DENSITY, ULTRASONIC VELOCITY AND VISCOSITY STUDIES OF BOVINE SERUM ALBUMIN IN AQUEOUS SOLUTIONS OF SOME ELECTROLYTES AT 298.15 K

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ABSTRACT

Densities (ρ), ultrasonic velocities (u) and viscosities (η) of solutions of bovine serum albumin (BSA) in concentration range 0.00125 to 0.04 g cm^{-3} at 298.15 K in water and water containing 0.1480 mol dm^{-3} of tri-sodium citrate ($Na_3C_6H_5O_7.2H_2O$), ammonium sulfate $(NH_4)_2SO_4$, and ammonium thiocyanate (NH_4CNS) have been measured . The same measurements have also been carried out of solutions containing 0.02 g cm^{-3} of BSA in water and the concentration of the salts (c) was varied in the range between 0.0125 mol dm^{-3} to 0.4 mol dm^{-3}. From the densities (ρ), ultrasonic velocities (u) and viscosities (η) of solutions the isentropic compressibilities (K_s) and relaxation times (τ) in all these aqueous systems have been estimated. Both isentropic compressibility (K_s) and ultrasonic velocity (u) gives linear plots with protein concentration indicating strong structural changes of the protein in all these aqueous systems. The relaxation time (τ) and viscosity (η) results show an increasing trend in their values with increasing tri-sodium citrate ($Na_3C_6H_5O_7.2H_2O$), ammonium sulfate $(NH_4)_2SO_4$, and ammonium thiocyanate (NH_4CNS) and BSA concentration.

Keywords: Viscosity, Bovine Serum Albumin (BSA), Trisodium citrate, Ammonium sulfate and Isentropic compressibility.

INTRODUCTION

The interaction of protein with metal ions, water, some organic solvents, including organic compounds, and sodium dodecyl sulphate and other surfactants has been investigated. Lot of measurements such as viscosity , ESR, dielectric relaxation at microwave frequency, pressure jump, ultrasonic absorption, NMR, diffusion coefficient and dilatometry require special attentions [1-3]. A large no. of physico-chemical investigations of bovine serum albumin (BSA) have been made in water and with other system under various conditions of ionic strength, pH, temperature and in the presence of different concentration of electrolytes [4-6]. Protein denaturation with sodium dodecyl sulphate, urea, substituted urea, and guanidine hydrochloride has been investigated. Gill and coworkers measured the ultrasonic velocities, isentropic compressibilites and viscosities of bovine serum albumin in water and water containing organic solvents, sodium lauryl sulphate and tetrabutylammonium iodide at 310 K in the protein concentration range 0.0022 to 0.0430 g cm^{-3} [7]. These authors also continued their studies and measured absorption coefficients in 10-100 MHz frequency range for 10 sugars, 3 polysaccharides, 5 amino acids, 2 amino acid mixtures, 15 native proteins, 7 denaturated proteins and 2 depolymerised proteins. Absorption increased with increasing molecular weight only in restricted molecular weight range, was indicated by the analysis of resulting data The data also indicated that in linear proteins it was dependent upon the amount of α-helix content whereas [absorption in globular proteins was insensitive to their structural characteristics [8].

Some work have been done to investigate their nature in aqueous solutions containing transition metal and inorganic metal salts. Kupke and Hodgins reported first result for the measurment of density and viscosity with a magnetic suspension instrument [10]. By using capillary viscometer Gaillard and Rovel measured the kinematic viscosity of purified plasma proteins [9]. The study of denaturation of bovine serum albumin was done by Ayranci et al. by urea in aqueous solutions by using the methods of apparent molal volume and viscosity at 298K [3]. In another study Barnes et al. measured ultrasonic absorption of the globular protein bovine serum albumin in aqueous solutions in the frequency range 60-160 KHz by using a spherical resonator [12]. Gill et al. studied the behaviour of bovine serum albumin in aqueous solution of some salts of organic acids tetraethyl ammonium bromide and dextrose by studying their ultrasonic velocity, viscosity and density measurements [12]. Takeda found

a single relaxation in the system of SDS and bovine serum albumin and SDS using a pressure jump method with conductance detection [13]. The studies have been done in the concentration range of 0.01 to 0.40 mol dm-3 at 308.15K [8]. The relaxation was observed in the concentration range of the surfactant below the critical micelle concentration. The relaxation time becomes fast with the stepwise formation of a complex between. Bae Jong Rim Chang, Seung Hycm studied the ultrasonic absorption in bovine serum albumin (BSA) in aqueous isolation (50g/L) in the frequency range from 100 kHz to 1600 MHz at neutral pH. Evans et al. studied the effect of solvation of the ultrasonic absorption of bovine serum albumin solutions [1]. SDS and albumin and finally attains the values compatible with those in the system of the pure surfactant micelle. In another study Barnes et al. measured ultrasonic absorption of the globular protein bovine serum albumin in aqueous solutions in the frequency range 60-160 KHz by using a spherical resonator [8]. Different measurement techniques have been used to get the correct information about the nature, strength and interactions present in the proteins. ultrasonic velocity, density, viscosity, isentropic compressibility and relaxation time measurement of protein with any system are used to find that whether there is protein-protein, protein-solvent or solvent- solvent interactions are present in the system. These interactions give an idea of structural and dynamic features of these proteins and show a greater dependence of volumetric data on the hydrophobicity as compared to other protein. This work also helps to understand nature of interactions of BSA in aqueous solutions of tri-sodium citrate ($Na_3C_6H_5O_7.2H_2O$), ammonium sulfate $(NH_4)_2SO_4$, and ammonium thiocyanate (NH_4CNS).

MATERIAL AND METHODS

Bovine serum albumin(BSA) (Fraction v. Fluka), tri-sodium citrate ($Na_3C_6H_5O_7.2H_2O$), ammonium sulfate $(NH_4)_2SO_4$, and ammonium thiocyanate (NH_4CNS) were purchased from Sisco Research Lab., Mumbai and used as received. Doubly-distilled water with conductivity $2\text{-}4\times10^{-7}$ Scm^{-1} was used for all measurements. Required concentrations of BSA were prepared by weighing the protein and dissolving it in the appropriate volume of water or in the desired salt solutions. Solution was not stirred vigrously to prevent foam formation during preparation of protein solution in all cases. The ultrasonic velocities and densities of various protein solutions were measured by using Anton Paar density and sound velocity analyzer DSA 5000 at 298.15 K.

Viscosities of various protein solutions were measured at the same temperature using an Ubbelohde suspended bulb viscometer having accuracy equal to 0.1%.

RESULT AND DISCUSSION

Tables shows an increase in the u, ρ and η with increase in BSA concentration in the former case (0.1480 mol dm^{-3} of salts) and also with increase in salt concentration (0.02 g cm^{-3} of BSA) in the latter case. This behaviour is due to the stronger interactions of salts with the globular BSA protein as compared to the protein-protein interactions. .The interactions shown by trisodium citrate and ammonium sulpate with the protein are much stronger as compared to the other salts. The interaction, as shown by the viscosity trend by cupric acetate under this condition is very high as compared to the other salts. This effect is probably due to the increase in the folding of the protein structure, making the structure more compact, resulting into the stabilization of the protein BSA in solution.Salts used in solutions were present in water in the range of 0.0125 to 0.4 mol dm-3 at 298 K. Ultrasonic velocity (u) density (ρ) and viscosity (η) of BSA solution have been measured in the protein concentration range between 0.00125 to 0.04g cm-3 at 298 k in water and water containing 0.1480 mol dm-3 salts of sodium benzoate, cupric acetate, trisodium citrate, ammonium sulfate, ammonium thiocyanate, with varying concentration of BSA and water containing 0.02 g cm-3 of BSA with varying concentration of above salts. In each case ultrasonic velocity (u), density (ρ) and viscosity (η), it found to increase with the increase in concentration of BSA or increasing concentration of salts in fixed amount of BSA as well in water. The value of these parameters in each case is reported in the table 1 to 11.

Ultrasonic velocity increases with the increases in BSA as well as salts concentrations, tables shows that strong interaction of BSA with salts. Values in tables of concentration Vs density shows that they follow the same trend as found in the ultrasonic velocity In aqoues system of BSA, aqueous system of different salts, with fixed concentration of salts and with fixed concentration of BSA, density is found to be increases with the increase in concentration which also shows that strong interaction of BSA with salts. The isentropic compressibilities (Ks) another derived

parameter which comes from the values of ultrasonic velocity and density. Ks of the protein solutions were calculated using the relation

$$K_S = 1/u^2\rho \qquad \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

The Ks values for cupric acetate is more than sodium benzoate. Decrease in Ks values in all the cases with increase in concentrations of protein and salt indicates structural changes in the globular protein BSA with increase in the respective concentrations. These structural changes may arise due to the interaction of the protein with different salts or due to protein-protein interactions. Plots of Ks versus molality (m) in the cases of many electrolytes are generally linear [8].

Table 1. Ultrasonic velocity (μ), density (ρ), viscosity (η), isentropic compressibility (K*s*) and relaxation time (τ) of BSA at different concentration in water at 298.15K.

$C\ (gcm^{-3})$	$\mu\ (ms^{-1})$	$\rho\ (gcm^{-3})$	$\eta\ (cp)$	$Ks\ (10^{-6}bar^{-1})$	$\tau\ (ps)$
0	1497.01	0.997055	0.9125	44.754	5.445
0.00125	1497.54	0.997347	0.9102	44.709	5.426
0.0025	1497.82	0.997747	0.9225	44.675	5.495
0.005	1498.44	0.998355	0.9426	44.610	5.606
0.01	1499.64	0.99961	0.9768	44.483	5.793
0.02	1502.22	1.002214	1.0451	44.215	6.161
0.04	1506.96	1.007378	1.2201	43.712	7.111

Table 2. Ultrasonic velocity (μ), density (ρ), viscosity (η), isentropic compressibility (K*s*) and relaxation time (τ) of trisodium citrate at different concentration in water at 298.15K.

$C\ (gcm^{-3})$	$\mu\ (ms^{-1})$	$\rho\ (gcm^{-3})$	$\eta\ (cp)$	$Ks\ (10^{-6}bar^{-1})$	$\tau\ (ps)$
0	1497.28	0.997004	89.37	44.740	5.330
0.0125	1500.62	0.999663	91.48	44.423	5.418
0.025	1503.14	1.00183	92.54	44.178	5.450
0.05	1507.94	1.006123	94.90	43.71	5.531
0.1	1518.81	1.015863	100.3	42.674	5.706
0.2	1537.98	1.033348	111.82	40.912	6.099
0.4	1576.54	1.067992	135.48	37.672	6.804

Table 3. Ultrasonic velocity (μ), density (ρ), viscosity (η), isentropic compressibility (K*s*) and relaxation time (τ) of ammonium thiocyanate at different concentration in water at 298.15K.

$C\ (gcm^{-3})$	$\mu\ (ms^{-1})$	$\rho\ (gcm^{-3})$	$\eta\ (cp)$	$Ks\ (10^{-6}bar^{-1})$	$\tau\ (ps)$
0	1496.97	0.997074	0.9184	44.755	5.480
0.0125	1497.33	0.997304	0.9169	44.723	5.467
0.025	1497.74	0.997528	0.9154	44.689	5.454
0.05	1498.48	0.997948	0.9133	44.626	5.434
0.1	1500.04	0.998839	0.9081	44.493	5.387
0.2	1503.03	1.000498	0.9011	44.243	5.315
0.4	1509.33	1.003887	0.8955	43.726	5.221

Table 4. Ultrasonic velocity (μ), density (ρ), viscosity (η), isentropic compressibility (K*s*) and relaxation time (τ) of ammonium sulfate in water at 298.15K.

$C\ (gcm^{-3})$	$\mu\ (ms^{-1})$	$\rho\ (gcm^{-3})$	$\eta\ (cp)$	$Ks\ (10^{-6}bar^{-1})$	$\tau\ (ps)$
0	1497.28	0.997004	89.37	44.740	5.342
0.0125	1500.62	0.999663	91.48	44.423	5.355
0.025	1503.14	1.00183	92.54	44.178	5.354
0.05	1507.94	1.006123	94.90	43.71	5.356
0.1	1518.81	1.015863	100.3	42.674	5.348
0.2	1537.98	1.033348	111.82	40.912	5.353
0.4	1576.54	1.067992	135.48	37.672	5.356

Table 5. Ultrasonic velocity (μ), density (ρ), viscosity (η), isentropic compressibility (K*s*) and relaxation time (τ) of BSA at fixed concentration (i.e. 0.02 g dm^{-3}) of ammonium thiocyanate at different concentration in water at 298.15K.

$C\ (gcm^{-3})$	$\mu\ (ms^{-1})$	$\rho\ (gcm^{-3})$	$\eta\ (cp)$	$Ks\ (10^{-6}bar^{-1})$	$\tau\ (ps)$
0	1496.97	0.997074	0.9184	44.755	5.480
0.0125	1497.33	0.997304	0.9169	44.723	5.467
0.025	1497.74	0.997528	0.9154	44.689	5.454
0.05	1498.48	0.997948	0.9133	44.626	5.434
0.1	1500.04	0.998839	0.9081	44.493	5.387
0.2	1503.03	1.000498	0.9011	44.243	5.315
0.4	1509.33	1.003887	0.8955	43.726	5.221

Table 6. Ultrasonic velocity (μ), density (ρ), viscosity (η), isentropic compressibility (Ks) and relaxation time (τ) of ammonium sulfate at different concentration in water at 298.15K.

$C\ (gcm^{-3})$	$\mu\ (ms^{-1})$	$\rho\ (gcm^{-3})$	$\eta\ (cp)$	$Ks\ (10^{-6}bar^{-1})$	$\tau\ (ps)$
0	1497.28	0.997004	89.37	44.740	5.342
0.0125	1500.62	0.999663	91.48	44.423	5.355
0.025	1503.14	1.00183	92.54	44.178	5.354
0.05	1507.94	1.006123	94.90	43.71	5.356
0.1	1518.81	1.015863	100.3	42.674	5.348
0.2	1537.98	1.033348	111.82	40.912	5.353
0.4	1576.54	1.067992	135.48	37.672	5.356

Table 7. Ultrasonic velocity (μ), density (ρ), viscosity (η), isentropic compressibility (Ks) and relaxation time (τ) of BSA at fixed concentration (i.e. 0.02 g dm^{-3}) of ammonium sulfate in water at 298.15K.

$C\ (gcm^{-3})$	$\mu\ (ms^{-1})$	$\rho\ (gcm^{-3})$	$\eta\ (cp)$	$Ks\ (10^{-6}bar^{-1})$	$\tau\ (ps)$
0	1538.94	1.023778	0.9674	41.243	5.320
0.00125	1539.57	1.023961	0.9659	41.201	5.306
0.0025	1539.96	1.024214	0.9722	41.170	5.336
0.005	1540.54	1.024823	0.9841	41.115	5.394
0.01	1541.98	1.025881	1.0103	40.996	5.522
0.02	1544.21	1.027983	1.0625	40.794	5.779
0.04	1548.87	1.033167	1.1857	40.345	6.378

Table 8. Ultrasonic velocity (μ), density (ρ), viscosity (η), isentropic compressibility (K*s*) and relaxation time (τ) of BSA at fixed concentration (i.e. 0.02 g dm^{-3}) of ammonium thiocyanate in water at 298.15K.

C (gcm^{-3})	*μ* (ms^{-1})	*ρ* (gcm^{-3})	*η* (cp)	*Ks* ($10^{-6}bar^{-1}$)	*τ* (ps)
0	1501.20	0.998984	1.0166	44.418	6.020
0.00125	1501.80	0.999975	1.0183	44.339	6.020
0.0025	1502.18	1.00031	1.0264	44.301	6.062
0.005	1502.78	1.000915	1.0347	44.239	6.103
0.01	1503.94	1.002167	1.0552	44.116	6.206
0.02	1506.24	1.004654	1.1064	43.872	6.472
0.04	1511.19	1.009598	1.211	43.372	7.003

Table 9. Ultrasonic velocity (μ), density (ρ), viscosity (η), isentropic compressibility (K*s*) and relaxation time (τ) of salt of trisodium citrate with fixed concentration of BSA (i.e. 0.02 g dm^{-3}) in water at 298.15K.

C (gcm^{-3})	*μ* (ms^{-1})	*ρ* (gcm^{-3})	*η* (cp)	*Ks* ($10^{-6}bar^{-1}$)	*τ* (ps)
0	1501.98	1.002247	1.0342	44.228	6.098
0.0125	1505.13	1.0047	1.0004	43.936	5.860
0.025	1511.01	1.011099	1.0204	43.318	5.893
0.05	1515.08	1.011544	1.0447	43.067	5.998
0.1	1523.83	1.021003	1.0971	42.179	6.170
0.2	1542.96	1.03822	1.2078	40.458	6.515
0.4	1583.07	1.073456	1.4762	37.172	7.316

Table 10. Ultrasonic velocity (μ), density (ρ), viscosity (η), isentropic compressibility (Ks) and relaxation time (τ) of salt of ammonium sulfate (i.e. 0.02 g dm^{-3}) with fixed concentration of BSA in water at 298.15K.

$C\ (gcm^{-3})$	$\mu\ (ms^{-1})$	$\rho\ (gcm^{-3})$	$\eta\ (cp)$	$Ks\ (10^{-6}bar^{-1})$	$\tau\ (ps)$
0	1501.99	1.002233	0.9988	44.228	5.890
0.0125	1503.71	1.003194	0.9981	44.085	5.866
0.025	1505.53	1.004381	1.0001	43.926	5.857
0.05	1508.77	1.006391	1.0047	43.650	5.847
0.1	1515.21	1.010435	1.0172	43.107	5.846
0.2	1526.92	1.017801	1.0409	42.141	5.848
0.4	1548.92	1.031520	1.0826	40.408	5.832

Table 11. Ultrasonic velocity (μ), density (ρ), viscosity (η), isentropic compressibility (Ks) and relaxation time (τ) of salt of ammonium thiocyanate with fixed concentration of BSA (i.e. 0.02 g dm^{-3}) in water at 298.15K.

$C\ (gcm^{-3})$	$\mu\ (ms^{-1})$	$\rho\ (gcm^{-3})$	$\eta\ (cp)$	$Ks\ (10^{-6}bar^{-1})$	$\tau\ (ps)$
0	1501.90	1.002179	1.0274	44.235	6.059
0.0125	1502.31	1.002407	1.0108	44.201	5.957
0.025	1502.62	1.002616	1.0076	44.174	5.934
0.05	1503.04	1.002997	1.0038	44.132	5.906
0.1	1504.83	1.003752	0.9969	43..994	5.848
0.2	1507.89	1.005433	0.9910	43.742	5.779
0.4	1514.10	1.008778	0.9875	43.241	5.693

Relaxation time (τ) is another interesting parameter from which better information regarding the behavior of BSA in the present solutions can be obtained. The relaxation times have been computed using the relat

$$\tau = 4/3(\eta.k_s) \quad \text{.....} \quad (2)$$

The relaxation time (τ) as a function of salt concentration is given in tables. It is evident from that the relaxation times increase in all cases with increase in BSA concentration in the presence of salts. This indicates strong interaction of the salts with the BSA protein. The similar studies involving the varying concentration of these salts in the presence of fixed amount of BSA, have also supported this trend i.e. the relaxation time of protein is again found to increase for both salts. In all the system the behaviour of viscosity is found to increase with increase in concentration of BSA and salts in water containing 0.01480 mol dm^{-3} of the salts and also in water containing 0.02 g dm^{-3} of BSA. But the system of ammonium thiocyanate shows somewhat different behaviour. Its viscosity found to decrease in aqueous system and salt containing 0.02g dm^{-3} of BSA with increasing salt conc. while in other cases viscosity increases with increase in concentration. The increase in viscosity value vs concentration in aqueous system and in inorganic metal salt solution indicates strong interaction of BSA with these metal salts. It shows strong protein- protein, protein- solvent and solvent-solvent interactions.

CONCLUSION

The variation of u, ρ and η as a function of protein concentration (C) in all cases are nonlinear indicating that BSA shows somewhat different behaviour as compared to simple molecule. The isentropic compressibility (Ks) and relaxation time (τ), dispersion of protein in all these system have been estimated. The isentropic compressibility of the protein also varies non-linearly with the protein concentration showing some conformational charges in the native protein in the presence of inorganic salts.The relaxation time in all these cases increase with increase of BSA concentration. The increase of relxation time with concentration indicates that there is strong interaction of protein with these salts ultrasonic velocity (u), density (ρ) and viscosity (η) shows an increasing trend in their values with increasing BSA and salts concentration. But in case of ammonium thiocyanate its viscosity

and relaxation time is found to decrease in aqueous medium and with fixed concentration of BSA indicating weak protein-solvent interactions.

ACKNOWLEDGEMENTS

Authors are thankful to UGC, New Delhi for providing financial assistance.

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