STUDY OF INTERACTIONS OF 2-{[2-(CYCLOHEXYLCARBAMOYL) BENZOYL] AMINO}-3-METHYLBUTANOIC ACID (2CA3MBA) WITH BSA: ULTRASONIC INTERFEROMETER

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A B S T R A C T

In this paper we account the interaction of the2-{[2-(cyclohexylcarbamoyl) benzoyl] amino}-3-methylbutanoic acid (2CA3MBA) with Bovine serum albumin (BSA) using ultrasonic interferometer technique. 2CA3MBA solution was added to aqueous solution of BSA and its ultrasonic velocity had been measured at different composition of BSA and 2CA3MBA on ultrasonic interferometer. Binding effect at various temperature viz. 25^0c, 30^0c and 35^0c shows that 2CA3MBA bound to the BSA more significantly at temperature 35^0c than at 30^0c and 25^0c. Scatchard analysis gives the values of association constants (K_f)0.5003, 0.5010 and 0.5011 at temperatures 25^0c, 30^0c and 35^0c respectively which confirms the binding is more efficient at higher temperature. Furthermore FT-IR study was done which showed the changes in secondary structure of BSA and confirms the binding of 2CA3MBA with BSA.

Keywords: Ultrasonic interferometer, BSA, Association constant, Scatchard analysis, Vant Hoffs plot

1. Introduction

Affinity of drug for protein in blood is one of the efficient biological characteristic of that drug. Human serum albumin (HSA), alpha acid glycoprotein (AGP) and lipoprotein are three major proteins in human blood also called as a plasma protein. Plasma proteins are most abundant protein it comprises 60% of total protein in blood. These proteins perform the function of transportation of drug. HSA primarily bind acidic drug (Albengrers and Urien, 1987) and glycoprotein bind basic drug (Otagiri, 2005). Binding of chiral drug to HSA protein is topic of interest as it is measure of metabolism of transportation of drug.

BSA in lieu of HSA was used in this study as it is easily available and showed similar structure bonding chemistry as HSA. BSA is alkaline having 7-8 pH range (Putnam, 1975). It is the moiety with large molecular weight approximately (M_r = 66,500) contains 583 amino acids. As BSA is a major protein in blood, any change in level of BSA produces effect on transportation of drug. There are various forces which are responsible for binding of drug to plasma protein they are hydrogen bonding, vander wall forces, electrostatic attraction etc.

Effect of binding on specific site of BSA for ciprofloxacin and captopril drugs in presence of specific site probe was studied using equilibrium dialysis (Mahbulal and Reza, 2004). The protein-protein and protein-ligand interactions involved in retinol transport in plasma were studied (Raz and Godmann, 1970). Drugs like i-bruprofen& naproxen show successive binding to protein (Rahman, 2004). Effect of arsenic on binding of protein with warfarin and acetaminophenol had also been observed (Alam and Uddin, 2008). Crystal structure analysis of binding of warfarin to BSA was also done (Petipas, 2001). NMR Spectroscopic approach reveals metabolic diversity of human blood plasma associated with protein drug interaction (Yuan, 2013). Effect of arsenic on binding of paracetamol with BSA was studied using equilibrium dialysis method (Riaz and Naddia, 2012). Thin layer chromatography technique used for study of protein binding interaction of daspone and

pyrimethamine (Ahmad and Roggers, 1980). Structure based approach for discovering protein–ligand binding affinity and drug designing from serum albumin model systems was studied using NMR technique (Fieldieng and Rutherford,). Affinity and specificity of ciprofloxacin-BSA interaction was studied by fluorescence spectrophotometry (Yan and Zang, 2010). Mass spectrometry based tools were used to investigate protein-ligand interactions for drug discovery (Perchloraz and Garlish, 2012). Interaction of propanolol with glycoprotein was also studied using micro liquid-liquid interface (Lopes and Kataky, 2012). Comparative study of various techniques for drug-protein binding gives informative knowledge (Busch and Carles, 1997). 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.

Study of binding of various ligand with serum protein using FT-IR technique also add valuable contribution in this study. Changes in secondary structure of protein due to addition of ligand confirm the binding of the ligand with serum protein. Study of interaction of the bioactive component Jatrorrhizine to human serum albumin shows significant change in secondary structure of HSA (Kang and Liu, 2004). Interactions of human serum albumin with chlorogenic acid and ferulic acid were studied (Li and He, 2005). Study binding of atrazine and 2, 4-D with HSA show partial unfolding (Purcell and Malonga, 2001). Effect of binding of mitoxantrone with HSA was successfully observed using FT-IR (Khanna and Islama, 2008). Quercetin and amantadine successfully binds with egg albumin which form new complex (Bakkilakshmi and Barani, 2013).

In this paper we report the simple and useful ultrasonic interferometer technique for the study of interaction of2CA3MBAwith BSA. 2CA3MBA showing antibacterial activity synthesized using known method (Pande and Utale, 2014) and characterized by spectral techniques viz. IR, NMR and Mass spectrometry. Moreover study of 2CA3MBA was also done using FT-IR technique and changes in secondary structure of BSA were observed.

2. Experimental

For synthesis, all the chemicals used were of A.R. grade of Merck India Limited and purchased from commercial suppliers. The purity of the synthesized compound was ascertain by thin layer chromatography on silica gel G in petroleum ether and ethyl acetate (7:3) mixture, Melting point was recorded using digital melting point apparatus Equiptronics (EQ 730) . ^{1}H NMR spectra of the compound were recorded in $CDCl_3$ on NMR instrument (500MHz) using TMS as an internal standard from SAIF, CDRI Lucknow.

For measurement of binding, ultrasonic interferometer (Vi-Microsystem, India), BSA (M_r =66,500) (Chemsworth chemical Ltd. India) were used. 0.1M sodium acetate buffer solution of 7.4 pH was used.

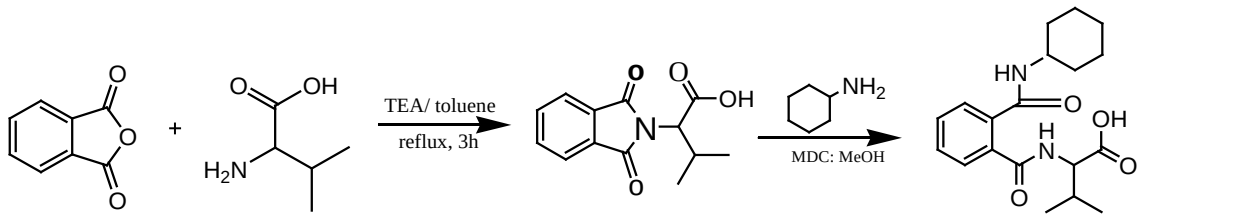

Fig.1- Scheme for the preparation of *2-{[2-(cyclohexylcarbamoyl) benzoyl]amino}-3-methyl butanoic acid*

2.1 Measurement of binding affinity:

Set up of ultrasonic interferometer was done at 1MHz frequency range and appropriate cell of the frequency was used. BSA solution of 50µM concentration in aqueous phase using acetate buffer of pH 7.4 was prepared and ultrasonic velocity of these solutions were measured at 25^0c, 30^0c and 35^0c in lack of 2CA3MBA. Later 0.001M solution of a 2CA3MBA prepared using a buffer solution of pH 7.4. Then the mixture of solution of BSA and 2CA3MBA in different composition viz. 9:1, 8:2, 7:3, 6:4, 5:5, 4:6 prepared and allow to stand for half an hour and then inserted into the cell of appropriate frequency. Ultrasonic velocities for this solution measured at different temperature 25^0c, 30^0c and 35^0c. Graph plotted between ultrasonic velocity and percent ligand fraction, from the values of ultrasonic velocity, the values of specific binding obtained. The graph of specific binding Vs percent ligand fraction plotted at different temperature, from which values of the association constant obtained at different temperatures 25^0c, 30^0c and 35^0c.

3. Result and Discussion

3.1 Experimental observations

50µ*M* Solution of BSA at buffer 7.4 pH were taken in a cell of 1MHz frequency of ultrasonic interferometer. The recorded values of ultrasonic velocity of these BSA solutions in absence of 2CA3MBA are 1484.240, 1492.160 and 1501.790 m/s at temperature 25^0c, 30^0c and 35^0c respectively. The values of ultrasonic velocities and specific binding for complex solution of BSA-2CA3MBA at varying composition and at different temperature are shown in table 1 and 2 respectively.

Table 1- Ultrasonic velocitiesof BSA-2C3MBA at different temperature and composition

	Ultrasonic velocity at different temperatures		
Composition of BSA-2CA3MBA	**25^0C**	**30^0C**	**35^0C**
10:0	1484.240	1492.160	1501.790
9:1	1484.278	1496.212	1507.412
8:2	1486.980	1497.960	1507.946
7:3	1488.349	1499.387	1508.329
6:4	1488.349	1500.206	1509.680
5:5	1487.286	1501.347	1509.680
4:6	1487.286	1501.347	1509.729

Table 2- Specific binding for BSA-2CA3MBA at different temperature and composition

	Specific Binding at different temperature		
Composition of BSA-2CA3MBA	**25^0C**	**30^0C**	**35^0C**
9:1	0.5001	0.5007	0.5009
8:2	0.5005	0.5010	0.5010
7:3	0.5007	0.5012	0.5011
6:4	0.5007	0.5013	0.5013
5:5	0.5006	0.5014	0.5013
4:6	0.5006	0.5014	0.5013

Measurement of ultrasonic velocity at varying composition of BSA and 2CA3MBA at temperature 25^0c,30^0c and 35^0c gives the value ofassociation constant (K_f) which is calculated from Scatchard graph. The value of association constant (K_f) for BSA-2CA3MBA complex at temperature 25^0c, 30^0c and 35^0care found to be 0.5003, 0.5010 and 0.5011 respectively.

Figure 2 Shows change in ultrasonic velocity and specific binding of complex solution at

different composition of BSA and 2CA3MBA at 25^0C. Likewise **Figure 3** and **Figure 4** Show change in ultrasonic velocity and specific binding of complex solution at different composition of BSA - 2CA3MBA at temperature 30^0c and 35^0c.

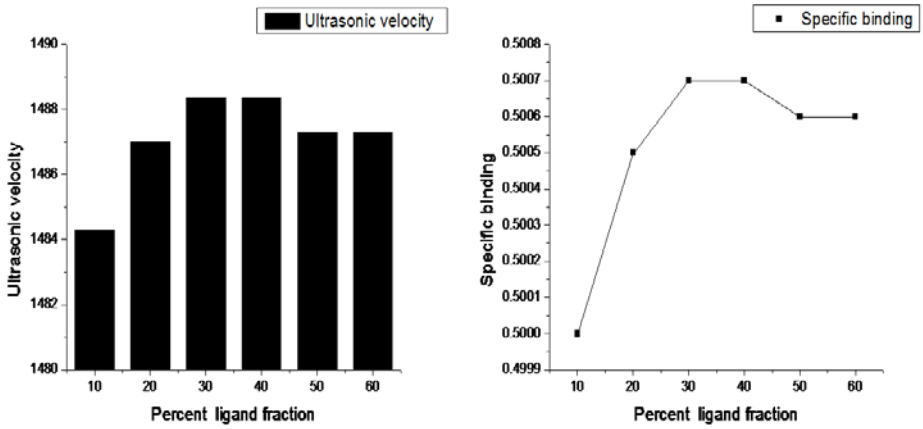

Fig.2-Graph of Ultrasonic velocity and Specific binding Vs Percent ligand fraction at temperature 25^0c

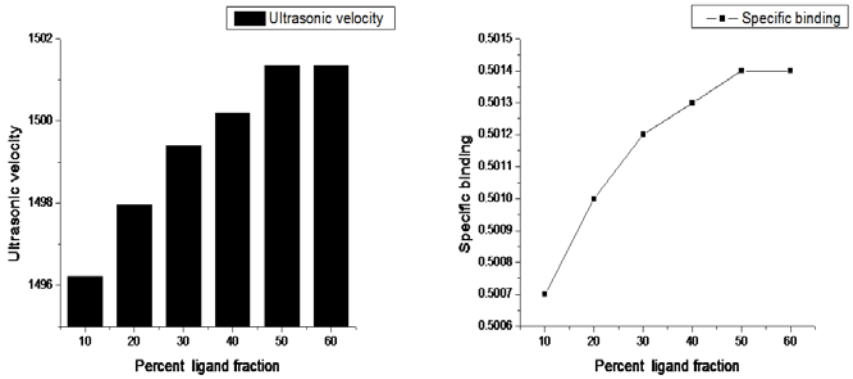

Fig. 3-Graph of Ultrasonic velocity and Specific binding Vs Percent ligand fraction at temperature 30^0c

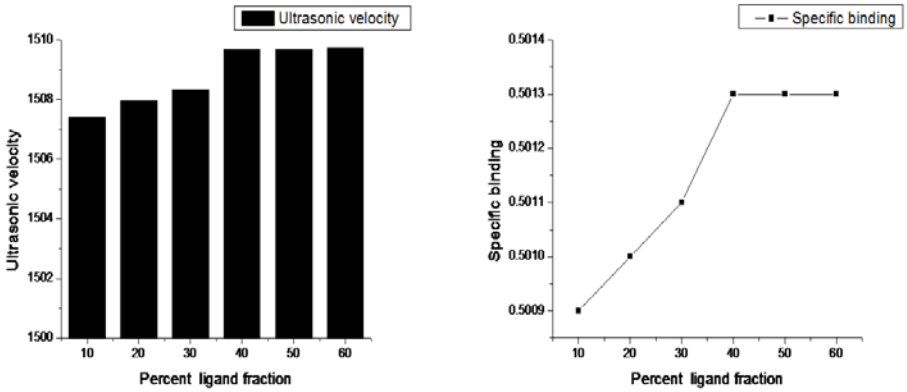

Fig. 4-Graph of Ultrasonic velocity and Specific binding Vs Percent ligand fraction at temperature 35^0c

VantHoffs equation used in order to calculate the value of Enthalpy, Entropy and Gibbs free energy associated with complex solution.

Graph plotted between **lnkVs 1/T** which gives straight line

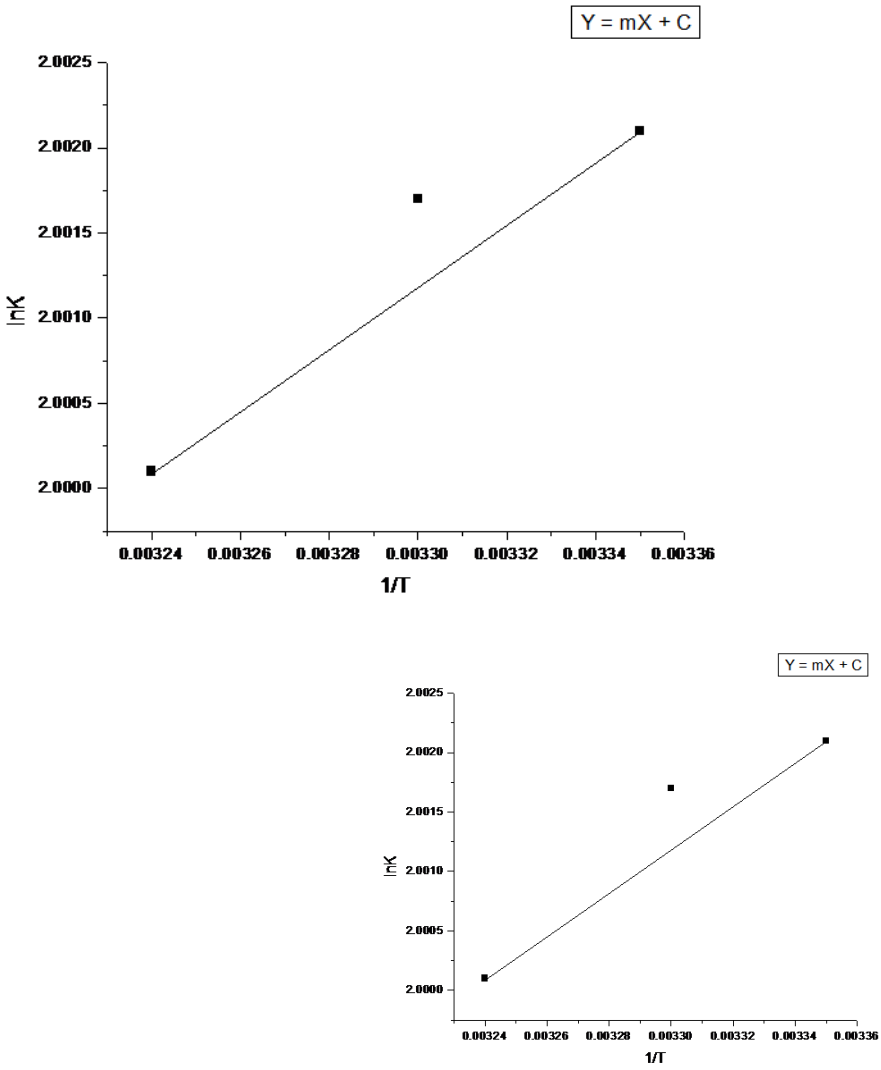

Fig. 6-Graph plotted between lnk Vs 1/T

Table 3: Values of enthalpy, free energy, entropy at different temperature

Sr. No.	Temp. (K)	Enthalpy	Free energy	Entropy
1	298		-4.961 KJ/Mol	
2	303	-151.16 J/Mol	-5.039 KJ/Mol	16.13 J/K.Mol
3	308		-5.119 KJ/Mol	

At all the temperature the value of free energy is negative indicating spontaneous reaction between drug and BSA. Negative value of Enthalpy shows the binding process is exothermic and positive value of entropy indicates unfolding of BSA. For unfolding process must be endothermic but as enthalpy negative so process is exothermic, it shows that value of enthalpy is low negative value indicating electrostatic force of attraction between drug and BSA for binding. Negative value of enthalpy also shows the possibility of Vander Waals forces and hydrogen bonding.

1. Conclusion:

Ultrasonic technique shows simple and effective method for the interaction of 2CA3MBAwith BSA. 2CA3MBA show antibacterial activity and efficiency towards the BSA. We studied the novel interaction of 2CA3MBA with BSA using ultrasonic interferometer technique. Study of interaction of

2CA3MBA at various temperature 25^0c, 30^0c and 35^0c shows the association constant (K_f) value is more at temperature 35^0c in comparison with temperature 25^0c and 30^0c. The values of association constant at temperature 25^0c 30^0c and 35^0c are found to be 0.5003, 0.5010 and 0.5011 respectively. It means that binding of 2CA3MBA with protein BSA is more efficient higher temperature. From VantHoffs plot the negative value of free energy indicates the process is spontaneous. Negative value of enthalpy showing process is exothermic which showing possibility that binding is due to Vander Waals forces and hydrogen bonding.

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