albumin. International journal of Pharmaceutical, chemical and biological sciences, 2013; 3: 559-564.

4. Tian TJ, Liu J, Chen X, Interaction of wogonin with BSA. FEBS Letters, 2005; 1: 995-1002.

5. Alam A, Uddin R, Protein binding interaction of warfarin and acetaminophen in presence of arsenic. J. Pharmacol, 2008; 3: 49-54.

6. Hu YJ, Yang OU, Zhang Y, Liu Y, Affinity & specificity of ciprofloxacin-protein interaction spectroscopic approach. Pubmed, 2010; 29: 234-241.

7. Ahmad RA, Roggers HJ, Protein binding interaction of dasponepyrimethymine. BCJP, 1980; 10: 519-524.

8. Erim FB, et al, Protein-drug bonding constant by pressure assisted capillary electrophoresis. Journal of Chromatography, 1988; 710: 205-210.

9. Jia Z, Ramstad T, Zhong M, Pharmaceutical and medical analysis Protein-drug bonding

constant by pressure assisted capillary electrophoresis. J. Pharm. Biomed. Anal, 2002; 30: 405-413.

10. Taylor RJ, et al, Mass spectrometry to investigate protein-drug interaction. Chemical society review, 2012; 41: 4335-4355.

11. Bajko B, et al, Investigations of acetaminophen binding to bovine serum albumin in the presence of fatty acid: fluorescence and 1H NMR studies. Journal of Molecular Structure, 2009; 924: 332-339.

12. Valko K, et al, HPLC method to determine binding to HSA. Journal of Pharmaceutical Sciences, 2003; 92: 2236-2248.

13. Xiangrong Li, Gongke W, Dejun C, Yan Lu, Spectroscopic approach for ciprofloxacin-protein interaction. Mol. BioSyst, 2014; 10: 326-337.

14. Zahra R, et al, Study of the interaction between popranolol and NSAIDS in protein binding by gel filtration method. Indian journal of clinical biochemistry, 2006; 21: 121-125.

15. Ghosh R, et al, Tangential flow ultrafiltration technique for studying protein-drug binding. Pharmaceutical sciences, 2013; 102: 2679-2688.

16. Hage DS, et al, Chromatographic and electrophoretic studies of protein binding to chiral solutes. Journal of chromatography, 2001; 906: 459-461.

17. Lopes P, Kataky R, Chiral interactions of the drug Propranolol and α-acid glycoprotein at a micro liquid interface. Analytical chemistry, 2012; 84: 2299-2304.

18. Hartshorn MJ, et al, Diverse, high-quality test set for the validation of protein ligand docking performance. J. Med. Chem, 2007; 50: 726-741.

19. Taufer M, et al, Study protein-ligand docking algorithm based on molecular dynamics. Concurr. Comput, 2005; 17: 1627-1641.

20. Sousa SF, et al, Virtual screening in drug design and development. Comb. Chem. High throughput Screen, 2010; 13: 442-453.