Studies Of Bovine Serum Albumin In Aqueous Solutions Of Some Salts Investigated By Ultrasonic Velocity, Viscosity And Density Measurements At 303.15K.

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Abstract:- Ultrasonic velocity (u), densities (ρ) and viscosities (η) of (BSA) have been measured in the protein concentration range between 0.00125 to 0.04 g cm^{-3} at 303.15 K in water and water containing fixed concentration 0.1480 mol dm^{-3} of salts like potassium oxalate , sodium benzoate, cupric acetate, ammonium thiocyanate. The variation of u, ρ, η and isentropic compressibility (K_s) as a function of protein concentration (C) in all cases is non linear. The relaxation time in all these cases increases with increase of BSA concentration which indicates that there is strong interaction of protein with these salts .The values of u, ρ and η increase with increasing concentration of BSA.

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INTRODUCTION

Although some efforts has been made in the literature to interpret the behaviour of protein in aqueous solutions by using different techniques but a little attempt has been made to investigate their behaviour in water containing some salts. The nature and strength of these interactions in the case of mixed solvents can be well understood by studying some solvent properties such as excess thermodynamic functions, viz; excess isentropic compressibility, excess molar volume, excess enthalpy and excess heat capacity. A thorough survey of literature reveals that a large number of physico-chemical studies of proteins have been made in water and in some other solvent systems with the intention to investigate the interactions of proteins with water [1,2], metal ions [3], urea [4,5], sodium dodecyl sulphate [6-9], guanidine hydrochloride [10], sulphonamide [11],sodium lauryl sulphate [12], tetrabutylammonium iodide [12] and dextrose [13]. The denaturation of protein with urea, substituted urea and sodium dodecyl sulphate and guanidine hydrochloride has been investigated. During the last several years emphasis has been laid on the ultrasonic absorption and dielectric relaxation studies of this protein. But ultrasonic velocity measurements of BSA in water containing some salts are still lacking. In the present note, therefore, we report ultrasonic velocities, isentropic compressibilities and viscosities of BSA in different water + organic solvent mixture. BSA was chosen for these studies because it has served as a model in many physicochemical as well as in chemical studies. Its solubility, stability and tendency to bind with many reagents was an additional advantages for carrying out these studies.

EXPERIMENTAL

Doubly distilled conductivity water was used throughout. By using a long vertical fractionating column containing ion-exchange resins (supplied by Ion-exchange India Ltd), it was distilled twice over acidified $KMnO_4$. Potassium oxalate 98%, Sodium benzoate 98%, Cupric acetate 97.5%, Ammonium thiocyanate 99% purity (all from S.D. Fine Chemicals, Mumbai). BSA fraction-V crystal was also obtained (from Sisco Laboratories, Mumbai). These salts were used without further purification. Desired concentration of BSA were prepared by weighing the protein and dissolving it in the appropriate volume of water or in desired salt solution.Vigorous stirring was avoided to prevent foam formation during preparation of protein solution in all these cases. The ultrasonic velocities (u) at 2 MHz frequency and densities (ρ) of various protein solutions were measured by using an Anton-Paar DSA 5000 density and sound analyzer instrument. DSA 5000 is the first oscillating U-tube density (accuracy, 0.000005 g cm^{-3}) and ultrasonic velocity (accuracy 0.5 m s^{-1}) meter which measures this parameter with the highest accuracy in wide viscosity and temperature ranges. Viscosity of

various protein and salt solutions were measured using an Ubbelohde suspended-level viscometer. Measurements were repeatedly made to check there producibility of results. The overall accuracy of viscosity measurements was ± 0.01%. The viscosity of the filled sample is measured by damping of the U-tube oscillation. The DSA 5000 automatically corrects the viscosity related errors in the density

All physicochemical measurements were made in a water thermostat bath maintained at 303.15± 0.01K.

RESULTS AND DISCUSSION

Ultrasonic velocity (u), densities (ρ) and viscosities (η) of BSA have been measured in the protein concentration range between 0.00125 to 0.04 g cm^{-3} at 303.15 K in water and water containing fixed concentration i.e 0.1480 mol dm^{-3} of salts like potassium oxalate $(COOK)_2H_2O$, sodium benzoate (C_6H_5COONa), cupric acetate Cu $(CH_3COO)_2.H_2O$, ammonium thiocyanate (NH_4SCN). The plots of ultrasonic velocity (u) versus BSA concentration (C) in the presence of fixed amount of salts are given in Fig. 1. The corresponding plots of density (ρ) and viscosity (η) for these case i.e fixed amount of salts are shown in figs. 2 and 3 respetively.

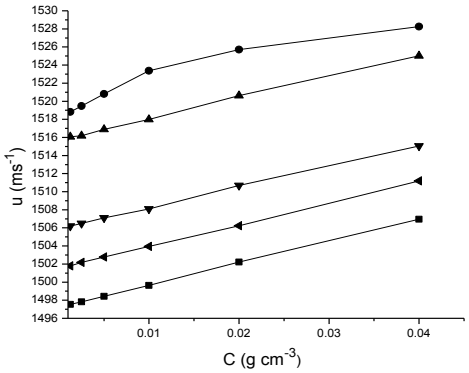

Fig. 1 Plot of ultrasonic velocity (u) vs BSA concentration (C) in water containing 0.1480 mol dm^{-3} of different salts at 303.15K. ■ water + BSA, ● potassium oxalate + BSA, ▲ sodium benzoate+ BSA, ▼ cupric acetate+BSA, ammonium thiocyanate + BSA.

All these plots are non-linear. The plots of ultrasonic velocity (u) versus molality (m) in the cases of many electrolytes in water and organic solvents on the other hand, were observed to be linear[15-16]. Similarly the plots of (ρ) versus m and η versus m for most of the electrolyte were also linear.

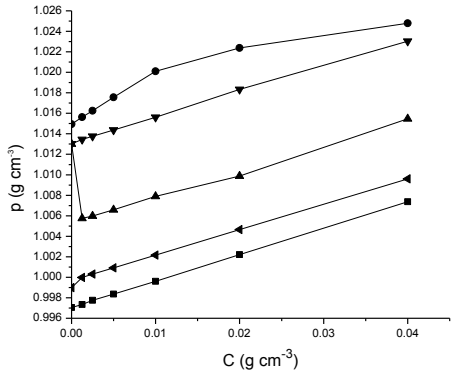

Fig. 2 Plot of density (ρ) vs BSA concentration (C) in water containing 0.1480 mol dm^{-3} of different salts at 303.15K. ■ water + BSA, ● potassium oxalate + BSA, ▲ sodium benzoate+ BSA, ▼ cupric acetate+BSA, ammonium thiocyanate + BSA.

The non- linearity of the plots of u versus BSA concentration shows that ultrasonic behaviour of protein in these solvent systems is not as simple as that of many electrolytes. The non linearity of the plots especially of u versus concentration of protein shows strong structural changes in the presence of salts. By using ultrasonic velocity (u) and density data for various solutions, the isentropic compressibility (K_s) for various solutions was calculated by using the equation $K, =1/u^2P$. (1)

The plots of K_s versus BSA concentration (C) are shown in Fig. 4.The K_s values for the salts investigated decreases in the order: potassium oxalate, sodium benzoate, cupric acetate ammonium thiocyanate.

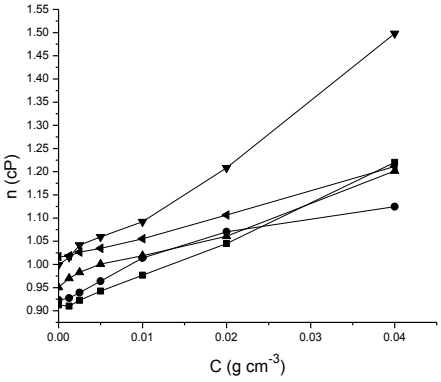

Fig. 3 Plot of viscosity (η) vs BSA concentration (C) in water containing 0.1480 mol dm^{-3} of different salts at 303.15 K. ■ water + BSA, ● potassium oxalate + BSA, ▲

sodium benzoate+ BSA, ▼ cupric acetate+BSA, ammonium thiocyanate + BSA.

The isentropic compressibility of the protein solutions decreases, also non linearly in all the cases with the increase in protein concentration. The decrease in K_s value in all the cases with the increase in protein concentration indicates increased structural effect in solution with the increase in protein concentration. These structural effects arise from the structural changes of the protein by the interaction of protein with these salts. . It is seen that a very interesting effect is obtain from Fig.4.The K_s values for that cupric acetate and ammonium thiocyanate fall on the more negative value side. More negative K_s value is due to more structural effects.

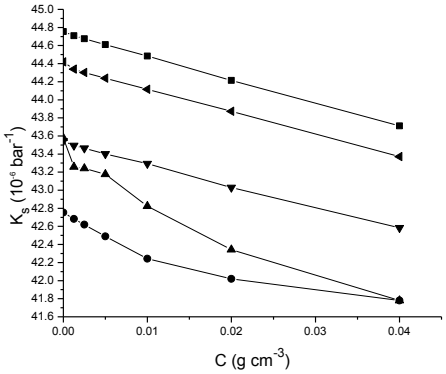

Fig. 4 Plot of isentropic compressibility (K_s) vs BSA concentration (C) in water containing 0.1480 mol dm^{-3} of different salts at 303.15 K. ■ water + BSA, ● potassium oxalate + BSA, ▲ sodium benzoate+ BSA, ▼ cupric acetate+BSA, ammonium thiocyanate + BSA.

When BSA denaturates or dissociates it occupies more volume of space and due to increased volume more structure are produced. On the other hand when BSA interacts with salts , structure becomes more compact only in certain region of solutions and the K_s value will be more positive or less negative. In the higher protein concentration range, all salts shows stabilisation effects on BSA with the result protein shows no denaturation effect. Similar results are also obtain from viscosity measurements in Fig.3 it shows the variation in viscosity with various salts. When BSA dissociates in the presence of the ions, it occupies more volume of space, then due to increased volume more structure are produced which causes increase in viscosity. Ammonium ion plays an important role in the interaction with BSA. This ion has mainly hydrophobic interactin with protein, so has strong effect on BSA due to hydrogen bonding enhancement. This enhancement is consistent with the ultrasonic velocity (u) , density (ρ) and viscosity (η) for many electrolytes in water and organic solvents.

Relaxation time (τ) is the most interesting parameter from which better information regarding the behaviour of BSA in the protein solutions can be obtained. The relaxation times have been computed from the relation $r = 4/3\ (nk_s)$ (2)

The results of relaxation times (τ) as a function of protein concentrations in the presence of fixed amounts of different salts are plotted Fig.5. These results show that the relaxation times (τ) increase in all the cases with increase in BSA concentration in the presence of fixed concentration of salts. This indicates strong interaction of the salts with the protein BSA. The plot indicate that sodium benzoate, cupric acetate, show stronger interaction with BSA.

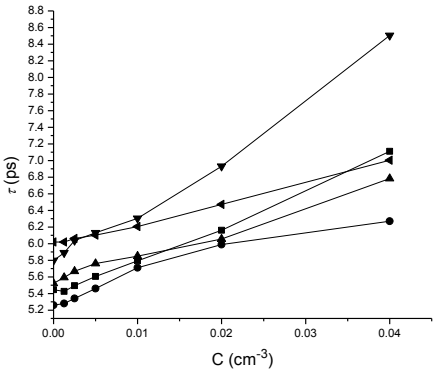

Fig. 5 Plot of relaxation time (τ) vs BSA concentration (C) in water containing 0.1480 mol dm^{-3} of different salts at 303.15 K. ■ water + BSA, ● potassium oxalate + BSA, ▲ sodium benzoate+ BSA, ▼ cupric acetate+BSA, ammonium thiocyanate + BSA.

The larger value indicates more structural effect due to strong interaction among protein and solvent molecule in these systems.The relaxation time of dispersion of BSA in various system increases with increase in protein concentration. But in most of the cases it remains in between 5 to 7 ps.

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