Structural Interactions of Globular Proteins—Bovine Serum Albumin, Egg Albumin, and Lysozyme, in Aqueous Medium, Elucidated with Molar Volumes, Viscosities, Energy Functions, and IR Spectra from 293.15 to 303.15 K

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ABSTRACT: Densities (ρ), apparent molar volumes (V_ϕ), viscosities (η), and IR spectra on 0.0010–0.0018% aqueous solutions of bovine serum albumin (BSA), egg albumin (E-Alb), and lysozyme at an interval of 0.0004% and at temperatures from 293.15, 298.15, and 303.15 K have obtained. The free energy (ΔG), entropy (ΔS), and enthalpy (ΔH) data with compositions and temperatures are calculated from the values of the flow velocity (v_f) of viscous flow, which decrease with temperature. The densities decrease with concentrations and temperatures except BSA, and the V_ϕ values slightly increase with concentrations for BSA and lysozyme, which depict structural reorientations and transition states of protein molecules with increase in viscosities and decrease in reduce viscosities. The reduce viscosities at 293.15 K for BSA, E-Alb, and lysozyme are noted positive, and for BSA and lysozyme remain positive at 298.15 and 303.15 K, whereas for E-Alb it is negative. Activation energies (E^*) for lysozyme remain almost constant, and are higher than those of the BSA and E-Alb, respectively, also slightly higher E^* values for the BSA than those of the E-Alb at 293.15 and 298.15, and lower than of the E-Alb at 303.15 K, are observed elucidating greater structural interactions for BSA at lower while weaker at temperatures. Stretching frequencies of amide (—NHCO—), —NH—, —CO, and —CH— groups of proteins are noted from IR spectra with broader stretching frequencies for —NH—. © 2006 Wiley Periodicals, Inc. J Appl Polym Sci 103: 1420–1429, 2007

Key words: intermolecular interactions; structure breaking; making; cage model; hydrodynamic volume; Newtonian flow; torsional forces

INTRODUCTION

In general, the proteins make non-Newtonian solutions in water, whose viscosities depend on the temperature and the compositions of them in aqueous solutions. Thus, the structural interactions of globular proteins and biopolymers have been of current interest and useful for their biochemical activities in several processes. Their thermodynamic (densities, apparent molar volumes)[1–3] and transport (viscosity) properties depict the structural state of proteins and are indispensable from the activity in biological processes. The several viscometric studies on the biopolymers in aqueous solutions have been conducted by Monkos and illustrated several conformational states of the proteins,[4–6] but no studies on molar volume and energy functions are made, as these do elucidate the compatibility and interacting feasibility of the molecules in different medium.

Basically Monkos[7,8] made a comparison of conformation and hydrodynamic properties of equine, porcine, and rabbit serum albumin with viscometry along with hydrodynamics and temperature dependence of the solution conformation of human serum albumin from viscometry approach, and extended his studies for the temperature dependence of the solution conformation of ovalbumin and comparative viscosity of human, bovine, and pig IgG immunoglobulins in aqueous solutions along with concentration and temperature dependence of viscosity in lysozyme aqueous solutions. Further, he focused the viscosities[9,10] of bovine serum albumin (BSA) aqueous solutions as a function of temperature and concentration, study of human, bovine, equine, and ovine hemoglobin in aqueous solution but molal volume and entropy, free energies were not undertaken. Thereby, apart from viscosities, other functions are focused, which are helpful to reveal interplay between the forces that influence behavior of cell functioning with respect to their hydrodynamic volume.[11,12] Additionally, the

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biological activities of proteins are also mediated by their interactions with water, salts, membranes, other proteins, nucleic acids, etc. in living systems[13] to understand the physical basis of life. Many of the processes in biological sciences are extremely complex and physical forces at play can be understood at a more basic or idealized level for structural changes of proteins. Hence, studies are chosen and serve as model to generalize the protein's behavior, the three temperatures have been chosen to study an effect of thermal energy on conformational changes of proteins. Enormous studies have already been done on amino acid–water and salt–water systems,[14] but there is a scarcity of densities, viscosities, and apparent molar volumes data of protein–water systems. However, Barry and Irving[15] have reported the viscosities of concentrated aqueous electrolytic solutions. Eisenberg and Pouyet[16] have noted the significance of intermolecular electrostatic interactions among polyions and polyvalent systems in solutions with some distortion[17] and coupling approximation[18,19] during viscous flow associated with reduced viscosity. Also, Rice and Kirkwood[20] found an influence of shield coulumbic potential between charged sites of macromolecules and role of counter ion[21] with temperature[22] under an influence of external forces like voltage and current, affecting the natural behavior of proteins. Consequently, here an investigation is initiated on volumetric and viscometric properties on the latter systems as it involves solvent's structure making and breaking effects on the solute–solvent interactions. Critically, the proteins do cause intramolecular hydrodynamic cage in water that contributes to the η_{sp}/c (the reduced viscosity); hence, $\eta_{sp}/c = \eta_{intra}/c + \eta_{inter}/c$ relation stands valid.[23] The η_{intra}/c and η_{inter}/c represent the values of the viscosities due to the intra and inter hydrodynamic cages formations, respectively. The structural behavior of globular proteins in single solvent will lead to estimate the activities[24,25] and hydrophobic interactions in mixed solvent, which play key role for interaction dynamics of biopolymers. In accordance to this, such functions determined here could be treated as prerequisite characteristics of proteins for studies of interstitial empty states and the analysis of densities of states.[26] The values of the V_ϕ, $(\eta_r - 1)/c$, B, ΔG, ΔE^*, and ΔS derived from the measured data could be related to chemical reactivity and helpful to elucidate electronic structure[27] and an existence of electronic unoccupied states in structural cavities caused by folding of polypeptides. The cavities associated in such systems are crucial for interactions[28] with water and other polar solvents, to determine the functions in several cellular and biochemical processes.[29] Several theoretical studies on proteins to determine the density functional theory with single calculation using silicon graphics implementation of the code[30] and local density approximation have been made. Thus, the data obtained on the present studies belong to a process, where molecules make a free movement detaining their natural behavior; hence, it is an element of great significance and relevance for proteins. The amino and carboxyl groups of proteins form intrahydrogen bonds[31–33] and contribute to the intrinsic viscosities[34] with the strength of 2–10 kcal mol^{-1} at 25°C. The intermolecular forces like van der Waals are often represented by energy potential, as a function of distance that includes both the attractive and repulsion forces at close range and represented by Lennard–Jones potential relation,[35] which accounts for repulsion and van der Waals attractions. This summary evinces the need of designed studies.

EXPERIMENTAL PROCEDURES

The bovine serum albumin (BSA), egg albumin (E-Alb), and lysozyme (Sigma, USA, A 6918, A 3014, L 6876) solutions were prepared in deionized triple distilled and degassed (by boiling off) water, w/v, and the densities and viscosities were measured with 20 $\times 10^{-3}$ dm^3 bicapillary pyknometer and Survismeter[36] (Fig. 1) to ±0.01°C, read with Beckman thermometer. The schematic drawing of Survismeters-type 1 is shown in Figure 1; it is very handy, simple in operation and competent technique for accurate flow time measurements. It consists bulbs, limbs, and capillaries for solution, pressure, and liquid flow control.

Its capillaries of 0.5 mm inner diameter adjoin the bulb numbers 9 to 7 (for drops counts) and the 6 to 7 (for times for viscous flow) are vertical in shape and shorter in length (almost 5 in.). The bulb num-

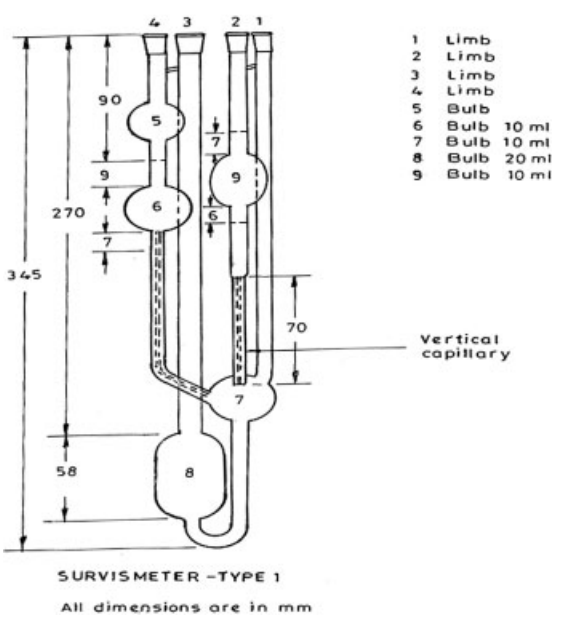

Figure 1 Survismeter, the numbers depicted on arrow-marked vertical lines illustrate the dimensions in millimeter.

ber 8 holds about 20×10^{-3} dm^3 solution, but 15×10^{-3} dm^3 solution is preferred for measurements with $\sim 5 \times 10^{-3}$ dm^3 empty volume for pressure equilibrium. The bulb number 8 is connected to limb number 3 and bulb number 7, respectively. The bulb number 7 holds about 10×10^{-3} dm^3 solution, and adjoins the lower ends of limb numbers 1, 2, and 4, respectively, and it controls an upward flow of solution being sucked to the bulb numbers 6 and 5, and 9 of limb 4th and 2nd, respectively. The bulb number 6 of 10×10^{-3} dm^3 is an important one as solution filled in is allowed to flow within the upper and lower marks made below and above it. The bulb number 5 stabilizes the flow by equilibrating a pressure and thermal stability. The bulb number 9 is of 10×10^{-3} dm^3; the solution is sucked above its upper mark and simultaneously allowed for downward flow, drop wise through vertical capillaries attached to its lower tube. The drops are counted at the lower end of the capillary, which opens to the bulb 7 and the upper end of limb number 1 is kept open for pressure control of the bulb. The tubes 1, 2, 3, and 4, respectively, are limbs, their upper ends are fitted with ground glass joints of B5 (except tube number 3 with B9). Here B depicts standard glass joint along with size of the B, the stoppers are also used to block the upper ends of tubes. The limb numbers 1 and 3 are directly attached to 8th and 7th bulb for pressure control of the bulbs during operation and 2nd and 4th limbs are attached to bulb numbers 5 and 6, and along with bulb number 9, they control air pressure applied overhead of the top ends of limbs for laminar flow of solution.

Usually, Survismeter is calibrated with any standard solution; however, water is preferred, and its calibration constant is known as Survismeter constant and found in the range of 1×10^{-5}. Unit number 4 is calibrated for viscosity and unit can measure surface tension.

About 15×10^{-3} dm^3 solution of known viscosity value is taken in the 8th bulb through 3rd limb keeping other ends of the limbs open. The Survismeter is mounted on the SS stand in thermostat at desired temperature. The solution is sucked upward from bulb number 8 through capillary by 50 mL capacity syringe fitted with airtight plunger. With needle of syringe, one end of teflon tube is attached and another end is attached to a movable stopper with hole. The stopper is fixed in the socket of 4th limb and pulling out the plunger sucks up solution till the bulb number 5 is filled up, thereafter the stopper is removed. During suck, the socket of 3rd limb is kept open, while that of 2nd and 1st are blocked with stopper and for down flow of solution, the syringe and stopper of limb number 1 is taken back. The flow time between upper and lower marks of the bulb number 6 is counted. The flow time with 1×10^{-2} s was recorded with electronic racer for several times for reproducibility in values, similarly the time data are collected for solutions. Air-free-filled pyknometer after thermostated for 20 min was taken out and wiped to absolute dryness with tissue paper, and weighed with 0.01 mg Dhona balance, model 100DS. FTIR spectra were recorded with Nicolet Protégé-460 spectrometer.

RESULTS AND DISCUSSION

The ρ data are calculated from the relation given below.

$$\rho = (w/w_0)\rho^0 + 0.0012(1 - w/w_0) \quad (1)$$

The ρ data are used to calculated the V_ϕ value from relation given below.

$$V_\phi = 1/\rho\ (M_2 - (1000/c)(w - w_0)/(w_0 - w_e)) \quad (2)$$

The w_e of pyknometer, w and $w_{0,}$ are the weights with the solution and solvent, respectively; 0.0012 g cm^{-3}, the air density; and $(1 - w/w_0)$ is buoyancy correction in the weights. The c is concentration in grams per 100-mL solution and M_2 the molecular mass; the relative viscosities (η_r) are calculated from the relation given below.

$$\eta_r = (\rho \times t)/(\rho_0 \times t_0). \quad (3)$$

The ρ and ρ_0 are densities of solution and solvents, and t and t_0 the flow times, respectively, and the η_r values are given in Table I. The ρ and V_ϕ values are regressed to first order equations given below.

$$\rho = \rho^0 + S_d\, c \quad \text{and} \quad V_\phi = V_\phi^0 + S_v c. \quad (4)$$

The ρ^0 and V_ϕ^0 denote the limiting densities and apparent molar volumes at infinite dilution $c \rightarrow 0$, and the S_d and S_v the slopes. The η_r values were fitted to Jones Dole equation given below.

$$(\eta_r - 1) = Ac^{1/2} + Bc \quad (5)$$

The A is Falkenhegan coefficient, B the Jones-Dole coefficient, for nonelectrolyte solutions, the A is negligible and is taken to be zero, thus an extended Jones Dole equation[33,34] is applied for our systems as under.

$$(\eta_r - 1)/c = Bc + Dc \quad (6)$$

The D is the concentration-dependent function and depicts protein–protein interactions, the values of the derived constants with eqs. (4) and (6) are given in the Table II. As the macromolecules on outset of

TABLE I
Densities with 2 ± 10^{-5} Standard Error, Molar Volume V_Φ ± 0.04/10^{-6} m^3 mol^{-1}, and Viscosity η ± 4 × 10^{-4} kg cm^{-1} s^{-1}

Concentration (% g mL^{-1})	ρ (10^3) (kg m^{-3})	V_Φ (10^{-6}) (m^3 mol^{-1})	η (kg cm^{-1} s^{-1})	η_r	$(\eta_r - 1)/c$ (dL g^{-1})
BSA[a]					
0.0010	0.99788	65017.48	1.0181	1.0162	16.1583
0.0014	0.99862	64994.76	1.1054	1.1033	73.7590
0.0018	0.99854	65036.11	1.1061	1.1040	57.7954
E-Alb[a]					
0.0010	0.99865	39910.20	1.0139	1.0119	11.9974
0.0014	0.99863	39956.24	1.0213	1.0194	13.8446
0.0018	0.99855	39996.57	1.0277	1.0257	14.2830
Lysozyme[a]					
0.0010	0.99861	28908.36	1.0107	1.0088	8.8258
0.0014	0.99849	28979.16	1.0231	1.0211	15.0848
0.0018	0.99847	28997.38	1.0245	1.0226	12.5467
BSA[b]					
0.0010	0.99698	65205.14	0.8929	1.0023	2.3315
0.0014	0.99686	65238.78	0.8956	1.0053	3.7983
0.0018	0.99673	65267.67	0.8991	1.0092	5.0992
E-Alb[b]					
0.0010	0.99699	40123.73	0.8774	0.9849	−15.0960
0.0014	0.99696	40132.08	0.8836	0.9918	−5.8442
0.0018	0.99684	40158.30	0.8876	0.9963	−2.0726
Lysozyme[b]					
0.0010	0.99700	29087.26	0.8785	0.9861	−13.9300
0.0014	0.99686	29128.04	0.9068	1.0179	12.7597
0.0018	0.99684	29123.43	0.9317	1.0459	25.4779
BSA[c]					
0.0010	0.99641	64907.39	0.7895	0.9891	−10.8953
0.0014	0.99622	65070.20	0.7992	1.0013	0.9124
0.0018	0.99619	65116.27	0.8085	1.0129	7.1896
E-Alb[c]					
0.0010	0.99625	39890.08	0.7614	0.9539	−46.0803
0.0014	0.99623	39970.16	0.7769	0.9733	−19.0983
0.0018	0.99623	40010.54	0.7917	0.9919	−4.4903
Lysozyme[c]					
0.0010	0.99634	39850.97	0.8039	1.0071	7.1395
0.0014	0.99621	39977.65	0.8041	1.0074	5.2987
0.0018	0.99607	40054.00	0.8065	1.0104	5.7810

[a] At 293.15 K.
[b] At 298.15 K.
[c] At 303.15 K.

viscous flow require some activation energy, thus the log (v_f) versus $1/T$ values were plotted as under.

$$Log(v_f) = \log(F) - (E^*/2.303R)1/T \quad (7)$$

The value v_f is the velocity of viscous flow and T is the experimental temperature; hence, this relation calculates the energies of viscous flow. The intercept F of the equation is referred to as frequency factor $= ekT/h.e^{\Delta S/R}$, whereas E^* is activation energy, R the gas constant (8.314 J mol^{-1} K^{-1}), the $E^*/2.303R$ = slope (S_{E^*}). The ΔH and ΔG are calculated from the $\Delta H = E^* - 2RT$ and $\Delta G = \Delta H - T\Delta S$ relations, the data are given in Table III and stretching frequencies of molecules in solutions in Table V.

The densities for 0.0010, 0.0014, and 0.0018% solutions at 293.15 K are found in order of E-Alb > lysozyme > BSA, E-Alb > BSA> lysozyme, and E-Alb > BSA > lysozyme, respectively. And this predicts a greater internal pressure by E-Alb on water molecules associated with its —NH— and —CO polar groups resulting in a contracted hydrated structure with greater densities, which infers stronger E-Alb–water interactions and enhances with composition. It also predicts a prominent caging of water around protein's molecules [Fig. 2(a)], while lysozyme and BSA predict comparatively less internal pressure of polar groups on water molecules and hence the weaker interactions and caging are evident. For their consecutive compositions, the interacting nature of the E-Alb remains similar but the strength of interaction of BSA becomes stronger than those of the lysozyme. Also at 298.15 K, with each concentration, their trend is noted as lysozyme > E-Alb > BSA, E-Alb > BSA = lyso-

TABLE II
Regression Constants of the ρ, V_ϕ, and $(\eta_r - 1)/c$ Data Fitted to First-Order, Masson, and Jones–Dole Equations Respectively

Temperature (K)	ρ^0 (10^3) (kg m^{-3})	S_d (10^3) (kg m^{-3} mol^{-1})	$V_\phi{}^0$ (10^{-6}) (m^3 mol^{-1})	S_v (10^{-6}) (m^3 kg mol^{-2})	B (10^{-3}) (m^3 kg^{-1})	D (10^{-3}) (m^3 mol^{-1})
BSA						
293.15	0.99720	0.8207	64984	23286	−23.627	52046.0
298.15	0.99730	−0.3149	65128	78162	−1.101	3459.7
303.15	0.99640	−0.2764	64666	261101	−32.580	22606.0
E-Alb						
293.15	0.99880	−0.1291	39803	10796	9.375	2856.9
298.15	0.99720	−0.1892	40078	43218	−30.462	16279.0
303.15	0.99630	−0.0220	39746	150571	−96.005	51987.0
Lysozyme						
293.15	0.99880	−0.1843	28806	111274	5.641	4651.1
298.15	0.99720	−0.1999	29050	45214	−60.861	49260.0
303.15	0.99670	−0.3383	28569	250047	8.450	−1698.1

zyme and E-Alb = lysozyme > BSA. And at 303.15 K, BSA > lysozyme > E-Alb, E-Alb > BSA > lysozyme, E-Alb > BSA > lysozyme, where in general, the densities of individual protein system with compositions decrease except BSA, which at 293.15 K first increases and then decreases for consecutive compositions. Also, at around 303.15 K, the densities of E-Alb around 0.0014 and 0.0018% are found to be equal and lower than those of around 0.0010% (Table I). Hence, it is predicted that a compact conformational structure of the proteins is developed at around 0.0014% in case of BSA because of stronger intramolecular interactions, which lead to greater densities, while the E-Albumin seems to form weaker hydrogen bonds with water molecules. It might be concluded that the concentration hinders the protein–water interactions and develops protein-protein–water interactions rather than protein–water interactions, hence accounting for deflection from the general trend (Table I). The decrease in densities with temperature concludes that the thermal energy does destabilize the protein–water interactions and their limiting densities with temperature are found in the order of 293.15 > 298.15 > 303.15 K except in case of BSA. An order of the values at 293.15 K is as (lysozyme = E-Alb) > BSA but at 298.15 K, the values of lysozyme and E-Alb are equal and lower than that of the BSA. It predicts almost same interacting strength of lysozyme and E-Alb at around 293.15 and 298.15 K, but the BSA at later temperature gains slightly greater interacting strength, which infer greater unfolding. The lysozyme > BSA > E-Alb at 303.15 K order of densities shows an effect of thermal energy on the their reactions carried out by the enzymes in the biological systems (Table I). The S_d values are found in order of BSA > E-Alb > lysozyme, E-Alb > lysozyme > BSA, and E-Alb > BSA > lysozyme at 293.15, 298.15, and 303.15 K, respectively. These infer an influence of compositions on the protein–water and protein–protein linkages and shows variation in densities at each temperature. The greater S_d values at 293.15 K prove larger concentration effect on protein–protein interactions with structure-breaking action of proteins on the hydrogen bonded water [Fig. 2(b)] molecule, while at 298.15 and 303.15 K, the lower values are found that predict slightly weaker composition effect on interactions (Table II) with unfolding of protein structures due to thermal energy.

TABLE III
Energy Functions Associated with Viscous Flow of Solutions

Temperature (K)	conc. % 10^3 kg m^{-3}	S_E (K)	E^* (10^{-5}) (J mol^{-1})	ΔH (J K^{-1})	v_f (10^{-2}) (m s^{-1})	log A (10^{-2}) (m s^{-1})	ΔS (J K^{-1})	ΔG (J mol^{-1})
BSA								
293.15	0.0010	−0.0010	1914.7	−4871.9849	9.77	0.98903	58.1868	−21920.7
298.15	0.0014	−0.0011	2106.0	−4955.1220	9.29	0.96573	57.9320	−22218.9
303.15	0.0018	−0.0008	1532.0	−5038.2687	9.20	0.96198	57.8407	−22564.0
E-Alb								
293.15	0.0010	−0.0009	1723.2	−4871.9868	9.77	0.98668	58.1673	−21915.0
298.15	0.0014	−0.0010	1914.7	−4955.1249	9.57	0.97781	58.0324	−22248.8
303.15	0.0018	−0.0010	1914. 7	−5038.2649	9.39	0.97094	57.9152	−22586.6
Lysozyme								
293.15	0.0010	−0.0011	2106.2	−4871.9830	9.45	0.97378	58.0599	−21883.5
298.15	0.0014	−0.0011	2106.2	−4955.1230	9.23	0.96332	57.9119	−22212.9
303.15	0.0018	−0.0011	2106.2	−5038.2623	9.08	0.95595	57.7906	−22548.8

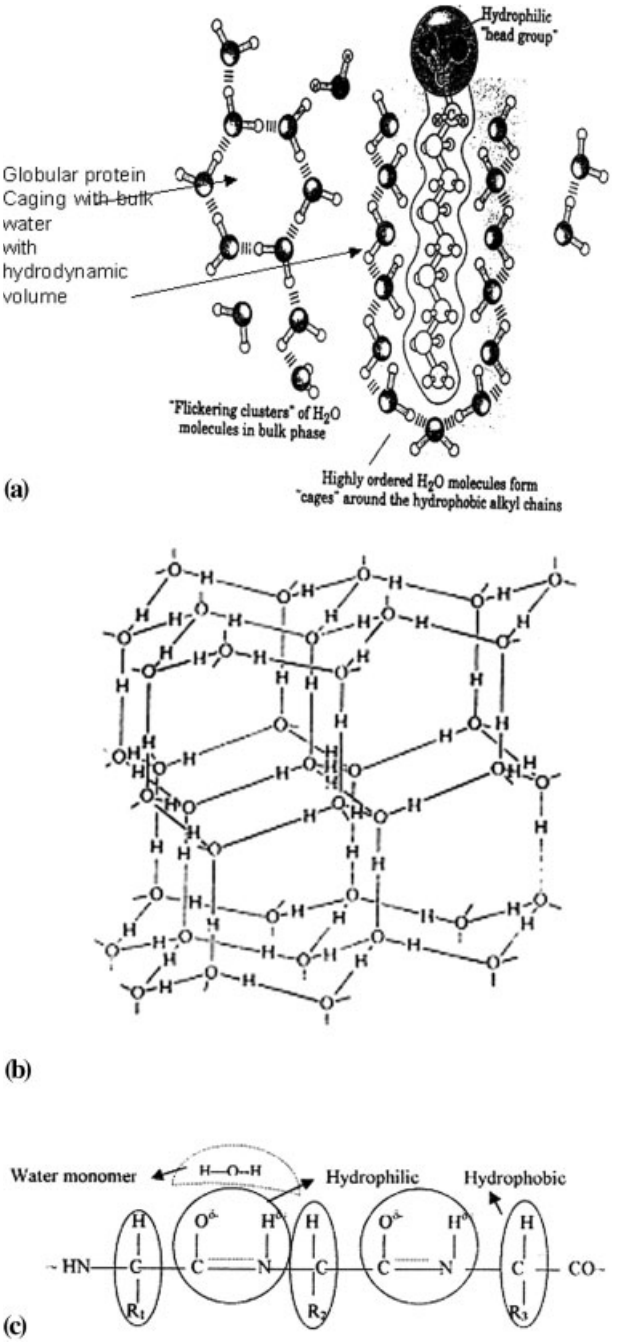

Figure 2 (a) Caging model, (b) structured water, and (c) Basic mechanism of protein–water interaction, R_1, R_2, and R_3 represent alkyl groups. Part (b) shows hydrogen-bonded clusters in liquid water (Reproduced with permission from Nemethy and Scheraga, J Chem Phys, 1962, 36, 3382, © American Institute of Physics, reproduced by permission).

The values of the V_ϕ function are a crucial tool to estimate a state of interactions and in general, its determination is unusual for globular proteins/macromolecules/biomolecules due to their extremely higher molecular weights. Thus, the V_ϕ values have been calculated after replacing m molality with g/100 mL (%), usually, the V_ϕ values increase with compositions confirming development of protein–protein–water interacting complex of the larger size except for the BSA at 293.15 K having an order of the V_ϕ values as 0.0018 > 0.0010 > 0.0014% and lysozyme at 298.15 K as 0.0014 > 0.0018 > 0.0010%, which illustrate clathrate formation with compositions and prove that presence of multipoles at close proximity may repel each other expanding their hydrated state. This is supposed to be a reason for such trends obtained for the systems, exceptions are attributed to temporary conformational states. The higher V_ϕ values of BSA around 0.0018% at 293.15 K and lysozyme around 0.0014% at 298.15 K show greater dominance of the BSA–BSA and lysozyme–lysozyme interactions. At around 0.0010% at 293.15 K, the V_ϕ values are found in order of BSA > E-Albumin > lysozyme with similar trends for the concentrations and temperatures (Table I). It concludes that the proteins of the larger size occupy larger V_ϕ, which is in contradiction with the V_ϕ values of the salts and polyelectrolytes.[37] Also the —NH—–water and —CO–water interactions are weaker than those of ions–water, and it can be inferred that attractive forces between multipoles of proteins and dipoles of water are weaker than those of between the ions and dipoles of water. Again, it seems that the proteins due to their multipoles form intrahydrogen bonds between the —NH— and —CO groups and contribute to the value of V_ϕ, which is absent in the ions. The trend of V_ϕ^0 values is found to be BSA > E-Alb > lysozyme at each temperatures. Since BSA is the bigger-sized molecule than those of the E-Alb and lysozyme, and the E-Alb is bigger than the lysozyme, but with temperature, each of them shows a trend as 298.15 > 293.15 > 303.15 K. This concludes that, at 298.15 K, they occupy larger volume and could be attributed to a dominance of the interactions with themselves, which could be extended to the fact that the globular proteins may have maximum activity at the normal temperature, and deviate below and above the latter temperature (Table II). An effect of concentration on the V_ϕ values is assessed from the S_v slope values (Table II), which are found in the order of lysozyme > BSA > E-Alb at 293.15 K, BSA > lysozyme > E-Alb 298.15 K, lysozyme > BSA > E-Alb 303.15 K, which can be amounted to the fact that E-Alb at all temperature has greater concentration effect on the V_ϕ values where considerably greater V_ϕ and V_ϕ^0 values reflect opening and packing of water molecules around the protein molecules. Such orders of the S_v values infer proteins weaker structure breaker and maker with temperature and composition, respectively.

The v_f values for a constant volume are different for the concentrations and temperatures, respectively, indicating that the hydrated complexes of proteins are disturbed on flow, while probably a primary hydra-

tion sphere detains its identity. This reflects that the viscosities increase with concentration, while decrease with the temperature (Table I), which confirms that the protein–protein complex of larger size hinders the viscous flow and develops torsional forces. This makes flow slow and results in higher values of viscosity and infer that the portions in aqueous medium make the non-Newtonian solutions.

An increase in the η_r values of polyelectrolyte solutions with dilutions[38] has been attributed to an expansion effect of polyion chains. Thus, the fundamental properties of the solutions show variations in trends as the alignment of counterions has been noted to weaken a screening effect with concentration of the biomolecules[39] because of an increase in molecular size. The latter has been correlated to an increase in intramolecular forces with an increase in the $(\eta_r - 1)/c$ values of the proteins, and the trend of the $(\eta_r - 1)/c$ values increase with concentrations that show greater structural reorientation in the systems. But at 293.15 K, the disparity is observed as in the case of BSA and lysozyme, where it first increases and then decreases as $16.1583 < 73.7590 > 57.7954$ and $8.8258 < 15.0848 > 12.5467$, whereas E-Alb follows a general trend. Also at 303.15 K, $(\eta_r - 1)/c$ values of lysozyme first decrease from 0.001 to 0.0014% and then increase at the next higher concentration, i.e., at 0.0018% as $7.1395 > 5.2987 < 5.7810$. Thus, the E-Alb is more prone to make effective non-Newtonian solutions, but at same temperature, the trends of these values at 293.15 K for 0.0010% are BSA > E-Alb > lysozyme, for 0.0014% are BSA > E-Alb < lysozyme, and for 0.0018% BSA > E-Alb > lysozyme (Table I). These data infer greater compositions influence on the interaction of the globular proteins because of the protein–protein pair wise interactions. At 209.15 K, the $(\eta_r - 1)/c$ values are found positive but at 298.15 and 303.15 K, the values for E-alb are found negative, which increase with its compositions, while for BSA and lysozyme, the values remains positive. In our calculation of the $(\eta_r - 1)/c$ values, the extended Jones-Dole's eq. (6) is applied, which gives positive values at lower temperature, but for 298.15 and 303.15 K, the E-alb gives negative values. The proteins, in general, give lower viscosities in aqueous solutions, which infer weaker structure breaking actions of the proteins on water and the water develop weaker cage around the protein molecules where entropies considerably increase; thus, for the selected proteins, the ΔS values are found about 58 J K^{-1}.

At 298.15 K and 0.0010%, the trend of $(\eta_r - 1)/c$ values is BSA > E-ALB < lysozyme, at 0.0014% it is lysozyme > BSA > E-Alb and at 0.0018% it is lysozyme > BSA > E-Alb. Similarly, at 303.15 K, starting from 0.0010 to 0.0018%, the orders are lysozyme > BSA > E-Alb, lysozyme > BSA > E-Alb, and BSA > E-Alb > lysozyme. These orders infer minimum hydrogen bonds formation with water at 298.15 K but reduced viscosities (Table I) decrease with the temperatures, which illustrate the weakening of their bonding with water along with heteromolecular forces. Thus, a lowering of the $(\eta_r - 1)/c$ values could be attributed to a compression of the chains due to repulsive interactions between them, as is proposed previously.[40] With protein, a long-range arrangement of water structure is destabilized and water monomers so formed get adhered to the charged sites of protein molecules and form protein–water complex [Fig. 2(c)]. The further addition of protein molecules possibly get engaged with unused monomer or get fitted into the protein–water complex, which expand the net size of the complex analogous to the behavior of polyelectrolytes in aqueous solution, where an increase in size[41] is reported to enhance the $(\eta_r - 1)/c$ values. Also on further increase in concentration, the protein molecules experience a competition with each other for water molecules and the spontaneity of interactions is enhanced. This seems to disrupt protein–water complex, thereby decreasing the overall size and hence decrease in the $(\eta_r - 1)/c$ values. The trends of the B values with temperature are observed as $298.15 > 293.15 > 303.15$, $293.15 > 298.15 > 303.15$, and $303.15 > 293.15 > 298.15$ K for the BSA, E-Alb, and lysozyme (Table II). These infer that the protein–water interactions do not deem fit to behave in a similar manner with temperature, therefore their denaturing mechanism differs in aqueous solutions, because with time and temperature, the viscosities of proteins changes, which infer that the chosen proteins make non-Newtonian fluids with water. This evinces the fact that their hydration is a specific function of temperature, which results in a variation in size of hydrated complex, for example, at 293.15, 298.15, and 303.15 K, the trends of the B values are as E-Alb > lysozyme > BSA, BSA > E-Alb > lysozyme, and lysozyme > BSA > E-Albumin. As the B values elucidate the state of hydration and disruption of hydrogen bonds with temperature thus these trends mark specific structural spontaneity of these proteins. Specifically, the negative B values for E-Alb infer greater unfolding in water at slightly higher temperatures than of 293.15 K with holding of bulk water in its interstitial spaces that decrease the torsion forces or the toque of the molecules. An order of the values of the D coefficient at 293.15, 298.15, and 303.15 K is found as BSA > lysozyme > E-Alb, lysozyme > E-Alb > BSA, and E-Alb > BSA > lysozyme (Table II), respectively. The latter trends with compositions indicate greater structural interactions for BSA and the E-Alb at lower and higher temperatures respectively, due to greater structural reorientation. In terms of temperature, D values for BSA, E-Alb, and lysozyme are in the order $293.15 > 303.15 > 298.15$ K, $303.15 > 298.15 > 293.15$ K, and $298.15 > 293.15 > 303.15$ K, respectively. Here, the lysozyme shows maximum

spontaneity at 298.15 K, an optimum temperature, perhaps this feature of it allows its uses as enzyme for several biological activities.

The values of the V_Φ^0 and B parameters are found maximum at 298.15 K for BSA, 298.15 and 293.15 K for E-Alb, and 298.15 and 303.15 K for lysozyme, respectively. This denotes that protein–water interaction complex is of larger size at 298.15 K, but the B values depict that on flow, the hydrated complex do develop maximum torsion forces resulting in larger B values for BSA, E-Alb, and lysozyme at 298.15, 293.15, and 303.15 K. And the S_v values are noted maximum for the proteins at 303.15 K, while the maximum D values for the BSA, E-Alb, and lysozyme at 293.15, 303.15, and 298.15 K. Thus, such S_v and D values illustrate stronger volumetric interaction at 303.15 K and stronger hydrodynamic volume contribution at 293.15, 303.15, and 298.15 K, respectively. The V_Φ^0, ρ^0, B, ΔH^0, ΔS, ΔE^*, and ΔG are regressed further against temperature to illustrate its influence on the interaction dynamics of proteins (Table IV). Their slope values are found negative for these proteins, which depict effective spontaneity for structural reorientation but a no set trend of values is noted, which need further investigations.

The IR spectra [Fig. 3(a–c)] depict stretching frequencies for amino, carboxyl, and alkyl groups present in the globular proteins. A decrease in stretching frequencies is found for amino groups with concentration, as the frequency of vibrations is inhibited due to protein–protein linkage. The BSA, lysozyme, and E-Alb show 3360.00, 3322, and 3304.58 cm^{-1} stretching frequency for amino group but degree of broadness is greater for lysozyme and E-Alb but lower foe BSA, which infer weaker hydrogen bonding for BSA and stronger for others. Similarly, the stretching frequency for —CO group is 1632.25 cm^{-1} for E-Alb and higher for others, but frequencies of the carboxyl groups increase for E-Alb and lysozyme but remain constant for BSA; these reveal a key role of amino groups in structural interactions of them because of a lone pair of electrons. A decrease and then increase in frequencies for alkyl groups in E-Alb, and decrease in BSA and lysozyme, evinces that, with the concentrations, the reorientations in bulk water occur as there might be some groups within the hydrophobic part, which are accountable for the values obtained (Table V). The magnitude of the values of each group remains almost within the same range with concentrations, which elucidate the deformation/reorientation/conformations due to hydration without losing the identity of the basic structure of proteins.

TABLE IV
Regression Constants of Aqueous Proteins Systems with Respect to Temperatures[a]

System	BSA	E-Alb	Lysozyme
V_Φ^0			
$V_\Phi^0 t$ (10^{-3}) (m^3 kg^{-1})	71543.0	41575.0	35874.0
$V_\Phi^0 s$ (10^{-3}) (m^3 kg^{-1} K^{-1})	−22.3	−5.7	−23.7
ρ^0			
$\rho^0 t$ (10^3) (kg m^{-3})	1.0208	1.0720	1.0602
$\rho^0 s$ (10^3) (kg m^{-3} K^{-1})	−8.00E−05	−0.0003	−0.0002
B			
$B^0 t$ (mL g^{-1})	−95750	−42590	231118
$B^0 s$ (mL g^{-1})	−1.0801	−0.5141	2.6035
$B^0 s_1$ (mL^2 g^{-2})	643.18	296.03	−1551.60
ΔH	2.6269	−4539.5	2.5153
$\Delta H^0 t$ (J K^{-1})	−16.6280	0.0002	−16.6280
$H^0 s$ (J K^{-2})			
ΔS			
$\Delta S^0 t$ (J K^{-1})	68.301	65.551	65.948
$S^0 s$ (J K^{-2})	−0.0346	−0.0252	−0.0269
ΔE^*			
$\Delta E^{*0} t$ (J g^{-1})	0.1327	−0.0386	0.0211
$E^{*0} s$ (J g^{-2})	−0.0004	0.0002	5.00E−17
ΔG			
$\Delta G^0 t$ (J g^{-1})	−3064.6	−6778.6	−2389.7
$G^0 s$ (J g^{-2})	−64.323	−50.525	−66.522

[a] t and s represent the values of intercepts and slopes.

Energy functions

The orders of the E^* values for BSA, E-Alb, and lysozyme with temperatures are listed as 298.15 > 293.15 > 303.15 K, 293.15 > 298.15 = 303.15 K, and 293.15 = 298.15 = 303.15 K, respectively, and the trends at 293.15, 298.15, and 303.15 K are lysozyme > BSA > E-Alb, BSA = lysozyme > E-Alb, and lysozyme > E-Alb > BSA, respectively. These depict an increase in the E^* values at 298.15 K and infer that at this temperature, the higher energy is required by the groups present in globular protein molecules to reach to the transition state, whereas at 303.15 K, the low values are noted. The E^* values of E-Alb firstly increases and later the values become constant and of the lysozyme, the value remains same at temperatures taken and shows (Table III) that at higher temperature, the E^* values either increases or remains same, supporting the reasons already mentioned. The ΔG decreases with temperature in order of 293.15 > 298.15 > 303.15 K for the proteins, the ΔG at 293.15, 298.15, and 303.15 K decreases as lysozyme > E-Alb > BSA, lysozyme > BSA > E-Alb, and lysozyme > E-Alb > BSA, respectively. Thus, an increase in temperature drives the reaction in favorable direction and an element of structured ness of water effect is responsible for a decrease in ΔG, which infers weakening of the hydrophobic interactions at low temperatures. Thus, the structured ness of water increases the solubility of nonpolar molecules in it and has opposite implications to a usual meaning of the term hydrophobic interaction. Basically, the ΔG determines the magnitude of hydrophobic interactions and ΔH the difference in the magnitude of noncovalent interactions of proteins with water molecules.

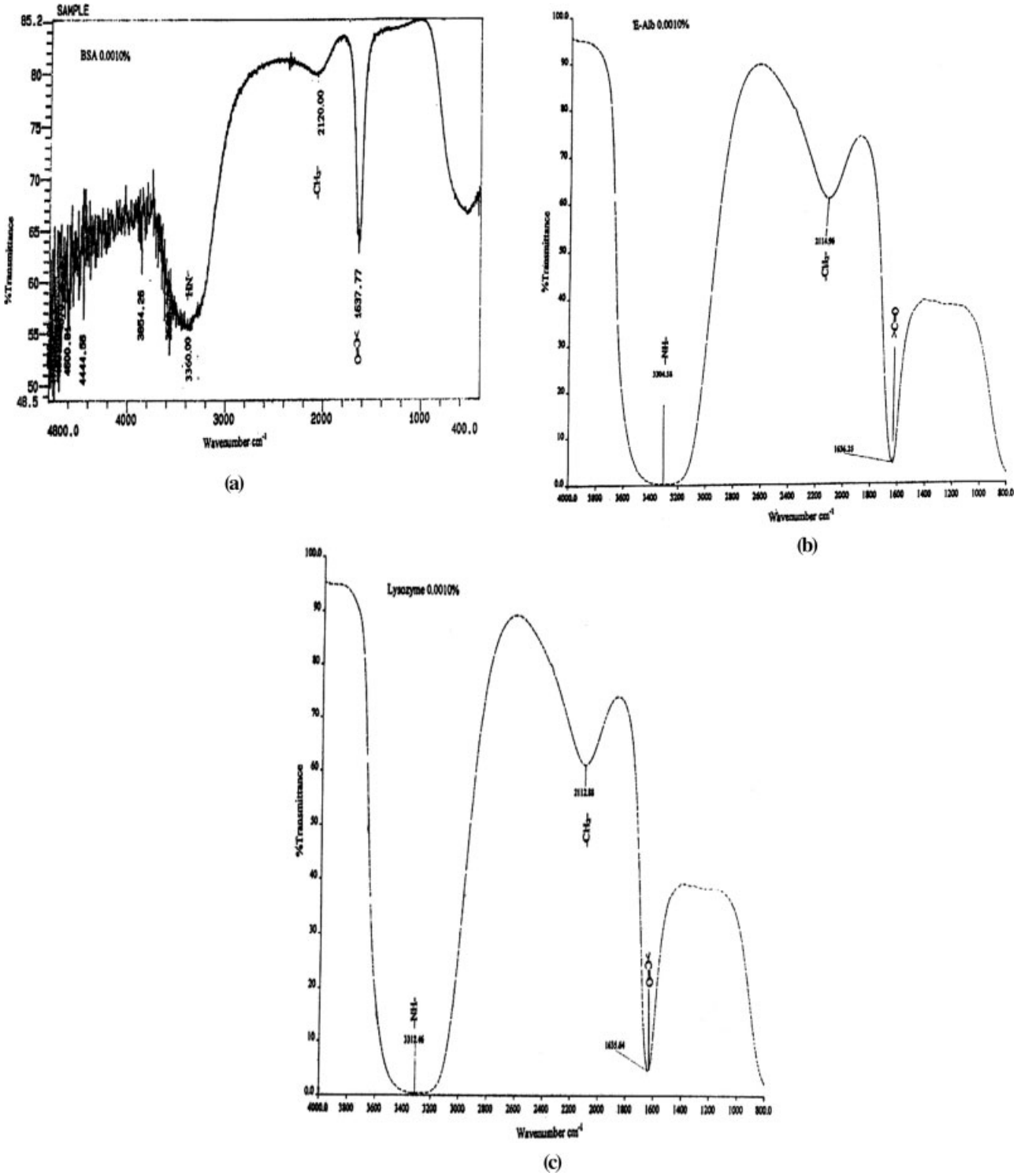

Figure 3 IR spectra for (a) 0.0010% BSA, (b) 0.0010% egg albumin, and (c) 0.0010% lysozyme.

The negative ΔG values favor the unfolding of proteins so that minimum energy is required for its stabilization under specific conditions of solvation (Table III). The ΔH of formation of hydrogen bonds in water produces a favorable decrease in enthalpy ΔH values in each protein with temperature as the molecules participating in hydrogen bonding, which might be relatively fixed in orientation and proximity. Incorporating the clathrate structures into the hydrotactoid results in a negative enthalpy of solvation change decreases with increase in temperature as 298.15 > 298.15 > 303.15 K. At higher temperature, there is the release of energy favoring the reaction in a favorable direction with temperature. The

TABLE V
Stretching Frequencies of Aqueous Protein Systems with Composition at 298.15 K

Proteins	Concentration (g mL^{-1})	—NH—	—CO	$—CH_2—$
BSA	0.0010	3360.00	1637.77	2120.00
	0.0014	3472.00	1637.77	2040.50
	0.0018	3489.67	1637.77	2040.00
E-Alb	0.0010	3304.00	1636.25	2114.96
	0.0014	3470.38	1637.77	2080.00
	0.0018	3570.69	1640.50	2380.00
Lysozyme	0.0010	3312.46	1635.64	2112.88
	0.0014	3303.19	1636.07	2112.37
	0.0018	3304.29	1636.56	2111.21

ΔH values of systems are listed as lysozyme > BSA > E-Alb, BSA > lysozyme > E-Alb, and lysozyme > E-Alb > BSA (Table III) at 293.15, 298.15, and 303.15 K, respectively. These depict the greater enthalpies for lysozyme at lower temperature perhaps due to changes in hydrogen bonding strength[42,43] and the entropies decreases with increase in temperature as 293.15 > 298.15 > 303.15 K. Thus, with the temperature, the proteins form clathrate surrounding their molecules, which in turn decreases the disorderliness. The Table III shows minor variation in entropies inferring relatively lower order of changes in orientation and proximity. At same temperature, the proteins show the variation at 293.15 K as BSA > E-Alb > lysozyme, at 298.15 K as E-Alb > BSA > lysozyme, and at 303.15 K as E-Alb > BSA > lysozyme. The v_f values decrease with temperature as 293.15 > 298.15 > 303.15 K, perhaps the larger heat content on solvation of proteins might break some of the bonds, which weakens the compact structure of protein–water complex. This in turn decreases the velocity of the flow but the order of values at 293.15, 298.15, and 303.15 K for proteins are as BSA = E-Alb > lysozyme, E-Alb > BSA > lysozyme, and E-Alb > BSA > lysozyme (Table III).

CONCLUSIONS

The temperatures and compositions are noted to influence the linkages of globular proteins with water where the negative enthalpies and positive entropies do infer several conformational states proving them to make non-Newtonian solutions. Notably, the enthalpies and entropies do compensate the free energies of viscous flow. The hydrophobic (structure making) or hydrophilic (structure breaking) activities of proteins have been rationalized with the densities and viscosities accompanied with effective spontaneity for solvalization. The B and $V_\phi{}^0$ values infer cage interactions due to hydrophobic interactions and reflect atomic volume of the molecule. An existence of broad bands in IR spectra infers that hydrophilic and hydrophobic interactions do occur.

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