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IN VITRO BINDING STUDY OF 4HDDD TO BSA AT PHYSIOLOGICAL PH: ACOUSTICAL AND THERMODYNAMIC STUDY

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ABSTRACT:
The present study showed the binding interaction of diethyl-4-(4-hydroxyphenyl)-2, 6-dimethyl-1, 4-dihydropyridine-3, 5-dicarboxylate in 1, 4-dioxane, DMSO and DMF to the bovine serum albumin (BSA) by acoustical study at physiological pH and its molecular modeling. Findings were interpreted by scatchard plot which showed an increase in association constants with increasing temperature and concentration. Binding supposed to be more in 1, 4-dioxane than DMSO and DMF, which may be due to aprotic and non-polar nature 1, 4-dioxane. The free energy (ΔG), enthalpy (ΔH) and entropy (ΔS) values were calculated from van't Hoff equation. The negative ΔG showed the spontaneous process and positive values of ΔH and ΔS showed endothermic interaction between drug and BSA. ΔG becomes more negative with increase in temperature, indicated feasibility of binding interaction at high temperature. The positive value of ΔH and ΔS also showed specific electrostatic and hydrophobic interaction of drug and BSA. Molecular modeling confirmed the binding interaction showing energy -217.66 kJ/mol, which concluded the stable complex formation between drug and BSA.

KEYWORDS: Acoustical study, molecular modeling, Scatchard analysis, association constants, BSA.

1. INTRODUCTION:

Human serum albumin (HSA) is the most abundant protein in blood serum with the concentration of 0.63 mM. It is single polypeptide chain of 585 amino acids with a large helical triple domain structure that forms heart shaped molecule. HSA binds a relatively a number of insoluble endogenous drugs such as fatty acids, billirubin, etc. and it facilitates their transport to target tissues. Human serum albumin shows various pharmacological importances, because of its broad interactions and abundance. The structure of HSA explains numerous physiological phenomena and provides further insight in pharmacokinetics[i]. A

variation in temperature is found to be a key factor in binding affinities of HSA[ii], as evident from the drugs Ligustrazine[iii], Ciprofloxacin[iv], methotrexate[v] and cisplatin[vi]. 1, 4-dihydropyridine derivatives shows a wide range of biological activities and medicinal properties[vii,viii]. Diethyl -4-(4-hydroxyphenyl) -2, 6- dimethyl-1, 4-dihydropyridine-3, 5-dicarboxylate (4HDDD) is one of the pyridine derivatives showing antimicrobial and anticonvulsant activities[ix]. A drug binding influences the metabolic activity of target tissues so the binding interactions between drugs and plasma protein are important to understand the pharmacokinetics and pharmacodynamics. Functional and physiological properties of these proteins extensively studied over decades[x]. In BSA varying binding sites are available for ligands[xi, xii]. Various techniques are available to monitor the binding interactions of ligands to protein like NMR[xiii], isothermal titration calorimetry[xiv], U.V. visible absorbance[xv], fluorescence[xvi], equilibrium and FT-IR[xvii] and CD spectroscopy[xviii]. Molecular modeling also shows important aspects about protein-drug interaction[, xix, xx,xxi], It is difficult to obtain HSA for experimental purposes. As HSA and BSA exhibit similar chemical properties, BSA in lieu of HSA was used in this study because of low cost and easy availability. In the view of above considerations, present study demonstrate the effect of drug concentrations, different temperatures and polar/non polar solvent on binding interaction of 4HDDD to BSA at physiological pH by acoustical properties along with thermodynamic parameters like free energy, enthalpy, entropy and molecular modeling study.

2. EXPERIMENTAL:

Materials and Methods: Ultrasonic interferometer (VI Microsystems, Chennai, India). BSA procured from Chemsworth Chemicals Ltd (India), drug 4HDDD, software HEX 8.0, basic buffer (7.4). For the synthesis, all the reagents used were of A.R. grade purchased from Merck India Limited.

Optimization study:

4HDDD was insoluble in basic buffer at physiological pH. Hence mixture of buffer with non aqueous solvent such as 1, 4-dioxane, DMF and DMSO were used to dissolve 4HDDD. Different ratio of buffer: non-aqueous solvents were tried, but the complete solubility of 4HDDD was obtained at optimum ratio 30: 70:: non-aqueous solvent: buffer.

Preparation of 4HDDD:

Diethyl-4-(4-hydroxyphenyl)-2, 6-dimethyl-1, 4-dihydropyridine-3, 5-dicarboxylate (4HDDD) synthesized by known method[xxii]. 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 recorded using digital melting point apparatus Equiptronics (EQ 730). 1H NMR spectrum of the compound recorded in $CDCl_3$ on NMR instrument (500MHz) using TMS as an internal standard from SAIF, CDRI Lucknow, India. UV spectra recorded on BioEra's spectrophotometer and FT-IR spectra on Brukers alpha at Jankidevi Bajaj college of science wardha, MS., India.

Measurement of binding affinity:

For the Scatchard analysis, binding affinity of BSA and 4HDDD is expressed as an equilibrium constant or association constant which is derived from the law of mass action. BSA (B) interacts with the 4HDDD (L) to form the complex is given as

$$B + L \rightarrow [BL]$$

Hence, association constant $K_a = \frac{[BL]}{[BL]+[B]}$

Binding strength of the ligand to BSA is a measure of association constants.

Ultrasonic study

Ultrasonic is a versatile non-destructive and highly investigatory technique. Ultrasonic absorption in a medium provides important tools for evaluation of the structural, chemical and physical properties of medium[xxiii]. Initially ultrasonic interferometer set at 1MHz. Different concentrations (1×10^{-3} to 3.5×10^{-3} M) of 4-HDDD in different solvents (1, 4-dioxane, DMSO, DMF (30:70:: solvent: buffer) were prepared at physiological pH. 0.15μM BSA also prepared at same pH and its ultrasonic velocity was measured. Different concentrations of 4HDDD mixed with BSA at 298K and allowed to stand for 1 hr for maximum binding. The ultrasonic velocities of complex solutions were recorded. Similar steps were performed at 303 and 308K and specific binding along with association constants were determined using Scatchard plot.

Molecular modeling study

Molecular modeling of BSA with 4HDDD was carried out on Hex 8.0 software. This gives value of an efficient energy. PDB file of the crystal structure of BSA obtained from the RCSB data bank having ID 4F5S and 3D structure for 4HDDD was developed. Initially, the structure of 4HDDD has been drawn using Chem Draw and its 3D structure is developed. The obtained 3D structure arranged in a minimized energy form. The PDB files runs on Hex 8.0 which gave the energy value of the newly formed complex showing its stability.

3. RESULT AND DISCUSSION:

Ultrasonic study:

In present study, ultrasonic velocities of 0.15 μM BSA in 1, 4- dioxane was measured at temperature 298, 303 and 308K. The ultrasonic velocities are 1496.899, 1503.163 and 1505.189 m/s respectively. Ultrasonic velocity of 4HDDD-BSA complex was also measured at varying concentrations and temperatures (Table 1). The scatchard graph is plotted for specific binding versus percent ligand fraction and from this plot binding parameters of 4HDDD to BSA have been determined. The Scatchard analysis gives different association constants at different temperatures. The association constants in 1, 4-dioxane are 0.5011 (±0.0005), 0.5018 (±0.0005) and 0.5027 (±0.0005) at temperature 298, 303 and 308K respectively. Similar analyses were also carried out in DMSO and DMF and association constants have been calculated. The association constants in DMSO and DMF are 0.5011 (±0.0005), 0.5008 (±0.0005), 0.5007 (±0.0005) and 0.5006 (±0.0005), 0.5007 (± 0.0005), 0.5008 (±0.0005) at temperature 298, 303 and 308 K respectively. The association constants for 4HDDD-BSA binding increases as the temperature increases. This increase association constants clearly indicates the exothermic nature of reaction. This supports the interaction of 4HDDD with BSA by means of van der Waals interactions and hydrogen bonds in the hydrophobic packet of binding sites. It is also observed that the binding affinity increases with increase in concentration of the drug; this probably enhances the pharmacological activity of the drug. The scatchard plot given below represents binding affinities of 4HDDD with BSA at different temperatures. Figures 1 to 3 shows the Scatchard plots of BSA-4HDDD binding in 1, 4-dioxane, DMSO and DMF respectively. The effect of temperature on BSA-4HDDD binding is summarized in van't Hoff equation.

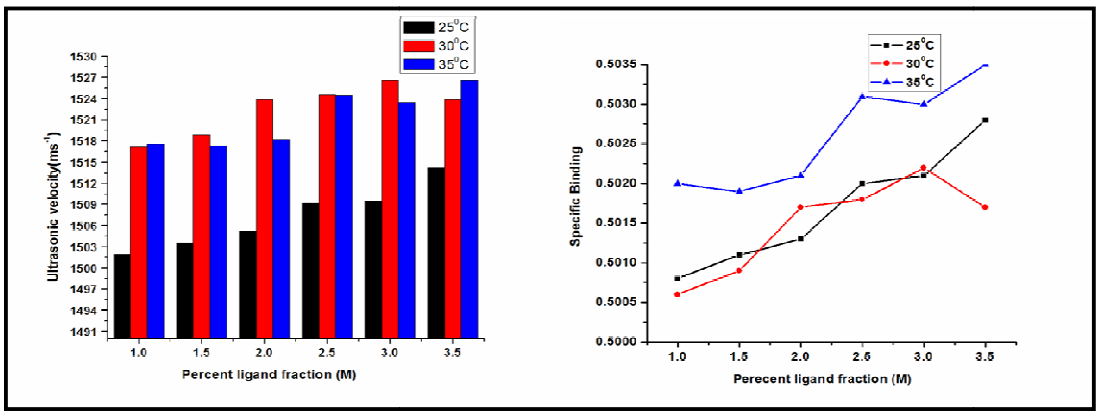

Figure 1: Graph of ultrasonic velocities and specific binding vs. conc. of 4HDDD in 1, 4 - dioxane.

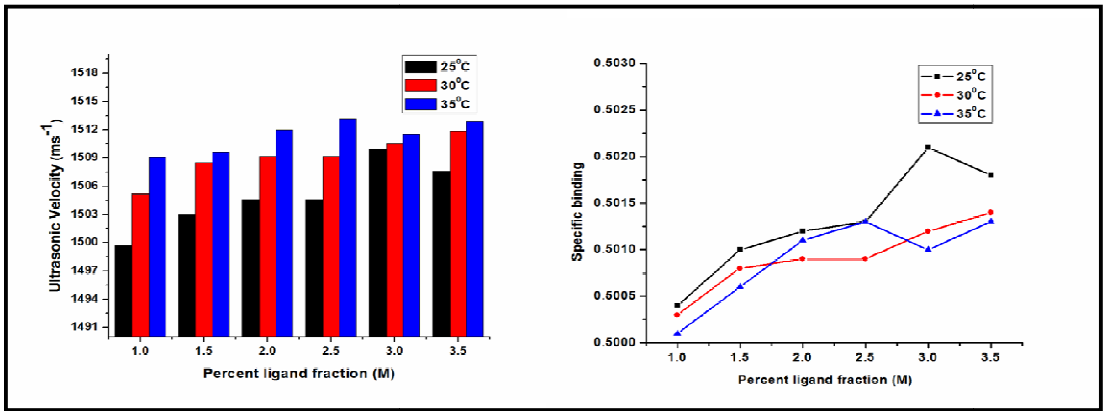

Figure 2: Graph of ultrasonic velocities and specific binding vs. conc. of 4HDDD in DMSO.

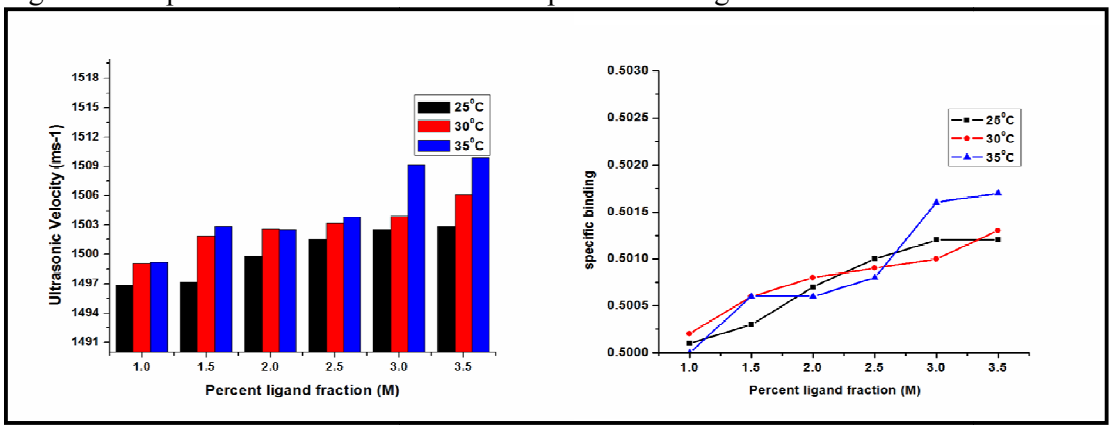

Figure 3: Graph of ultrasonic velocities and specific binding vs. conc. of 4HDDD in DMF.

Table 1: Ultrasonic velocities of 4HDDD-BSA complexes at different conc. and temperatures

Temp Conc.	**1,4 Dioxane**			**DMSO**			**DMF**		
	25^{0}C	30^{0}C	35^{0}C	25^{0}C	30^{0}C	35^{0}C	25^{0}C	30^{0}C	35^{0}C
1	1496.894	1499.083	1499.187	1499.696	1505.189	1506.091	1501.921	1517.167	1517.566
1.5	1497.17	1501.8	1502.8	1502.9	1508.5	1509.6	1503.5	1518.8	1517.27

	3	76	60	59	29	14	66	60	7
2	1499.850	1502.576	1502.559	1504.614	1509.189	1511.959	1505.277	1523.860	1518.211
2.5	1501.499	1503.277	1503.835	1504.614	1509.189	1513.163	1509.211	1524.534	1524.503
3	1502.529	1503.876	1509.167	1509.959	1510.512	1511.519	1509.503	1526.559	1523.503
3.5	1502.870	1506.085	1509.876	1507.594	1511.836	1512.876	1514.191	1523.860	1526.534
BSA	1495.229	1497.697	1499.085	1496.899	1503.269	1505.189	1496.899	1503.163	1517.566

Thermodynamic study

In order to elucidate the interaction of 4HDDD with the BSA, the thermodynamic parameters (ΔG, ΔH and ΔS) have been calculated from van't Hoff equation at the temperatures 298, 303 and 308 K. The enthalpy change is calculated from the slope of the van't Hoff relationship.

$$\ln k = -\frac{\Delta H}{RT} + \frac{\Delta S}{R} \quad \text{————} \quad (1)$$

Graph plotted between **lnk vs 1/T** shows straight line with positive slope (figure 4).

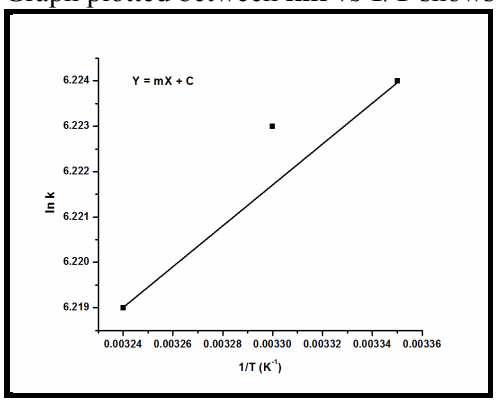

Figure 4: Graph of lnk vs 1/T in 1,4dioxane

Table 2: Thermodynamic parameters at different temperature in 1, 4-dioxane.

Sr. No.	**Temp.** (k)	**ΔH** J/mol	**ΔG** kJ/mol	**ΔS** J/mol
1	298 k	332	-14.735	50.56
2	303 k		-14.988	
3	308 k		-15.240	

Positive values of ΔH and ΔS indicates that drug interaction with BSA are enthalpy and entropic driven. Positive value of entropy indicates that there is unfolding of BSA. For unfolding, process must be endothermic which is indicated by positive values of enthalpy and entropy (table 2). The specific electrostatic interaction is also characterized by the positive values of enthalpy and entropy. The negative value of ΔG indicates that the 4HDDD-BSA complexation is a spontaneous process. As the temperature increases the negative value of ΔG is also increases, which concluded the 4HDDD-BSA interaction is more feasible at high temperature. So, the hydrogen bonding, electrostatic and hydrophobic interactions are supposed to be possible factors contributing binding of 4HDDD to BSA. The thermodynamic parameters in DMSO and DMF are found to be close but not significant with respect to 1, 4 dioxane.

Molecular modeling study

Molecular modeling is also an efficient method for measurement of interaction between protein and drug. The binding interaction between BSA and 4HDDD was also studied by molecular modeling. The obtained energy is a measure of binding of 4HDDD to BSA. The energy obtained is -217.66 kJ/mol, shows an efficient binding of 4HDDD with BSA. Diagrammatic representation of interaction between BSA and 4HDDD is as shown in figure 5.

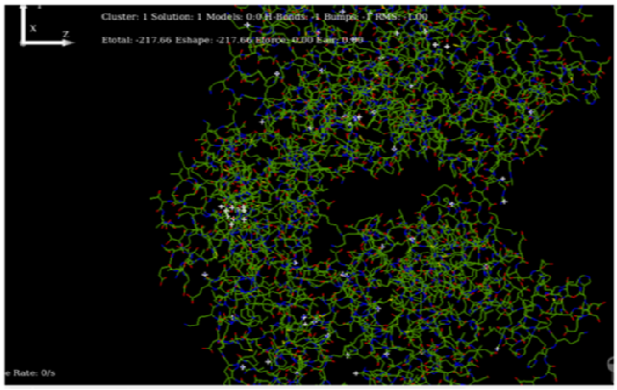

Figure 5: Molecular modeling interaction between BSA and 4HDDD.

4. CONCLUSION:

Current investigation was an attempt to study the interaction of diethyl-4-(4-hydroxyphenyl)-2, 6-dimethyl-1, 4-dihydropyridine-3, 5-dicarboxylate in 1, 4-dioxane, DMSO and DMF to BSA by acoustical method at physiological pH and molecular modeling. It is found that binding is more significant in 1, 4-dioxane as compared to DMSO and DMF. It may be due to aprotic and non polar nature of the 1, 4-dioxane. The scatchard plot at all temperatures found to be a non-linear indicating the presence of at least two binding sites on BSA. The values of thermodynamic parameters indicate that hydrogen bonding, electrostatic and hydrophobic interactions induce alterations in secondary structure of the BSA. Molecular docking also supports the binding having energy -217.66 kJ/mol, concluding the stable binding of 4HDDD with BSA.

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