INTERNATIONAL JOURNAL OF RESEARCHES IN BIOSCIENCES, AGRICULTURE & TECHNOLOGY
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www.vmsindia.org

# INTERACTIONS OF BSA WITH 2-{[2-(CYCLOHEXYLCARBAMOYL) BENZOYL] AMINO}-3-METHYLBUTANOIC ACIDS AT VARIABLE pH: ULTRASONIC AND FT-IR STUDY

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**Abstract:**
In this paper we accounted the interaction of 2-{[2-(cyclohexylcarbamoyl) benzoyl] amino}-3-methylbutanoic acid (ligand) with protein Bovine serum albumin (BSA) on multifrequency ultrasonic interferometer and FT-IR spectrophotometer. Solutions of ligand and BSA were prepared at different pH and their ultrasonic velocities have been measured on multifrequency ultrasonic interferometer. Differences in the ultrasonic velocities with different compositions of protein and ligand are measure of binding capacity of the drug with BSA. Binding analysis shows that the ligand bound more significantly at pH 3 than pH 4 and 5 i.e. at more acidic pH. From Scatchard analysis association constants ($K_f$) have been calculated and they are 0.5107, 0.5064 and 0.5046 at pH 3, 4 and 5 respectively. This confirms the significant binding at pH 3 as compared to other pH. Furthermore binding was confirmed by FT-IR spectroscopy. It shows changes in peak positions of amide bands of BSA, which shows the changes in secondary structure of BSA in presence of ligand. This indicates hydrophobic interactions played major role in the binding of ligand with BSA.

**Key words:** Ultrasonic interferometer, FT-IR, BSA, Association constant, Scatchard analysis, 2-{[2-(cyclohexylcarbamoyl) benzoyl] amino}-3-methylbutanoic acid (ligand).

**Introduction:**

Binding of drug to plasma protein is one of the efficient biological characteristic of that drug. Human serum albumin (HSA), alpha acid glycoprotein and lipoprotein are three major proteins in human blood. These proteins perform the function of transportation of drug. HSA is most abundant protein. HSA primarily bind acidic drug and glycoprotein bind basic drug [1-2]. Binding of chiral drug to BSA protein is topic of interest as it is the measure of metabolism of transportation of drug.

Effect of binding on specific site of BSA for ciprofloxacin and captopril drugs in presence of specific site probe was studied using equiliblium dialysis [3]. The protein-protein and protein-ligand interactions involved in retinol transport in plasma were studied [4]. Drugs like i-bruprofen & naproxen show successive binding to protein [5]. Effect of arsenic on binding of protein with warfarin and acetaminophenol had also been observed [6]. Crystal structure analysis of binding of warfarin to BSA was also done [7]. NMR spectroscopic approach reveals metabolic diversity of human blood plasma associated with protein drug interaction [8].

Effect of arsenic on binding of paracetamol with BSA was studied using equilibrium dialysis method [9]. Thin layer chromatography technique used for study of protein binding interaction of daspone and pyrimethamine [10].

Comparative study of various techniques for drug-protein binding gives informative knowledge [11]. Study of protein-drug interaction using ultrasonic interferometer can also add valuable contribution in the field of drug metabolism however only few observations are seen in drug metabolism using ultrasonic interferometer.

In this paper we report the use of multifrequency ultrasonic interferometer for the study of interaction of titled ligand (L) with BSA. The ligand (L) shows antibacterial activity was synthesized using known method [12] and characterized by spectral techniques viz. IR, NMR and Mass spectrometry.

The binding affinity of the ligand with BSA was measured using ultrasonic interferometer and FT-IR spectrophotometer. Also effect of varying pH on binding capacity has been studied using ultrasonic interferometer.

**Materials and Methods:**

For synthesis, all the chemicals used were of A.R. grade of Merck India Limited make and purchased from commercial suppliers. Multifrequency ultrasonic interferometer (Vi Microsystem Pvt. Ltd. Chennai), BSA ($M_r$ = 66,500) (make-chemsworth chemical Ltd. India) were used. 0.1M sodium acetate buffer solution of 3, 4 and 5 (±0.05) pH were used. All the FT-IR spectral measurements were done on a Bruker alpha IR Spectrometer (Germany) at room temperature at department of chemistry J.B. College of science Wardha. All spectra were taken via the Attenuated Total

Reflection (ATR) method with resolution of 4 $cm^{-1}$ and 60 scans. Solutions of 50µM BSA and 0.01M ligand solution were prepared in acetate buffer solution with various pH.

**Measurement of binding affinity:**

Multifrequency ultrasonic interferometer was set at 1MHz. 50µM BSA was prepared in acetate buffer of pH 3, 4 and 5 and ultrasonic velocity of these solutions were measured. Then the 0.01M solution of ligand was prepared in buffer at pH 3, 4 and 5. Ultrasonic velocity of BSA-ligand complex at various pH in different composition viz. 9:1, 8:2, 7:3, 6:4, 5:5, 4:6 were measured. From scatchard analysis specific binding of ligand (L) with BSA at different composition is calculated from which association constants has been calculated.

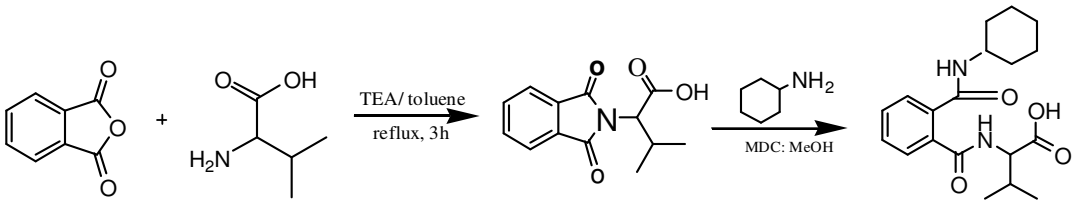


Figure 1: Scheme for the preparation of 2-{[2-(cyclohexylcarbamoyl) benzoyl]amino}-3-methyl butanoic acid

**Results:**

**Ultrasonic interferometer study:**

The ultrasonic velocities of 50µM BSA in absence of ligand are 1428, 1435 and 1453 m/s at pH 3, 4 and 5 respectively. Ultrasonic velocity at varying composition of BSA and ligand at pH 3, 4 and 5 gives the association constants ($K_f$). The association constants ($K_f$) for BSA-ligand complex at pH 3, 4 and 5 are found to be 0.5107, 0.5064, and 0.5046 respectively. Figure 2, 4 and 6 Shows change in ultrasonic velocity of complex solution at different composition of BSA and ligand at pH 3, 4 and 5 respectively. Figure 3, 5 and 7 Shows Scatchard graph for the specific binding of drug with BSA at pH 3, 4 and 5 respectively.

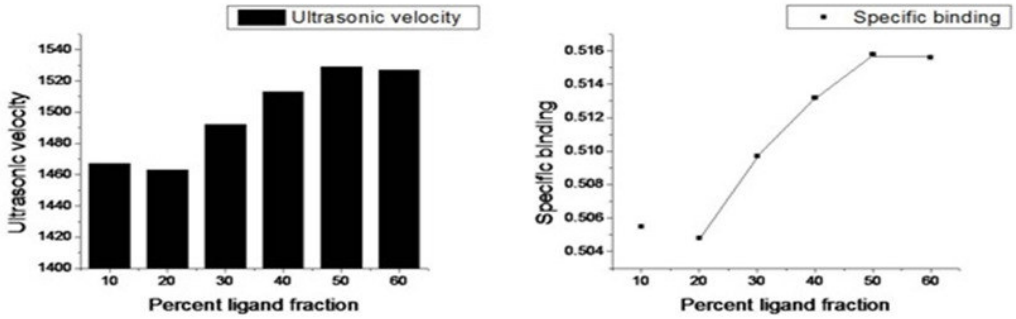


Figure 2 & 3: Ultrasonic velocity/ Specific binding Vs % ligand fraction at pH 3

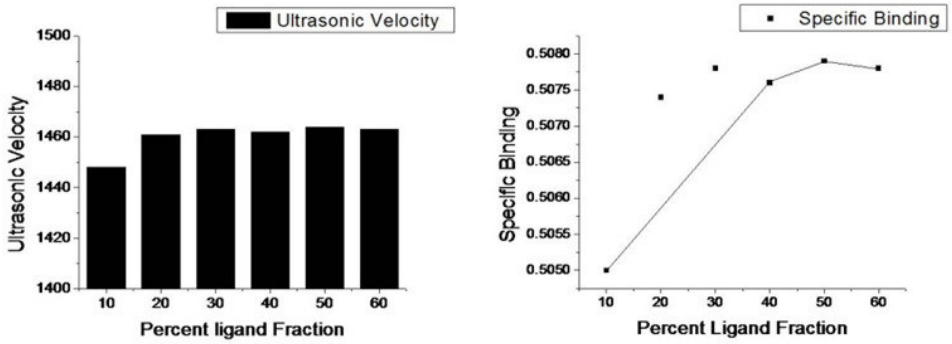


Figure 4 & 5: Ultrasonic velocity/ Specific binding Vs % ligand fraction at pH 4

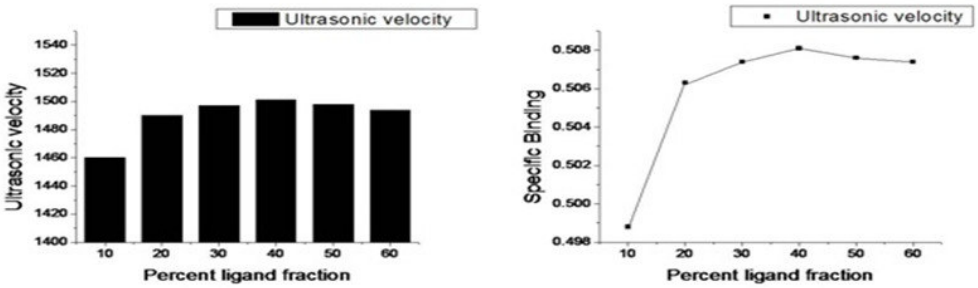


Figure 6 & 7: Ultrasonic velocity/ Specific binding Vs % ligand fraction at pH 5

**FT-IR spectroscopy:**

Binding study of ligand (L) with BSA was further confirmed using FT-IR spectroscopy. Secondary structure of BSA in absence of ligand shows mainly two bands. Amide I at 1633 $cm^{-1}$ which is due to C=O stretching and amide II at 1556 $cm^{-1}$ due to C-N stretching coupled with N-H bending mode. After binding of ligand with BSA, changes in secondary structure of BSA are observed. Amide I band shift to 1643 from 1633 $cm^{-1}$ however there is no change has been observed in band frequency of amide II. Fig 8 & 9 shows FT-IR spectrum of pure and BSA-ligand complex respectively.

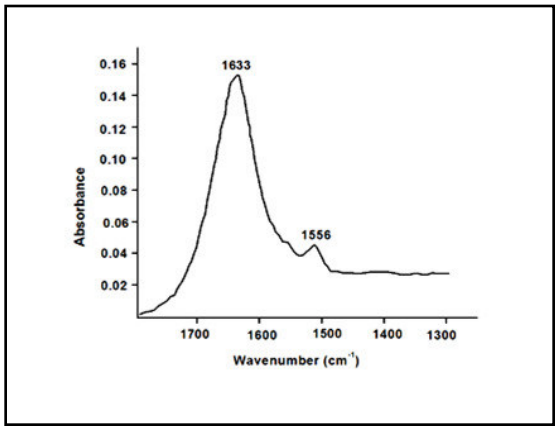


**Fig: 8 FT-IR spectrum of BSA**

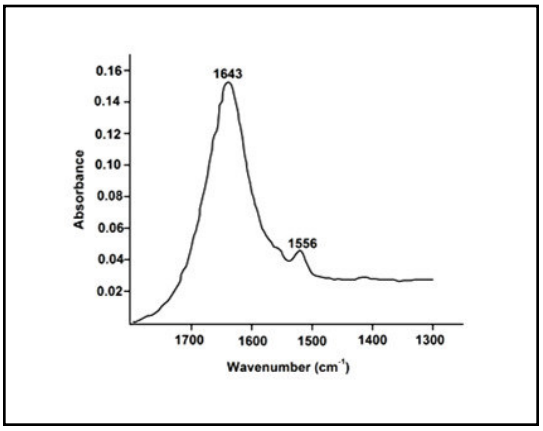


**Fig: 9: FT-IR spectrum OF BSA-ligand complex**

**Discussion:**

Ultrasonic technique shows an effective method for interaction of ligand with BSA. 2-{[2-(cyclohexylcarbamoyl) benzoyl]amino}-3-methyl butanoic acid having antibacterial activity show efficiency to bind with BSA . It is the novel interaction of the ligand (L) with BSA on ultrasonic interferometer. Study of interaction of ligand with BSA show successful binding with BSA. Study of interaction of this ligand (L) at pH 3, 4 and 5 shows different association constants. Association constant is more at pH 3 than other pH. It means that binding of ligand (L) with BSA is more efficient at pH 3 i.e. at more acidic pH. FT-IR study further confirms that ligand shows binding affinity towards BSA. However, at pH 3 and 5 no significant changes in position of amide bands were noted. A change in secondary structure of BSA in presence of ligand is observed at pH 4 which confirms its binding.

**Conclusion:**

Study of binding of ligand 2-{[2-(cyclohexylcarbamoyl) benzoyl]amino}-3-methyl butanoic acid with BSA shows that, the binding is more efficient at more acidic pH. FT-IR study concludes that the hydrophobic interaction played a major role in binding of ligand with BSA, which changes the secondary structure of protein.

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