Physicochemical Studies of Globular Proteins—Bovine Serum Albumin, Egg Albumin, and Lysozyme—in Some Aqueous Iodide Salts Solutions of IA Group and Cetyltrimethyl Ammonium Bromide Systems

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ABSTRACT: Densities (ρ, kg m^{-3}), and viscosities (η, 0.1 kg m^{-1} s^{-1}) of Bovine Serum Albumin (BSA), Egg Albumin, and Lysozyme in aqueous iodide salts of lithium, sodium, and potassium, along with cationic surfactant-cetyltrimethyl ammonium bromide (CTAB) were measured at a temperature of 303.15 K. The 0.0010–0.0018 g %, w/v of each protein at an interval of 0.0002 mol L^{-1} in 0.2, 0.4, and 0.8 millimol L^{-1} of salt and CTAB are studied. Data are used for apparent molar volumes (V_ϕ, 10^{-6} m^3 mol^{-1}) and intrinsic viscosities ([η], dL kg^{-1}), respectively. Data are regressed and extrapolated to zero concentrations for ρ^0, η^0, and V_ϕ^0 limiting values and S_d, S_η and S_V corresponding slopes for protein–salt structural interactions. With size of cations, the densities decrease as CTAB > LiI > NaI > KI and increase with salts concentrations, with salts the densities are as Lysozyme > BSA > Egg Albumin, viscosities and V_ϕ as BSA > Egg–Albumin > Lysozyme. The ρ and η values with CTAB higher and [η] are lower and converse at around 0.4 mmol L^{-1} salt and is effective for greater stability of proteins. The [η] in CTAB are higher than other salts and decreases with size of cations with stronger intermolecular forces. J Appl Polym Sci 110: 2293–2304, 2008

Key words: globular proteins; survismeter; molar volume; intrinsic viscosity; conformation

INTRODUCTION

Physicochemical functions, densities, apparent molar volumes, and viscosities of proteins were measured with few members of halide salts of IA group cations[1–4] of modern periodic table of elements. The study illustrates a protein interactions profile[5,6] in aqueous LiI, NaI, and KI salts medium, and the studies with remaining members of the IA group, with similar proteins[2] were studied elsewhere.[2] The studies in combine are assumed to furnish a better understanding of salt–protein interactions, with salts of the IA group. To elaborate the studies, a member of quaternary ammonium salt-based surfactant CTAB is chosen to provide larger spectrum of behavior with other members of the surfactants. These interactions[1-3] of proteins in mixed solvents might be useful for several purposes.[7,8] As there is a scarcity of density, molar volumes, and viscosity data on various proteins in mixed solvents where our data may furnish useful information. Rheological,[9-12] viscosities along with densities depict the conformational and optimized states of biopolymers[2-7] in absence of external forces applied on the molecules during measurements. The protein's interaction along with salt play key role in biochemical and biophysical processes, for example BSA, Egg Albumin, and Lysozyme are important for several metabolic processes. Also the studies with K^+, Na^+ and Li^+, and CTAB are not reported earlier, which can focus conformational changes because of generation of torsional force on Newtonian flow.[13–15] The values of thermodynamic (ρ and V_ϕ) and transport (η and B) functions focus structural changes of biomolecules[6,16] because of hydrophilic and hydrophobic interactions. Reportedly van der Waals, intermolecular and electrostatic forces[16-21] are fundamental and elucidate water–protein–salt interaction. The salts crucially influence such forces, hence our studies are relevant because the ρ, η, V_ϕ^0, and [η] parameters infer better insight of structural changes because of formation and reorientation of ''protein–water–salt complex''[9] or ''solute–solvent'' and ''solute–cosolute–solvent'' interactions.[2,22-27] The proteins experience an effect of cations size on ionic interactions with them because of differential intermolecular and residual forces.[2] Such behavior remains effective in various physiological and biochemical activies[1] and

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could be extended to different streams as pharmacological, biophysical, and life science. The proteins molecule with alkali salts solution of lower concentrations and CTAB[28] are surrounded by an ionic atmosphere, with an excess of ions of charge, opposite to net charge of the protein molecule. Such arrangements in response to ions of low ionic strength[24] perhaps increase an internal pressure on protein molecules leading to shrinkage in the size.

EXPERIMENTAL PROCEDURE

The BSA (B 4287), Egg Albumin (A 5253), and Lysozyme (L 6876) and LiI, NaI, KI, and CTAB (AR, Sigma) before use were dried and stored for 24 h in P_2O_5 vacuum dessicator. Solutions (w/v) were prepared in deionised, triple distilled water (conductivity $1 \times 10^{-6}\ \Omega^{-1}\ cm^{-1}$) with $KMnO_4$ and KOH, and degassed by boiling off before use. Densities and flow times were measured with 20×10^{-3} dm^3 bicapillary pyknometer of 14.63453 g, and viscosities with Survismeter[29] at 303.15 K with ±0.01 K controls in temperature at 1 atm pressure. An efflux time with a digital electronic timer with 1.0×10^{-2} sec and weights were obtained with 0.01 mg Dhona balance model 100 DS. The solutions were thermostated for 30 min before weighing.

Calculations and overview of data

The ρ values were calculated from weights of the solutions measured with pyknometer like in our earlier communication,[2] and five parallel determinations were made for each solution assuming to be representative samples of an infinite number of observations. The mean value was determined to calculate standard deviation (σ) with the following equation,

$$\sigma = \sqrt{\frac{\sum_{i=1}^{n} (X_i - \bar{X})^2}{n-1}} \tag{1}$$

The σ values are treated with normal distribution, and a mean is located at the center of the distribution curve to estimate an accuracy of data. The measurements were taken approximately with 95.5% confidence level [$100 \times (1 - \alpha)$%], assuming $\alpha = 0.05$ and degree of freedom $\nu = \infty$, it infers an ''interval'' bound by −1.96 and +1.96. A probable error in individual ρ data was calculated by the following relations.

$$\sqrt{\left(\frac{W - W_0}{10^5}\right)^2 + \left(\frac{W_e}{10^5}\right)^2} \approx \pm S_n \tag{2}$$

$$\sqrt{\left(\frac{W_0 - W_e}{10^5}\right)^2 + \left(\frac{W_e}{10^5}\right)^2} \approx \pm S_v \tag{3}$$

$$\sqrt{\left(\frac{\pm S_n}{W - W_e}\right)^2 + \left(\frac{S_v}{V_{\text{pyk}}}\right)^2} \approx \frac{\Delta\rho}{\rho} \tag{4}$$

The V_ϕ data are computed from the ρ values with the help of Eq. (5).

$$V_\phi = 1/\rho(M - (100/g)(\rho - \rho_0)/(\rho_0)) \tag{5}$$

The M is molar mass of proteins in g mol^{-1}, concentration is in g %, the ρ and ρ_0 are solution and solvent densities, respectively, an error in V_ϕ data was computed from the following equation.

$$V_\phi(\text{Error}) = (100/g)\Delta\rho/\rho \tag{6}$$

The $\Delta\rho = \rho - \rho_0$, then η is calculated by Eq. (7).

$$\eta = ((\rho t)/(\rho_0 t_0))\eta_0 \tag{7}$$

The t and t_0 are the flow times, and η and η_0 are viscosities of solution and solvent, respectively, and relative viscosity, $\eta_{\text{rel}} = \eta/\eta_0$. The errors in η data were obtained as of the ρ values from eqs. (2)–(4) and the η data along with errors are given in Table I.

The values of ρ and η are in polynomial, and V_ϕ is linear with concentrations c, which depict the proteins in salt systems as weak electrolytes. The values on plots were extrapolated to zero concentration ($c \rightarrow 0$) to obtain their limiting values as shown below.

$$\rho = \rho^0 + S_d c + S_d' c^2 \tag{8}$$

$$V_\phi = V_\phi^0 + S_v \tag{9}$$

The ρ^0 is the limiting density and S_d and S_d' are the first and second degree slopes, likewise, the V_ϕ and η values were fitted for their limiting V_ϕ^0 and η^0 values along with corresponding S_v, S_η, and S_η' the first and second degree slopes, respectively. These regression constants are given Tables II–IV, which illustrate the solute–solvent and solute–cosolute–solvent interaction. T2–T4

The V_ϕ^0 denotes ion-solvent and S_v ion–ion/protein–protein interactions,[28,30,31] the η^0 the hydrodynamic interactions and the S_η and S_η'compositional effect of salts and proteins, respectively. The η_{rel} values are fitted to extended Jones–Dole equation.[32]

$$(\eta_{\text{rel}} - 1)/c = [\eta] + D_c \tag{10}$$

The [η] (mL g^{-1}) is intrinsic viscosity or Jones–Done coefficient and D (mL $g^{-1})^2$ is slope to measure pairwise interactions on Newtonian flow, their data

TABLE I
The ρ, η, and V_ϕ for BSA, Egg Albumin and Lysozyme with salts (KI, NaI, LiI, and CTAB) Calculated with Eqs. (5) and (7) and Ref. 2

Con. of protein (g %)	$\rho \pm 6 \times 10^{-5}$ (g cm^{-3})	$\eta \pm 6 \times 10^{-5}$ (10 g cm^{-3} s^{-1})	$V_\phi \pm$ (cm^3 mol^{-1})
BSA			
KI (0.0008*M*)			
0.0018	0.99584	0.71022	65284.76 ± 3.29
0.0016	0.99583	0.70555	65285.77 ± 3.40
0.0014	0.99582	0.70262	65287.72 ± 6.13
0.0010	0.99583	0.70013	65288.05 ± 4.41
KI (0.0004*M*)			
0.0018	0.99567	0.73596	65305.86 ± 3.29
0.0016	0.99568	0.73687	65305.35 ± 3.66
0.0014	0.99580	0.73763	65291.03 ± 6.26
0.0010	0.99574	0.73743	65302.02 ± 4.35
KI (0.0002*M*)			
0.0018	0.99586	0.70809	65282.93 ± 3.80
0.0016	0.99529	0.71979	65356.18 ± 3.46
0.0014	0.99535	0.72287	65352.51 ± 6.23
0.0010	0.99532	0.72160	65373.83 ± 4.45
Egg Albumin			
KI (0.0008*M*)			
0.0018	0.99507	0.82381	40161.95 ± 3.89
0.0016	0.99515	0.82795	40160.91 ± 3.86
0.0014	0.99512	0.82791	40160.77 ± 6.24
0.0010	0.99515	0.83186	40160.75 ± 4.48
KI (0.0004*M*)			
0.0018	0.99113	0.66598	40103.78 ± 3.19
0.0016	0.99109	0.66573	40097.59 ± 3.66
0.0014	0.99158	0.66508	40102.73 ± 6.53
0.0010	0.99115	0.66445	40169.04 ± 4.41
KI (0.0002*M*)			
0.0018	0.99786	0.68820	40268.64 ± 3.29
0.0016	0.99765	0.68997	40272.97 ± 3.43
0.0014	0.99785	0.69279	40275.83 ± 6.23
0.0010	0.99778	0.69425	40312.19 ± 4.45
Lysozyme			
KI (0.0008*M*)			
0.0018	0.99586	0.88731	29192.96 ± 3.29
0.0016	0.99586	0.87281	29190.69 ± 3.46
0.0014	0.99585	0.87277	29200.08 ± 6.83
0.0010	0.99583	0.87496	29217.20 ± 4.45
KI (0.0004*M*)			
0.0018	0.99606	0.69805	29530.59 ± 3.89
0.0016	0.99608	0.69790	29567.41 ± 3.86
0.0014	0.99598	0.69703	29560.37 ± 6.23
0.0010	0.99573	0.69970	29740.52 ± 4.45
KI (0.0002*M*)			
0.0018	0.99362	0.82266	29813.38 ± 4.45
0.0016	0.99364	0.82087	29889.27 ± 3.46
0.0014	0.99370	0.82330	29943.50 ± 6.33
0.0010	0.99363	0.83737	30188.96 ± 3.89
NaI (0.0008*M*)			
0.0018	0.99499	0.72627	65388.09 ± 3.46
0.0016	0.99498	0.72160	65395.07 ± 4.05
0.0014	0.99487	0.71862	65481.60 ± 3.89
0.0010	0.99531	0.71633	65373.80 ± 6.33
NaI (0.0004*M*)			
0.0018	0.99537	0.78346	65342.37 ± 3.89
0.0016	0.99537	0.78436	65346.54 ± 3.06
0.0014	0.99548	0.78515	65333.71 ± 6.23
0.0010	0.99543	0.78493	65351.98 ± 4.15
NaI (0.0002*M*)			
0.0018	0.99518	0.73079	65364.83 ± 3.46
0.0016	0.99550	0.74250	65444.74 ± 3.89
0.0014	0.99458	0.74555	65445.29 ± 4.65
0.0010	0.99464	0.74431	65485.54 ± 6.03
Egg Albumin			
NaI (0.0008*M*)			
0.0018	0.99388	0.96621	40173.62 ± 3.46
0.0016	0.99479	0.97055	40173.42 ± 3.09
0.0014	0.99383	0.97041	40174.39 ± 6.23
0.0010	0.99416	0.97441	40176.42 ± 4.55
NaI (0.0004*M*)			
0.0018	0.99544	0.64898	40153.95 ± 3.46
0.0016	0.99540	0.64875	40150.79 ± 3.89
0.0014	0.99589	0.64808	40160.24 ± 4.15
0.0010	0.99545	0.64745	40241.80 ± 6.33
NaI (0.0002*M*)			
0.0018	0.98991	0.69369	40389.52 ± 3.80
0.0016	0.98971	0.69547	40403.25 ± 3.46
0.0014	0.98876	0.69828	40416.77 ± 4.75
0.0010	0.98979	0.69973	40489.14 ± 6.73
Lysozyme			
NaI (0.0008*M*)			
0.0018	0.99598	1.07421	29295.30 ± 3.40
0.0016	0.99598	1.05976	29279.69 ± 3.79
0.0014	0.99597	1.05967	29331.74 ± 4.45
0.0010	0.99595	1.06185	29347.15 ± 4.75
NaI (0.0004*M*)			
0.0018	0.99658	0.71225	29613.50 ± 6.23
0.0016	0.99649	0.71205	29656.77 ± 3.46
0.0014	0.99649	0.71123	29658.56 ± 3.89
0.0010	0.99589	0.71388	29867.41 ± 4.45
NaI (0.0002*M*)			
0.0018	0.99547	0.96466	30539.81 ± 3.46
0.0016	0.99549	0.96077	30674.75 ± 3.09
0.0014	0.99555	0.96530	30808.53 ± 6.83
0.0010	0.99547	0.97737	31298.82 ± 4.85
BSA			
LiI (0.0008*M*)			
0.0018	0.99508	0.74232	652364.95 ± 3.36
0.0016	0.99508	0.73765	65370.69 ± 3.89
0.0014	0.99526	0.73462	65351.49 ± 4.75
0.0010	0.99593	0.73253	65254.67 ± 6.25
LiI (0.0004*M*)			
0.0018	0.99567	0.83096	65305.86 ± 3.46
0.0016	0.99568	0.83185	65305.35 ± 3.59
0.0014	0.99580	0.83266	65290.47 ± 4.25
0.0010	0.99576	0.83243	65298.69 ± 6.93
LiI (0.0002*M*)			
0.0018	0.99586	0.75349	65282.88 ± 3.16
0.0016	0.99529	0.76521	65356.18 ± 3.89
0.0014	0.99535	0.76823	65352.45 ± 4.45
0.0010	0.99531	0.76702	65373.83 ± 3.16
Egg Albumin			
LiI (0.0008*M*)			
0.0018	0.99527	1.10861	40161.95 ± 6.22
0.0016	0.99539	1.11315	40161.00 ± 4.45
0.0014	0.99472	0.92743	40160.77 ± 3.89
0.0010	0.99515	1.11696	40159.61 ± 6.53
LiI (0.0004*M*)			
0.0018	0.99490	0.63198	40103.48 ± 3.46
0.0016	0.99486	0.63177	40097.59 ± 3.59
0.0014	0.99537	0.63108	40102.93 ± 4.44
0.0010	0.99492	0.63045	40169.04 ± 6.23
LiI (0.0002*M*)			
0.0018	0.99212	0.69918	40268.63 ± 3.66

TABLE I
Continued

Con. of protein (g %)	$\rho \pm 6 \times 10^{-5}$ (g cm^{-3})	$\eta \pm 6 \times 10^{-5}$ (10 g cm^{-3} s^{-1})	$V_\phi \pm$ (cm^3 mol^{-1})	Con. of protein (g %)	$\rho \pm 6 \times 10^{-5}$ (g cm^{-3})	$\eta \pm 6 \times 10^{-5}$ (10 g cm^{-3} s^{-1})	$V_\phi \pm$ (cm^3 mol^{-1})
0.0016	0.99194	0.70097	40272.97 ± 3.89	0.0010	1.00993	0.69377	60979.75 ± 6.20
0.0014	0.99211	0.70377	40275.83 ± 4.55	Egg Albumin			
0.0010	0.99202	0.70521	40312.16 ± 6.23	CTAB (0.0008*M*)			
Lysozyme				0.0018	1.00205	0.69470	37642.45 ± 6.83
LiI (0.0008*M*)				0.0016	1.002097	0.69523	37466.62 ± 5.12
0.0018	0.99598	1.26111	29175.58 ± 3.46	0.0014	1.001576	0.69530	37229.14 ± 4.75
0.0016	0.99598	1.24671	29168.69 ± 3.89	0.0010	1.002142	0.69056	36487.15 ± 6.73
0.0014	0.99597	1.24657	29240.28 ± 4.45	CTAB (0.0004*M*)			
0.0010	0.99595	1.24874	29216.75 ± 6.23	0.0018	1.00521	0.69635	37658.73 ± 5.12
LiI (0.0004*M*)				0.0016	1.00482	0.69641	37477.62 ± 6.03
0.0018	0.99659	0.72645	29530.76 ± 3.46	0.0014	1.00519	0.69821	37271.61 ± 4.65
0.0016	0.99659	0.72620	29567.41 ± 3.09	0.0010	1.00430	0.70340	36477.91 ± 4.49
0.0014	0.99649	0.72543	29558.33 ± 4.45	CTAB (0.0002*M*)			
0.0010	0.99587	0.72806	29740.39 ± 6.02	0.0018	1.00173	0.69088	37675.00 ± 5.12
LiI (0.0002*M*)				0.0016	1.00207	0.69711	37462.86 ± 4.49
0.0018	0.99507	1.10666	29813.38 ± 3.86	0.0014	1.00167	0.69739	37234.11 ± 6.23
0.0016	0.99510	1.10067	29886.46 ± 3.89	0.0010	1.00183	0.68667	36472.42 ± 4.95
0.0014	0.99515	1.10730	29943.18 ± 4.85	Lysozyme			
0.0010	0.99508	1.11737	30187.50 ± 6.28	CTAB (0.0008*M*)			
BSA				0.0018	1.00218	0.68384	26896.68±6.83
CTAB (0.0008*M*)				0.0016	1.00210	0.67989	26709.05±6.23
0.0018	1.00030	0.69131	62105.43 ± 3.56	0.0014	1.00211	0.67979	26524.19±4.45
0.0016	1.00040	0.69290	61909.62 ± 3.66	0.0010	1.00201	0.68179	25712.49±6.23
0.0014	1.00038	0.69297	61677.16 ± 3.40	CTAB (0.0004*M*)			
0.0010	1.00039	0.68954	60918.03 ± 3.66	0.0018	1.00200	0.67829	26888.75±6.23
CTAB (0.0004*M*)				0.0016	1.00199	0.67803	26739.71±4.15
0.0018	1.00037	0.68566	62097.84 ± 3.36	0.0014	1.00172	0.67801	26470.51±5.22
0.0016	1.00037	0.68007	61913.79 ± 3.46	0.0010	1.00208	0.68247	25826.91±4.45
0.0014	1.00038	0.67864	61675.85 ± 4.55	CTAB (0.0002*M*)			
0.001	1.00037	0.68788	60921.83 ± 4.45	0.0018	1.00183	0.68612	26922.72±6.33
CTAB (0.0002*M*)	0.0008M			0.0016	1.00213	0.69726	26711.07±4.65
0.0018	1.01029	0.69149	62097.49 ± 4.85	0.0014	1.00206	0.69723	26514.79±5.10
0.0016	1.01025	0.68984	61918.46 ± 4.45	0.0010	1.00212	0.70507	25751.68±4.40
0.0014	1.01125	0.68991	61550.92 ± 4.45				

The cp = 10 g cm^{-1} s^{-1}, the M is in mol l^{-1} along with their concentrations. The values written in table after ± sign, calculated by eqs. (2–4) and (6), denotes standard errors approximately with 95% CL. The 10^{-5} is accuracy in weights, and 6×10^{-5} [g cm^{-3}] error in the ρ and η values.

ρ, densities; η, viscosities; V_ϕ, apparent molar volumes; BSA, bovine serum albumin; CTAB, cetyltrimethyl ammonium bromide systems; LiI, lithium iodide; NaI, sodium iodide; KI, potassium iodide; g, gram; M, molar; c.p., centi poise; s, second; cm, centimeter.

are given in Table V. Their primary data are given in Table I and while Tables II–V contain the regression constants ρ^0, V_ϕ^0, η^0, and [η] data. The ρ^0 and [η] values for individual salts were obtained by regressing the ρ^0 and [η] values of proteins against salt concentrations are given in Tables VI and VII, which depict net effect of each salt on individual protein. The ρ, η, and [η] values are plotted against concentrations in Figures 1–3, the bar chart drawn between the ρ^0 and [η] values is shown in Figure 4 and represents the salt–protein interactions at a glance.

RESULT AND DISCUSSION

The primary and derived data of the systems are focused to illustrate the protein–salt interactions along with hydrophobic influence of the globular proteins. Briefly the functions along with their trends and magnitudes are discussed in the following sections.

Densities

The densities of the present systems calculated as per Ref. 2 along with earlier studies of these proteins with RbI and CsI salts, the members of the IA group are noted to decrease with the size of cations. Both of the studies focus the physicochemical characterization of the globular proteins with all the members of the said group with increasing order of the size and the orbitals. It infers that electronic structure scrutinizes the protein–salt interactions, perhaps the size due to weakening the ionic charge develops

TABLE II
Regression Constants of Densities for Proteins with Salts, Calculated with Eq. (8)

Salts	BSA				Egg albumin				Lysozyme			
(m M)	$\rho^0 \pm 6 \times 10^{-5}$	$S_d \times 10^2 \pm 10^{-3}$	$S_d' \times 10^2 \pm 10^{-3}$	R	$\rho^0 \pm 6 \times 10^{-5}$	$S_d \times 10^2 \pm 10^{-3}$	$S_d' \times 10^2 \pm 10^{-3}$	R	$\rho^0 \pm 6 \times 10^{-5}$	$S_d \times 10^2 \pm 10^{-3}$	$S_d' \times 10^2 \pm 10^{-3}$	R
KI												
0.8	0.9958	0.001	−0.309	0.54	0.9951	0.010	−1.972	0.62	0.9959	−0.001	0.601	0.97
0.4	0.9914	0.054	−16.038	0.69	0.9984	−0.051	20.393	0.48	0.9951	0.010	−4.134	1.00
0.2	0.9928	0.010	−3.754	0.83	0.9949	0.005	−1.972	0.50	0.9984	−0.051	20.393	0.78
NaI												
0.8	0.9958	0.002	−0.603	0.96	0.9949	−0.010	2.912	0.57	0.9983	−0.045	14.687	0.99
0.4	0.9922	0.053	−15.663	0.60	0.9921	0.053	−19.442	0.49	0.9950	0.008	−3.225	1.00
0.2	0.9948	0.011	−3.922	0.81	0.9902	−0.006	2.566	0.54	0.9978	−0.052	20.804	0.84
LiI												
0.8	0.9959	0.001	0.001	0.94	0.9982	−0.049	18.813	0.39	0.9999	−0.056	−0.056	0.99
0.4	0.9922	0.052	0.052	0.73	0.9915	0.055	−20.108	0.49	0.9952	0.009	−3.748	1.00
0.2	0.9944	0.011	−3.907	0.82	0.9923	−0.004	1.612	0.48	0.9984	−0.051	20.387	1.00
CTAB												
0.8	1.0019	0.001	0.249	0.69	1.0062	−0.064	22.956	0.52	1.0000	0.007	−2.904	0.89
0.4	1.0049	−0.044	15.470	0.76	1.0014	0.055	−16.100	0.75	1.0003	0.002	−0.701	0.67
0.2	1.0007	0.023	−9.348	0.61	1.0014	0.007	−2.268	0.61	1.0003	0.150	−53.067	0.74

The ρ^0 (g cm^{-3}), S_d (10^3 g^2 cm^{-3} mol^{-1}), S_d' (10^6 g^3 cm^{-3} mol^{-1}) values written in table after ± sign, denotes standard errors approximately with 95% CL, and Correlation coefficient R.

ρ^0, limiting density; S_d, first degree slope constant; S_d', second degree slope constant.

TABLE III
Regression Constants of Viscosity for Proteins with Salts, Calculated with Eq. (8)

Salts	BSA				Egg albumin				Lysozyme			
	$\eta^0 \pm 6 \times 10^{-5}$	$S_\eta \times 10^2 \pm 10^{-3}$	$S_\eta' \times 10^2 \pm 10^{-3}$	R	$\eta^0 \pm 6 \times 10^{-5}$	$S_\eta \times 10^2 \pm 10^{-3}$	$S_\eta' \times 10^2 \pm 10^{-3}$	R	$\eta^0 \pm 6 \times 10^{-5}$	$S_\eta \times 10^2 \pm 10^{-3}$	$S_\eta' \times 10^2 \pm 10^{-3}$	R
KI												
0.0008	0.7173	−0.336	164.917	1.00	0.8317	0.052	−51.868	0.92	0.9770	−1.655	639.402	0.98
0.0004	0.7270	0.135	−54.830	0.99	0.6630	0.012	2.957	0.98	0.7187	−0.287	96.307	0.89
0.0002	0.6379	1.391	−554.027	0.98	0.6883	0.137	−77.297	0.98	0.9334	1.388	429.624	1.00
NaI												
0.0008	0.7350	−0.356	171.451	1.00	0.9735	0.064	−56.697	0.92	1.1635	−1.650	637.982	0.90
0.0004	0.7770	0.135	−55.148	0.99	0.6459	0.013	2.502	0.97	0.7330	−0.289	96.875	0.97
0.0002	0.6606	1.388	−553.107	0.98	0.6937	0.139	−77.809	0.98	1.0702	−1.346	420.533	0.98
LiI												
0.0008	0.7524	−0.376	177.985	1.00	1.2249	−4.061	165.158	0.71	1.0532	−1.284	496.518	0.51
0.0004	0.8244	0.136	−55.467	0.99	0.6288	0.014	2.048	0.99	0.7473	−0.290	97.443	0.99
0.0002	0.6835	1.385	−552.186	0.98	0.6990	0.141	−78.320	0.98	0.9656	−1.044	329.151	0.99
CTAB												
0.0008	0.6581	0.476	−161.856	1.00	0.6574	0.489	−156.783	0.99	0.7186	−0.583	215.790	0.99
0.0004	0.7824	−1.455	509.750	1.00	0.7330	−0.411	114.622	1.00	0.7122	−0.435	137.365	1.00
0.0002	0.7282	−0.517	−0.517	1.00	0.5808	1.615	−556.877	1.00	0.6870	0.007	−219.450	0.99

The η^0 (10 g^2 m^{-1} s^{-1}), S_η (10 g^2 cm^{-1} s^{-1} mol^{-1}), S_η'(10 g^2 cm^{-1} s^{-1} mol^{-1}) values written in table after ± sign, denotes standard errors approximately with 95% CL, and correlation coefficient R.

η^0, limiting viscosity; S_η, first degree slope constant; S_η', second degree slope constant.

TABLE IV
Regression Constants of Apparent Molar Volume of Protein Solutions with Salts Calculated with Eq. (9)

	BSA			Egg albumin			Lysozyme		
Salts	$V_\phi^0 \pm 5$	$S_v \times 10^2 \pm 10$	R	$V_\phi^0 \pm 5$	$S_v \times 10^2 \pm 10$	R	$V_\phi^0 \pm 5$	$S_v \times 10^2 \pm 10$	R
KI									
0.0008	65272.46	19.38	0.51	40159.35	12.71	0.57	29248.31	−331.56	0.97
0.0004	65291.15	68.41	0.54	40243.58	−864.10	0.76	29974.38	−2583.86	0.86
0.0002	65479.72	−954.18	0.66	40362.38	−551.55	0.88	30638.75	−4689.48	0.97
NaI									
0.0008	65348.96	231.75	0.72	40179.98	−38.05	0.90	29427.37	−785.53	0.73
0.0004	65357.18	−93.32	0.87	40343.74	−1152.06	0.82	30154.86	−3143.45	0.88
0.0002	65627.16	−1324.52	0.80	40607.51	−1260.95	0.94	32217.29	−9564.22	0.97
LiI									
0.0008	65103.24	1523.40	0.92	40156.80	27.81	0.97	29294.77	−651.30	0.83
0.0004	65283.85	112.00	0.88	40244.03	−867.37	0.76	29972.96	−2577.53	0.85
0.0002	65479.75	−954.57	0.66	40362.35	−551.38	0.88	30636.58	−4682.38	0.97
CTAB									
0.0008	59472.90	1539.21	0.98	35076.18	1469.76	0.97	24292.38	1495.29	0.96
0.0004	59487.90	1493.23	0.98	35052.43	1495.86	0.96	24507.87	1361.01	0.98
0.0002	59551.90	1438.46	0.99	35009.65	1518.44	0.98	24338.94	1473.87	0.97

The V_ϕ^0 [cm^3 mol^{-1}], S_v [10^3 g cm^3 mol^{-2}], values written in table after ± sign, denotes standard errors approximately with 95% CL, and correlation coefficient R.
V_ϕ^0, limiting apparent molar volume; S_v, first degree slope constant; S_v', second degree slope constant.

TABLE V
Regression Constants of Intrinsic Viscosity of Proteins with Salts Calculated with Eqs. (9) and (10)

	BSA			Egg albumin			Lysozyme		
Salts	B	$D \times 10^2$	R	B	$D \times 10^2$	R	B	$D \times 10^2$	R
KI									
0.0008	−198.44	778.58	0.99	71.28	−302.12	0.99	137.71	−2782.81	0.84
0.0004	−115.93	417.32	0.97	−259.78	959.77	0.98	−189.48	682.31	0.98
0.0002	−135.64	439.77	0.85	−195.12	680.39	0.97	86.99	−416.63	0.88
NaI									
0.0008	−167.05	662.76	0.99	346.75	−1310.80	0.98	498.74	−1782.32	0.95
0.0004	−24.16	81.43	0.92	−292.63	1080.04	0.97	−162.09	582.07	0.98
0.0002	−91.77	279.18	0.76	−184.55	641.77	0.96	356.63	−1395.43	0.95
LiI									
0.0008	−135.67	546.93	0.99	546.35	−2082.34	0.97	859.76	−3103.41	0.96
0.0004	67.61	−254.45	0.98	−325.47	1200.29	0.97	−134.69	481.82	0.99
0.0002	−135.67	546.93	0.85	−173.97	603.14	0.96	626.26	−2374.23	0.96
CTAB									
0.0008	−211.98	787.53	0.96	−210.83	−2.11	0.98	−226.87	−2.27	0.98
0.0004	−212.77	−2.13	0.99	−177.34	604.34	0.98	−220.61	779.93	0.98
0.0002	−200.27	714.18	0.99	−407.92	3723.31	0.92	−165.02	510.33	0.99

The B value (10^3 g mol^{-1}), D [10^3 (g mol^{-1})^2] approximately with 95% CL, and correlation coefficient R.
B, limiting intrinsic viscosity; D, first degree slope constant; D', second degree slope constant.

TABLE VI
Absolute Limiting Density of Proteins with Salts, with respect to Each Salt

	BSA			Egg albumin			Lysozyme		
Salts	ρ_0^*	$S_d \times 10^2$	$S_d^* \times 10^2$	ρ_0^*	$S_d \times 10^2$	$S_d^* \times 10^2$	ρ_0^*	$S_d \times 10^2$	$S_d^* \times 10^2$
KI	0.9945	0.032	−50.417	0.9905	−0.191	304.167	0.9964	−0.238	284.583
NaI	0.9940	0.014	17.917	0.9705	0.507	−255.417	0.9985	−0.288	314.583
LiI	0.9956	−0.057	107.083	0.9917	−0.288	457.083	0.9985	−0.285	312.083
CTAB	1.0002	0.005	−9.167	1.0030	−0.121	201.667	0.9928	0.489	−470.000

The ρ_0^* (g cm^{-3}), S_d [10^3 g^2 cm^{-3} mol^{-1}], S_d^* [10^6 g^3 cm^{-3} mol^{-1}] values approximately with 95% CL.
*Represent the values of the corresponding coefficient for limiting concentration of each salt.
ρ_0^*, absolute limiting density; S_d, first degree slope constant; S_d^*, second degree slope constant.

TABLE VII
Absolute Limiting Intrinsic Viscosities of Proteins with Salts, with respect to Each Salt

	BSA		Egg albumin		Lysozyme	
Salts	B^*	$D^* \times 10^2$	B^*	$D^* \times 10^2$	B^*	$D^* \times 10^2$
KI	−94.39	−1191.77	−360.65	4988.03	−76.61	1893.12
NaI	−90.21	−0.22	−504.24	9873.46	373.32	−0.71
LiI	65.91	0.02	−1386.71	4147.70	683.94	−1.17
CTAB	−201.16	−146.67	391.17	2695.92	−266.02	0.31

The B^* (10^3 g mol^{-1}), D^* (10^3 g mol^{-1})^2 and D^* (10^3 g mol^{-1})^2 approximately with 95% CL.

*Represent the values of the corresponding coefficient for limiting concentration of each salt.

B^*, limiting intrinsic viscosity; D, first degree slope constant; D^*, second degree slope constant.

weaker polar electrostatic. The latter causes weaker protein–ion interaction with less shrinkage in the volume and hence CTAB > KI > NaI > and LiI order of densities (Table I) infer weaker ion–protein interaction, which denote weakening of hydrophilic and strengthening of hydrophobic interactions.

The order shows stronger hydrophobic[9,10] interaction of CTAB with protein rather than hydrophilic, and it predicts stronger force/pressure on molecules attributed to longer alkyl chain of CTAB. The Lysozyme > BSA > Egg Albumin, order of ρ^0 calculated with Eq. (8) (Table II) infer stronger interaction of the Lysozyme with each salt while the values for lower concentration of the salts the ρ values for BSA and Lysozyme are almost equal and lower For Egg Albumin. It tells that comparatively Lysozyme behaves as stronger polyvalent protein. Notably with salt composition, initially the values increase but slightly decrease for NaI-Lysozyme, LiI-Lysozyme, and KI-Lysozyme. Around higher salt concentrations effect is reverse, which infer stronger hydrophobic–hydrophobic interactions among proteins rather than ion–protein. This also depicts that structured water moves away from the hydrophobic cage of proteins where ions seem to prefer to home this water (Fig. 1). The densities and viscosities of proteins in water are used from literature[2] and noted higher then those of proteins in salt systems, but for the CTAB systems, the values are higher (Tables I and II).

Thus salts weaken the intermolecular forces among the ternary systems, in nutshell the salt weakly destabilize the hydrogen bonded water which effectively interact with proteins, hence cage of the water develop stronger hydrophobic interactions destabilized the protein–salt interaction resulting the lower ρ values. Thus in water, slightly stron-

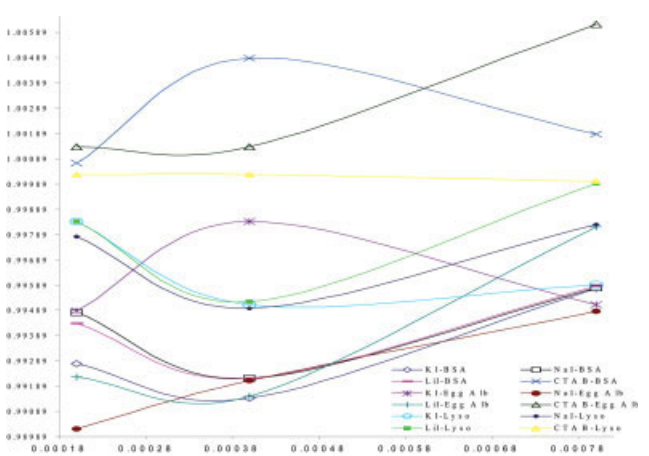

Figure 1 Densities ρ (g cm^{-3}) on *Y*-axis and composition (w/v, w in g and v in mL) of additives on *X*-axis. The composition of proteins from 0.0002 to 0.0008 g % w/v at an interval of 0.0002% for each salt concentration. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

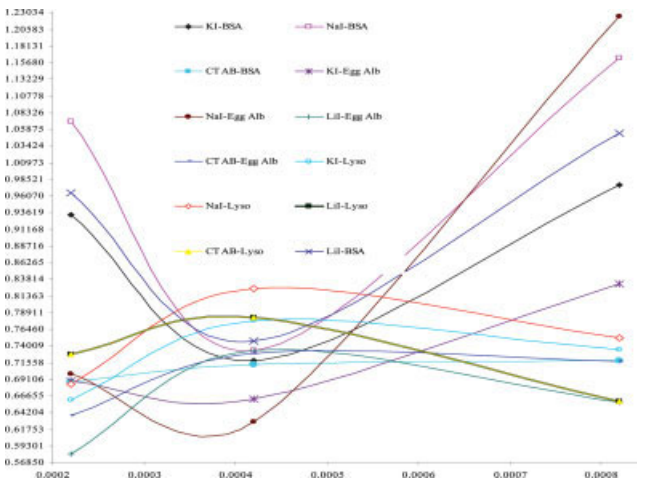

Figure 2 Viscosities, η (10 g cm^{-1} s^{-1}) on *Y*-axis and composition (w/v, w in g and v in mL) of additives on *X*-axis. The composition of proteins from 0.0002 to 0.0008 g % w/v at an interval of 0.0002 for each salt concentration. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

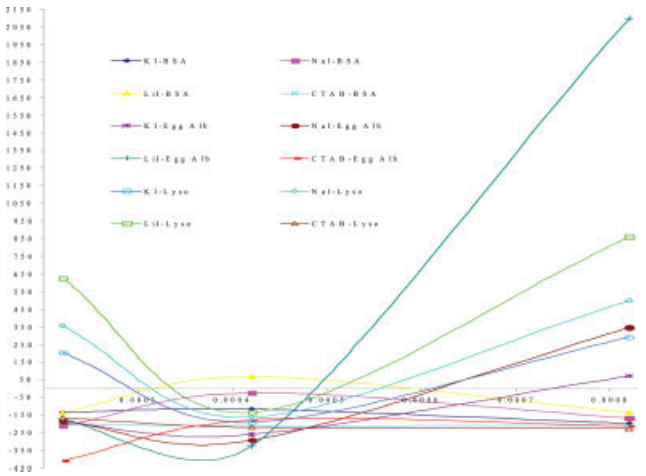

Figure 3 Intrinsic viscosities B (mL g^{-1}) on Y-axis and composition (w/v, w in g and v in mL) of additives on X-axis. The compositions of proteins from 0.0002 to 0.0008 g % w/v at an interval of 0.0002, for each salt concentration. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

ger forces between the dipoles of the water and the electrostatic centers of peptide bond of protein are developed. The lower density values of BSA in salts predict weaker heteromolecular hydrogen bonding which weaken with concentration, destabilizing the protein conformational states. It overcomes the influence of protein concentrations with weaker BSA-BSA interactions and seems to enhance the cage formation of water and the restructured water could not be used in protein system.[16,33]

Thereby partly the cations might be interacting with polar centers of protein and dipole of water, thus the cation–water interaction seems to be disrupted by protein molecules. The Lysozyme > BSA > Egg Albumin, order of density with salts predict stronger intermolecular forces between protein–water than those of protein–protein molecules, which get strengthen with concentration of protein, the density values for proteins from 0.0010 to 0.0018% slightly increases in salts solutions and indicate that proteins weaken the Van der Waals forces[3,25] of water due to the interactions with broken water. These values indicate stronger Van der Waals forces with water molecule this could be estimated that proteins are water structure breakers.

The higher density for Lysozyme with the additives, infer stronger intermolecular forces, slightly higher density values of Lysozyme than those of BSA predict similar interaction mechanism with polar mixed solvents. Perhaps, Lysozyme because of its ellipsoidal structure causes stronger intermolecular forces with each other. So estimations of the activity of Lysozyme with respect to ions can be assessed in various biochemicals, drug delivery and other processes for binding purpose.

Such associations of water-CTAB-protein perhaps cast stronger intermolecular forces resulting in higher densities, with reverse behavior of Lysozymes in CTAB system, it appears that weaker CTAB-protein interactions exist and salts might stabilize the protein structure, it depicts that cations and anions of salts at lower concentration interact, partly with unengaged and the solvated water. It appears that salt concentrations cause unrest in the system increasing its entropy leading to the unfolding of protein molecules.[6,7] It proves that ion–water, ion–protein, and ion–water–protein interactions reorient the bulk water with hydrophobic interactions. It could be asserted that salt-based interactions structurally influence the hydrophobic interactions of proteins. The action of salt remains similar for Egg Albumin and Lysozyme (Tables I and II). The CTAB-BSA-water system report maximum density values.

The densities were fitted in polynomial relation with salt concentrations from 0.0008 to 0.0002M, the densities decrease. Thus at lower concentration, effective rearrangements of intermolecular forces occur, for Egg Albumin in NaI and CTAB. A sharp increase in density infers stronger hydrophobic–hydrophobic interactions. The CTAB-BSA system at lower concentration produce lower values, which sharply increase at around 0.0004M and further decrease at 0.0008M additive concentrations. Around lower alkali salt and CTAB concentrations the proteins cause weaker intermolecular forces with CTAB, this effect is stronger than those of salts. It infers the hydrophobic–hydrophobic interactions between CTAB and proteins with promotion of the changes to be brought about by varying protein concentra-

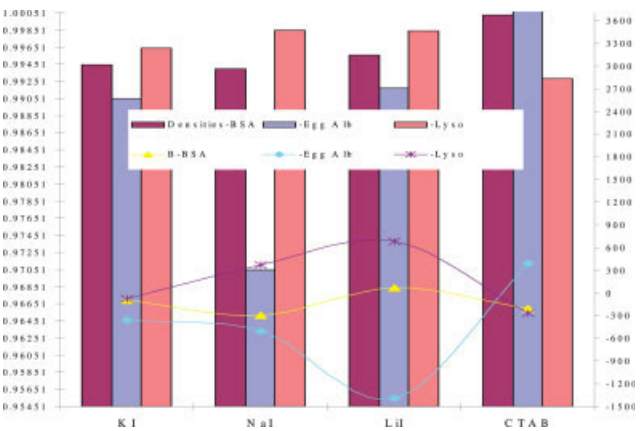

Figure 4 On LHS-Y-axis densities (g cm^{-3}) and on X-axis the additives, RHS-Y-axis intrinsic viscosities, the B value in mL g^{-1} are plotted. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

tions. Probably CTAB nullifies protein–protein interacting mechanism, it seems that hydrophobic alkyl chain of CTAB occupies the hydrophobic backbone of protein, which plays a deciding role in reorienting the protein molecule in cationic surfactant aqueous solutions.

The overview of these interactions illustrates that the Lysozyme in CTAB brings about a smooth change in the intermolecular forces with respect to its own and protein concentrations. It establishes the relation between the concentration of proteins and the hydrophilic–hydrophobic interactions of CTAB. Such studies of CTAB and salts can be used as prove for the estimation of hydrophobic and hydrophilic interactions.

Viscosities

The viscosities calculated by Eq. (7) (Table I) of BSA in NaI, LiI, and KI are higher at 0.0002*M* and decreases around 0.0004*M* with maximum value at 0.0008*M* salt concentrations. Although for other salt protein systems a reverse trend of values is marked. It infers stronger interaction with salt-BSA and weaker with Egg Albumin and Lysozyme at around lower salt concentration. Although reverse order of interaction is noted for other salt concentration. This illustrates that at around higher salt concentration the larger number of ions develop interaction with each polar center of the BSA, while Egg Albumin and Lysozyme weaker with larger unfolding of BSA structure at higher salt concentration. Such interaction mechanism favors generation of stronger frictional forces for proteins at higher concentration, for moderate salt concentration (0.0004*M*).[30]

In fact the latter forces generate electrostatic dipoles of water restrict viscous flow by applying additional frictional forces on adjacent layers and is responsible for lower η values. Taking into account the overall η values (Tables I and III) are higher at around 0.0008*M* additive concentrations. For BSA except in CTAB the η values are approximately higher by 0.20021×10 g cm^{-1} s^{-1} than those of others. However NaI-Egg Albumin has maximum η values among the systems while in CTAB, the values remain lower, this proves that the BSA interaction in NaI, Li, and KI with hydrodynamic forces are higher due to stronger torque or frictional forces. Because of large number of amino acids in BSA it behaves as polyelectrovalent protein and is responsible for this effect with salts. Such forces associated with BSA in NaI, Li, and KI systems get stabilized at 0.0004*M* and further increase with concentration.

Thus the pairwise interaction[30,31] of globular proteins with concentrations can monitor the structural changes crucially. These systems show maximum protein–protein interactions than those of the others, while CTAB-BSA interaction remains almost constant but slightly increases with concentrations, perhaps because of Newtonian flow. At lower concentrations the viscosities of BSA with each salt are higher (Fig. 2) than those of other systems, the order of η values is as LiI-Egg Albumin < CTAB-Egg < KI-Lysozyme < NaI-Lysozyme < CTAB-BSA < NaI-Egg Albumin < Li-Lysozyme < CTAB-Lysozyme systems. While at 0.0008*M* of salt, the values scatter to a broader range with maximum values for NaI-Egg Albumin and lowest for CTAB-Lysozyme. At this salt concentration the trends are as NaI-Egg Albumin > NaI-BSA > LiI-BSA > KI-BSA > KI-Egg Albumin > NaI-Lysozyme KI-Lysozyme > CTAB-Egg Albumin > CTAB-BSA > LiI-Lysozyme > LiI-Egg Albumin > CTAB-Lysozyme. It marked that because of larger size Lysozyme generate weaker torsional forces in CTAB, and the viscosities of Lysozyme remain higher at 0.0002*M* salts concentrations depict weaker protein interaction because of concentration and influence of additives.

Thus at around 0.0004*M* salts concentrations, the behavior of proteins are most stabilized, perhaps with conformational structure[28] (Fig. 2). The latter concentrations resist major changes in viscosities, thus termed as buffer zone. The order of viscosities in this zone are as CTAB-Lysozyme > LiI-Lysozyme > KI-Lysozyme KI-BSA > NaI-Lysozyme > CTAB-Egg Albumin >KI-BSA > CTAB-BSA > KI-Lysozyme > NaI-Egg Albumin. This illustrate the strength of their interaction with additive, the viscosity of proteins increase with salt concentration except 0.0004*M*, proves that the frictional forces monitor protein–salt interactions.

Intrinsic viscosities

The *B* values, calculated with Eq. (10) (Table V) illustrate the structural behavior of proteins at hydrodynamic level. Thus the *B* values for LiI-Lysozyme, NaI-Lysozyme, and KI-Lysozyme are higher for 0.0002*M* and denotes that the size of cation affect (Fig. 3). The *B* values in order of LiI-Lysozyme> NaI-Lysozyme > KI-Lysozyme could be attributed to the charge on cations and effect in respect of proteins. While other *B* values except CTAB–Egg Albumin, are narrowed from –130 to –200 mL g^{-1} with the trend as (Fig. 3) LiI-BSA > Na-BSA > KI-BSA > LiI-Lysozyme > NaI-Lysozyme > CTAB-Egg Albumin > KI-Lysozyme > CTAB-BSA > CTAB-Lysozyme > KI-Egg Albumin > NaI-Egg Albumin > LiI-Egg Albumin. Thereafter the *B* values at 0.0004*M* salt concentration converge to a range of 50 to -400 mL g^{-1}, and at 0.0008 concentration the values increase in order of LiI-Egg Albumin > LiI-Lysozyme > NaI-Lysozyme > NaI-Egg Albumin > KI-Lysozyme > KI-Egg Albumin > LiI-BSA > Na-BSA

> KI-BSA > CTAB-Egg Albumin > CTAB-Lysozyme.

Comparatively it illustrates the strength of proteins interactions[10,11] with concentrations of salt. Notably the B values of LiI-BSA, Na-BSA, and CTAB-Egg Albumin remain lower at 0.0002M concentration, and slightly increase at around 0.0004M and further decrease at around 0.0008M salts concentrations. This visualizes that the systems at around 0.0002 and 0.0008M salts concentrations have similar torque, B values of CTAB-BSA and CTAB–Lysozyme are almost constant. Thus with concentrations of salts, the mechanism of interactions for these protein systems remain unchanged. The LiI-BSA system lists a remarkable difference around three concentrations with maximum magnitude among the systems. It proves stronger LI-BSA interaction with no change in hydrodynamic structure.[2–5]

Apparent molar volumes

The BSA > Egg Albumin > Lysozyme order of V_ϕ and V_ϕ^0 values calculated with eqs. (5), (6), and (9) (Tables I and IV) elucidate their stronger volumetric interactions. The orders of V_ϕ^0 values of BSA, Egg Albumin, and Lysozyme in ternary systems[2–5] are as NaI > KI > LiI > CTAB, NaI > LiI > KI > CATB and NaI > LiI > KI > CTAB, respectively, it depicts stronger binding of CTAB with each protein, especially with Lysozyme. The higher V_ϕ^0 values of BSA depict, that the latter is comparatively less packed than other two proteins, because of the number and nature of amino acids present in its molecule. The CTAB-Lysozyme in combine interacts with water causing maximum shrinkage in volume due to stronger ion–protein interactions[8,9] than others.

Almost similar sequence of data for each protein with salts concentrations register similar compositional effect on intermolecular and hydrophobic forces of proteins. In general, the rate of decrease of V_ϕ^0 values of the proteins in CTAB is lower than those of the IA alkali metals, thus the stronger hydrophobic interactions develop stronger heterommolecular forces, which are stabilized by the restriction of rearrangement of bonds.[17]

Absolute salt–protein interactions

Limiting densities the ρ^0 and intrinsic viscosities the B values of proteins at zero concentrations of the salts are plotted against individual salt (Tables VI and VII) in the form of bar charts and free lines respectively (Fig. 4). Both the parameters depict net effect of salts on the conformational structure of the proteins under electrostatic condition and Newtonian flow.[2,3]

The bar charts registers the strength of salt–protein linkage, whose preknowledge could render assistance to the scientists engaged in the field of protein engineering and the influence of individual salt on protein behavior. The Figure 4 reports stronger interaction of BSA with salts and CTAB in order of CTAB > LiI > KI > NaI, while Egg Albumin comparatively weaker in order of CTAB > LiI > KI > NaI. However the Lysozyme make stronger binding in order of NaI > LiI > KI > CTAB. It observes weaker interaction of Lysozyme with CTAB. These orders reflect an influence of cation size on salt–protein interaction with maximum unfolding of Egg Albumin with NaI. Thus CTAB do leave nonformidable structural changes with Lysozyme, thus the bar chart is very informatory and could be ready recur to biochemists.

Similarly the B values give insight picture of the hydrodynamic behavior of the proteins and show maximum activity for Lysozyme on Newtonian flow with respect to cation size. The CTAB shows maximum affinity because of static behavior and weaker hydrodynamic forces. Like Lysozyme, the Egg Albumin shows an effect of cation size in a decreasing order due to weaker hydrodynamic forces or torque with lower size of cations and visa versa for cation of larger size with maximum affinity for CTAB.

Thus the ion–protein interactions remain unaffected on viscous flow, BSA as compared with other two do not show any fix trend with cation size. However for KI to NaI the B values decrease for CTAB due to stronger affinity of Lysozyme for LiI, the smallest cation.

Constants

The limiting values ρ^0, η^0, V_ϕ^0, and B constants calculated with eqs. (8–10) reveal protein–salt/solute–solvent interactions in infinitely dilute salt solutions. The higher the ρ^0 values of proteins in CTAB than those of alkali salts with their CTAB > KI > NaI > LiI order (Table II) infer stronger hydrophobic interaction of quaternary ammonium salt based on surfactant and the effect of $2s^1$, $3s^1$, and $4s^1$ shell of the IA alkali metals. The Lysozyme > BSA > Egg Albumin and Egg Albumin > Lysozyme > BSA orders of the ρ^0 and S_d values for the proteins in the salts, find least folding of Lysozyme and maximum concentration effect of Egg Albumin due to stronger Egg Albumin–Egg Albumin interaction than those of BSA-BSA and Lysozyme–Lysozyme. Thus lower S_d and S_d' with concentrations indicate a weaker compositions effect of salts due to micelle formation.

The Lysozyme > BSA > Egg Albumin order of η^0 of the proteins in salts (Table III) reflect stronger Lysozyme–salt interactions than those of BSA-Salt and Egg Albumin–salt where hydrophobic and

hydrophilic interactions[9] of Lysozyme decide their fate. The order of η^0 values of proteins with salts is as NaI > LiI > KI > CTAB and of their slope as Lysozyme > BSA> egg Albumin, reveals stronger frictional force and compositional effect for Lysozyme in salt. It marks that hydrophilic and hydrophobic effects remains functional during the flow, because of their specific hydrodynamic sphere formation.

The order of S_η and S'_η values for BSA, Egg Albumin, and Lysozyme in salt are as KI > NaI > LiI, NaI > KI > LiI, and LiI > NaI > KI, which denote stronger effect of size of cation in BSA interaction, while other proteins do not show any specific effect of size. The S_η for 0.0004*M* salt solution are as KI > NaI > LiI with reverse order for 0.0002*M* salt concentration. This shows weakening of salt-BSA interaction with size of cation. Likewise interactions with size of cation are stronger for Egg Albumin while weaker for Lysozyme with salt concentration.

The S_η values for Egg Albumin for lower (0.0002*M*), moderate (0.0004*M*), and higher (0.0008*M*), concentrations are as LiI > NaI > KI, KI > LiI > NaI, and NaI > KI > LiI, respectively, while for Lysozyme the trend as KI > LiI > NaI, KI > NaI > LiI, and LiI > NaI > KI. In CTAB Egg Albumin the S_η values depicted higher compositional effect of Egg Albumin than those of Lysozyme and BSA with a trend as Egg Albumin > BSA > Lysozyme.

The BSA > Egg Albumin > Lysozyme order of V_ϕ^0 (Table IV) of proteins demonstrate stronger intermolecular forces[5] for Lysozyme, which reveal the size of the molecule and stronger unfolding for BSA than those of others. The higher and positive S_v values for ternary systems infer stronger pairwise interactions[32,34–36] and its negative values, the weaker, perhaps the ions engage water salting out the proteins. The S_v values depict weakening of salt–proteins interactions around lower while stronger around higher concentration. Such trends of their values depicts protein nonelectrolyte. The V_ϕ^0 of the proteins with CTAB concentrations shows a linear relation with S_v for BSA and Egg Albumin at higher and lowers concentration of CTAB. The latter trend reflects stronger CTAB-BSA and CTAB-Egg Albumin interaction for corresponding concentrations.

The higher S_η and S'_η values of proteins for fixed concentrations of salts and vice versa indicate enhancement of interactions with their corresponding concentrations. The Lysozyme > BSA > Egg Albumin, trend of the *B* values for proteins in salts find stronger hydrodynamic hydration for Lysozyme. The negative *B* values for BSA and Egg Albumin demonstrates larger pressure of ions on proteins with a unique behavior of proteins[2] with larger alkyl chain and Iodide anions. While the Lysozyme–salt with highest *B* values show trend as LiI > NaI > KI > CTAB with stronger intermolecular forces, but for CTAB the B values fall down rapidly and remain almost similar, thus the proteins get stabilized in a particular range of concentrations.

The S_d, $S'_{\eta'}$, S'_v, and *D* values calculated as per eqs. (8) and (9), (Tables II–V) depict the stabilizing influence of compositions of proteins and salts on the interactions in binary and ternary systems respectively. It seems that ion–ion pairwise interactions also monitor the ion–protein interactions.

CONCLUSION

Proteins are noted to develop stronger heteromolecular interactions in binary systems and the salts weaken them, which are further weekend with size of their cations. Thus small sized cation cause stronger ion–protein bonds perhaps due to their higher ionization potential. Their first ionization potential (KJ mol^{-1}) is in order of 520.2 > 495.8 > 418.8 > 403.0 > 375.7 (these values are taken from standard periodic table of element) to the corresponding size as Li >Na >K > Rb > Cs. Here, each member of IA group metals is compared as the studies made earlier with Rb and Cs show similar trend. Thus smaller ions are stronger water structure breaker rather than larger, it seems that large sized cations do maximum unfolding of proteins as their V_ϕ^0 values are higher. Specifically the Lysozyme causes higher concentration effect on interactions with salt than that of with water. The BSA > Egg Albumin > Lysozyme order of the V_ϕ^0 values with salts reveal a lower internal pressure on the molecules. It visualizes that larger is the size of cation weaker is the internal pressure of molecule and agrees to Hofmeister series. The higher and lower viscosities for Lysozyme and Egg Albumin, respectively, illustrate the degree of torsional forces and the *B* values of proteins are lower for aqueous salts as compared to in water, which again recognizes the contribution of ions for strengthening the intermolecular forces. Thus the optimization and globular maxima of proteins with iodide salts of IA group metals in solutions at pH = 7, could be useful for understanding of hydration process, protein–substrate interactions, and drug delivery system.

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