African Journal of Biotechnology Vol. 7 (12), pp. 1807-1820, 17 June, 2008
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2008 Academic Journals

Full Length Research Paper

Volumetric and viscometric behaviour of soya bean and gram proteins in aqueous methotrexate (anticancer drug) solution at 298.15 to 308.15K

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Accepted 28 March, 2006

Apparent molar volumes (V_ϕ) and viscosities (η) for 0.00005 to 0.0004 kg.mol^{-1} (50 to 400 μmol kg^{-1}) aqueous methotrexate (MTX), gram (Gp) and soya bean (SBp) proteins, and similar compositions of Gp and SBp each in 50 to 400 μmol kg^{-1} aqueous MTX were determined at 298.15, 303.15 and 308.15K. The V_ϕ values are positive except aqueous MTX and listed as SBp > Gp > MTX in aqueous and SBp > Gp in MTX solutions, respectively. It infers weaker hydrophobic heteromolecular interaction of SBp in binary and ternary systems. The higher η values of SBp and Gp in MTX than those of aqueous MTX prove strengthening of hydrophobic interactions of proteins by MTX. It illustrates the conformational changes of proteins; slightly higher V_ϕ values of SBp than of Gp. The MTX confirm higher structural activity in biological process. The V_ϕ^0 of MTX increases with K and decrease with composition. But with compositions the values continuously decrease with lower magnitude.

Key words: Density, Apparent molal volume, viscosity B coefficient, methotrexate, soya bean, gram protein.

INTRODUCTION

Currently thermodynamics and transport functions of naturally occurring proteins in aqueous solution are of biophysical significance. Due to dominance of spectroscopic techniques, only limited physico-chemical studies are available in literature; soya and gram proteins have never been focused for such studies. Majority of proteins responds to aqueous and mixed solvent due to polyionic nature. Thus solvation has become an interesting tool for solute-solvent interactions, which gives some insights of structural interactions useful for biological activities due to conformational states. Therefore protein-drugs interactions have become the nucleus of biotechnological and pharmaceutical innovations. The physico-chemical characterizations of proteins in drug solutions have not been paid adequate attention and scarcity of such data with protein-drug solutions is noted. However such studies could render substantial help for drug design and understanding the physical basis of their structural interactions. Thereby in the present work the model studies with methotrexate (MTX), an anticancer drug was chosen for the studies of the ρ, V_ϕ, η and B data which depict internal state of the molecule in solution. This approximately calculates the state of intermolecular forces, hydrophilic and hydrophobic interaction. Additionally Several biological effects and action of MTX are given elsewhere (Farber et al., 1956; Weinblatt et al., 1985; Bookbinder et al., 1984; Baggott et al., 1993; Cronstein et al., 1991, 1993; Morabito et al., 1998;), and biochemical mechanism (Gao et al., 1998; Dolhain et al., 1998), influence on production in lymphocytes (Morgan et al., 1998; Nesher et al., 1997; Genestier et al., 1998) have dealt with, with biological aspects where no cognizance of physico-chemical studies is made. However HPLC studies of MTX with reverse phase are quoted elsewhere (Smolenski et al., 1990, 1993, 1991). Thus a brief literature review on studies of MTX with respect to biological relevance has found no physico-chemical reference yet, along with

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Table1[a]. Experimental and literatures density values along with apparent molal volumes and viscosities at 303.15k.

303.15K						
	Literature	**Experimental**	**Literature**	**Experimental**	**Literature**	**Experimental**
BSA(g%)	$\rho\pm/g.cm^{-3}$	$\rho\pm/g.cm^{-3}$	$V_\phi\pm/cm^3mol^{-1}$	$V_\phi\pm/cm^3mol^{-1}$	$\eta\pm/cp$	$\eta\pm/cp$
0.0010	0.99641	0.99642	64907.39	64907.41	0.7895	0.7894
0.0014	0.99622	0.99621	65070.20	65070.19	0.7992	0.7993
0.0018	0.99619	0.99619	65116.27	65116.27	0.8085	0.8085
Egg albumin						
0.0010	0.99625	0.99625	39890.08	39890.08	0.7614	0.7614
0.0014	0.99623	0.99622	39970.16	39970.14	0.7769	0.7770
0.0018	0.99623	0.99623	40010.54	40010.54	0.7917	0.7917
Lysozyme						
0.0010	0.99634	0.99633	39850.97	39850.95	0.8039	0.8040
0.0014	0.99621	0.99621	39977.65	39977.65	0.8041	0.8041
0.0018	0.99607	0.99607	40054.00	40054.00	0.8065	0.8065

a = Man Singh, Hema and K. C. Gupta; Chemistry & Biodiversity in press 2004.

effects of concentrations as gross elevation of uric acid due to excess nucleic acid degradation from dead cells (Pui et al., 1997; Simmonds et al., 1988; Herhert et al., 1999; Smolenski et al., 1998; Barankiewicz et al., 1991; Kalsi et al., 1998; Smits et al., 1998; McKendry et al., 1997; Kremer, 1995; Yamanaka et al., 1998). Soya bean and gram have been described (Beretta et al., 2000; Denten et al., 1998; Su et al., 1998) based on biological properties. Thus an estimation of intermolecular forces with water and proteins in aqueous solution is of interest to protein engineering (Mulder et al., 1839; Leiberg et al., 1841). Soya protein has cholesterol lowering effect in human serum (Carroll et al., 1995, 1980) with lysine and arginine rich soya protein exerting hypocholesterolemic effect (Potter, 1995; Sirtori and Lovati, 2001). Thus the present systems investigate a relevance of thermodynamic and transport functions ρ, V_ϕ and η for conformational states of proteins rationalizing a role of solvent for molecular forces between hydrophilic groups on protein backbone (Nagele et al., 1996; Plazzar et al., 1998; Dhont, 1996; Beretta et al., 1997; Durell et al., 1994). Such values focus size of solvent-protein complexes and torsional forces on Newtonian (Sear, 1999, Neal et al., 1999, Petsev and Vekilov, 2000). An electrostatic, covalent and van der Waals forces of proteins play key role in monitoring the conformationals (Eberstein et al., 1994; Hunter et al., 1907; Kossel, 1928; Park et al., 1992; Aparicio et al., 2003). Hence the studies substantiate the residual forces responsible for reorientation during solute-solvent and solute-cosolute-solvent interactions. Intensive functions ρ^0, V_ϕ^0 and B represent an internal state of protein molecule influence of concentration, and MTX due to a physical basis of interactions with water (Burley et al., 1998, Kollman et al., 1990) in relation to biological importance (Mahadevan and Hall, 1990; Kollman, 1977). A description of functional groups of proteins that monitor the interactions are explained elsewhere (Stiillinger et al., 1980, Mottonen et al., 1992, Ippolito, 1990). Comprehensively, such interactions of MTX may illustrate an optimization of globular proteins (McPherson et al., 1992; Muschol et al., 1997, 1995; Poon, 1997; Rosenbaum et al., 1996), which has stimu-lated for this work.

MATERIAL AND METHODS

Soya bean and gram were extracted from raw seed powder of soya and gram, respectively, and their purity was checked (David et al., 2000). The MTX (purity 98%, Aldrich), Gp and SBp solutions were prepared, while cleaned bicapillary pyknmometer and viscometer were calibrated with water with $\pm 0.00003/10^3$ kg mol^{-1} and $\pm 0.00004/0.1$ kg $m^{-1}s^{-1}$ accuracies, respectively, agreeing with reported values. The solutions were filled in them for density and viscosity, with special attention to prevent an evaporation of solutions. The aqueous BSA, egg albumin and lysozyme were run as controlled system and an agreement with our data given in Table 1. The densities, apparent molar volumes and viscosities of systems are measured and found as a linear function of temperature. The measurements were carried out in a thermo-statically controlled and well-stirred water bath with a temperature accuracy of ± 0.01°C, read on Beckmann thermometer.

Measurements

Calibration of Pyknometer and viscometer was checked with aqueous NaCl solutions (Mohammad et al., 2002) at 298.15K at a thermal stability of bath better than ± 0.01°C. A Hewlett-Packard quartz thermometer calibrated with gallium temperature standard measures the bath temperature and an accuracy of concentration of solutions was better than 1×10^{-5} m. The 0.99705, 0.99565 and $0.99404/10^3$ kg m^{-3} density of water (Alexander and Manzurola. 1999) at 298.15, 303.15 and 308.15K, respectively, were used. The calibration was repeated immediately before and after each measurement of density and reproducibility was better than 1×10^{-3} kg m^{-3}. The kinetic correction to energy of viscometer was calculated and found to be 1.8977×10^{-4}, 2.32647×10^{-5} and 2.32647×10^{-6} at

Table 2. Densities (ρ) of aqueous Methotrexate, Gram and Soya Bean Proteins with uncertainty in each data after ± σ sign at 298.15, 303.15 and 308.15K.

Aqueous Methotrexate			
	298.15K	**303.15K**	**308.15K**
m/ μ.mol.kg^{-1}	ρ±/10^{3}kg.m^3	ρ±/10^{3}kg.m^3	ρ±/10^{3}kg.m^3
50	0.99775±0.00029	0.99613±0.00029	0.99430±0.00029
100	0.99775±0.00029	0.99614±0.00029	0.99430±0.00029
200	0.99776±0.00029	0.99614±0.00029	0.99431±0.00029
400	0.99776±0.00029	0.99615±0.00029	0.99432±0.00029
Aqueous Gram protein			
50	1.00055±0.00029	0.99593±0.00029	0.99419±0.00029
100	1.00059±0.00029	0.99594±0.00029	0.99421±0.00029
200	1.00067±0.00029	0.99595±0.00029	0.99426±0.00029
400	1.00082±0.00029	0.99597±0.00029	0.99435±0.00029
Aqueous Soya Bean protein			
50	0.99742±0.00029	0.99586±0.00029	0.99409±0.00029
100	0.99742±0.00029	0.99587±0.00029	0.99410±0.00029
200	0.99744±0.00029	0.99588±0.00029	0.99412±0.00029
400	0.99747±0.00029	0.99591±0.00029	0.99414±0.00029

298.15, 303.15 and 308.15K, respectively. It depicts a negligible shear and not able to interfere the natural flow of the solution.

Theoretical

The ρ values were calculated from $\rho=((W-W_e)/(W_0-W_e))\rho_0+0.0012(1-(W-W_e)/(W_0-W_e))$ relation. The ρ solution, ρ_0 solvent and 0.0012x10^3 kg mol^{-1} air densities, respectively. The $(1-(W-W_e)/(W_0-W_e))$ is buoyancy correction for air, m molality, W_e, W_0 and W are weights of empty, solvent and solution filled pyknometer, respectively. Errors in ρ data are calculated from equations given below.

The V_{pyk} is pyknometer volume calculated from $(W_0-W_e)/\rho_0$ and V_ϕ data are computed with ρ from following equation:

$$V_\phi = M/\rho + 1000/(\rho_0-\rho)/\rho_0\rho\, m$$

The M is molar mass of solute. An uncertainty in V_ϕ is computed from the equation

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

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

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

$$V_\phi = (1000/m)\Delta\rho/\rho$$

The $\Delta\rho = \rho-\rho_0$, the viscosity η is calculated from relation $\eta = \eta_0(\rho.\, t)/(\rho_0.t_0)$

The t and t_0 flow times and η and η_0 are viscosity of solution and solvent and the η_{rel} (relative viscosity) is calculated from η/η_0. Like ρ, the errors in η data were obtained.

RESULTS AND DISCUSSION

The ρ, V_ϕ and η data are least square fitted against m for values at infinite dilution referred to as limiting values) from the following equation:

$$\rho = \rho^0 + S_d\, m$$

The ρ^0 is limiting density and S_d slopes. The V_ϕ is fitted in equation

$$V_\phi = V_\phi^0 + S_v\, m + S_v^*\, m^2$$

The V_ϕ^0 is limiting constant, S_v and S_v^* are slopes. The V_ϕ^0 focuses solute-solvent and the S_v and S_v^* the solute-solute and charge-charge interactions, respectively. The η_{rel} is fitted to extended Jones-Dole equation (Singh et al., 2005) given below:

$$(\eta_{rel}-1)/m = B + Dm + Cm^2$$

The B (kg mol^{-1}) Jones-Dole coefficient, D (kg mol^{-1})^2 and C (kg mol^{-1})^3 are slopes measuring heteromolecular interactions. Tables 2-4 and 7-12 contains primary data,

Table 3. Aparent molal volumes (V_ϕ) of aqueous Methotrexate, Gram and Soya bean proteins with uncertainty in each data after ± σ sign at 298.15, 303.15 and 308.15K.

Aqueous Methotrexate			
	$V_\phi \pm \sigma / 10^6 m^3 mol^{-1}$	**$V_\phi \pm \sigma / 10^6 m^3 mol^{-1}$**	**$V_\phi \pm \sigma / 10^6 m^3 mol^{-1}$**
m/ μ.mol.kg^{-1}	298.15K	303.15K	308.15K
50	-13594.80±0.00004	-9317.01±0.00002	-4724.14±0.00001
100	-6597.43±0.00007	-4459.39±0.00005	-2167.22±0.00003
200	-3094.16±0.00014	-2030.58±0.00001	-888.77±0.00005
400	-1336.79±0.00029	-808.12±0.00002	-249.54±0.00011
Aqueous Gram protein			
50	-4223.87±0.00017	60632.85±0.00001	63374.60±0.00001
100	30478.13±0.00035	63391.05±0.00003	64640.12±0.00002
200	47825.32±0.00072	64768.4±0.00006	65270.54±0.00004
400	56491.30±0.00151	65456.46±0.00013	65581.06±0.00013
Aqueous Soya Bean protein			
50	73327.33±0.00002	76565.11±0.00001	140635.20±0.000003
100	76948.43±0.00004	78632.51±0.00002	110733.10±0.000006
200	78758.15±0.00008	79663.07±0.00005	95781.22±0.000015
400	79661.35±0.00017	80177.87±0.0001	88303.50±0.000042

Table 4. Viscosity (η) of aqueous Methotrexate, Gram and Soya Bean Protein with uncertainty in each data after ±σ sign, cp= 10g cm^{-1} s^{-1}, at 298.15, 303.15 and 308.15K.

Aqueous Methotrexate			
m/μ.mol.kg^{-1}	**298.15K**	**303.15K**	**308.15K**
	η±σ/cp.	η±σ/cp.	η±σ/cp
50	0.9096±0.0033	0.8113±0.0024	0.7290±0.0016
100	0.9088±0.0015	0.8106±0.0010	0.7284±0.0006
200	0.9072±0.0006	0.8093±0.0004	0.7277±0.0003
400	0.9040±0.0002	0.8065±0.0002	0.7274±0.0002
Aqueous Gram protein			
50	1.0017±0.0239	0.8697±0.0170	0.7364±0.0037
100	0.9976±0.0114	0.8690±0.0083	0.7357±0.0016
200	0.9973±0.0056	0.8676±0.0040	0.7343±0.0006
400	0.9967±0.0027	0.8646±0.0018	0.7314±0.0002
Aqueous Soya Bean protein			
50	1.0533±0.0355	0.8779±0.0191	0.7393±0.0044
100	1.0525±0.0176	0.8778±0.0094	0.7383±0.0020
200	1.0510±0.0086	0.8775±0.0046	0.7364±0.0008
400	1.0480±0.0041	0.8770±0.0022	0.7328±0.0003

13-15 the regression constants, 5 and 6 regression constants of systems.

MTX > Gp > SBp sequence of ρ^0 values for binary systems at each K with 0.00162 and 0.00184 decreases for MTX, the 0.00157 and 0.00459 for Gp, and 0.00459 and 0.00175/10^3kg.m^{-3} for SBp from 298.15 to 303.15K and from latter to 308.15K (Table 5), respectively. It depicts a generation of higher internal pressure on the solutions due to stronger heteromolecular forces in sequence of MTX > Gp > SBp. The higher ρ^0 values than of water at each K conclude their stronger hydrogen bond formation with water. Firstly, the hydrogen bonded water is broken and interact strongly with MTX and proteins; thus are referred to as water structure breaker. It reveals stronger MTX structural interactions than those of Gp and weaker of the SBp with water, due to its one –CONH-

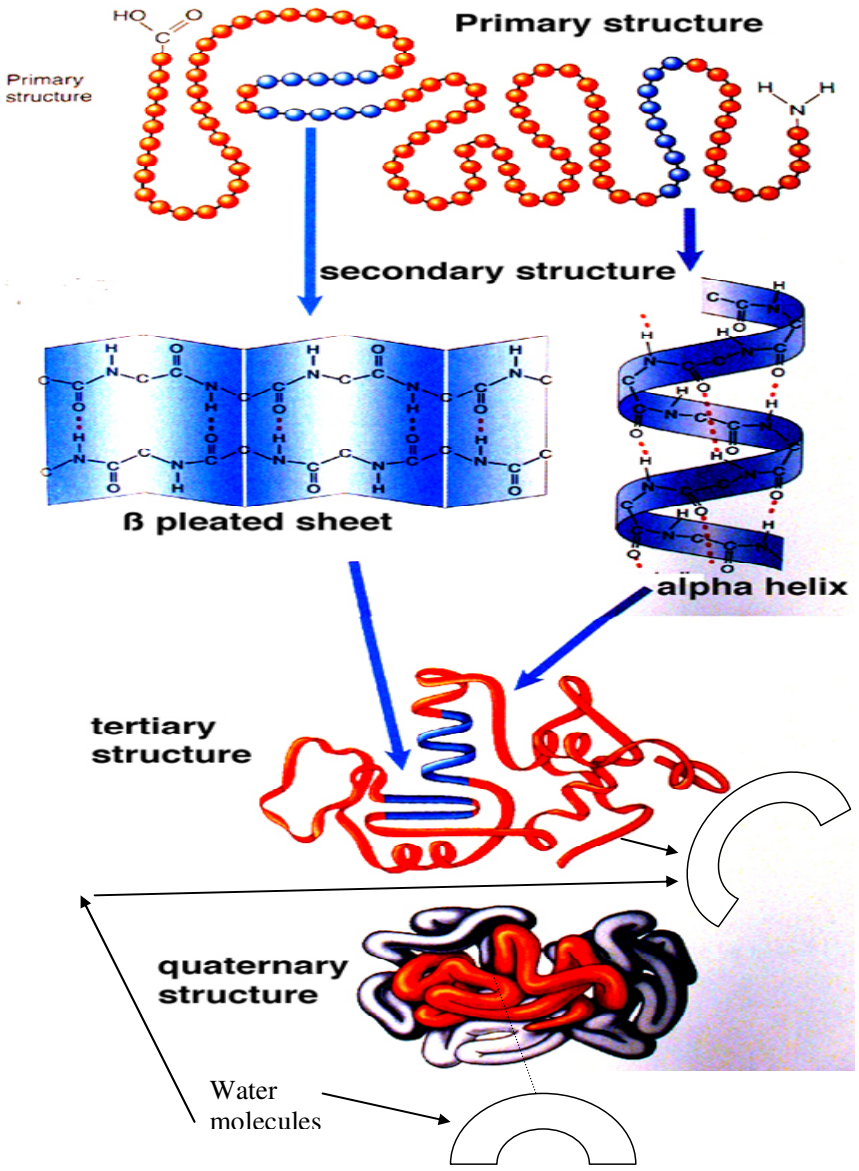

Figure. 1a. Protein-water-Methotrexate interaction.

(amide) and two -COO^- (carboxylic) groups at one (Figure 1) and two $–NH_2$ (amino) at another ends cause such behaviour. Secondly as compared to SBp, the Gp exert much internal pressure on water due to stronger structure breaker. As compared to MTX the hydrophilic interactions of the proteins are weaker than those of their hydrophobic. It infers that the amino acid residues in Gp are more polar with larger activities than of SBp and MTX. The Gp>SBp>MTX order of S_d values at each K, comparatively show a larger activity of Gp with concentration predicting Gp-Gp intermolecular interactions. The S_d values of Gp signify larger reorientation structure followed by destabilization with composition and conformational states. Such arrangement in their structures favors stronger Gp-Gp hydrophobic intermolecular interactions generating much of the internal pressure on the solutions. The Gp-Gp interactions weaken electrosrtiction of the water, causing mild water-protein interactions. Secondly such electrostatic changes could favor a cage formation around the Gp molecule applying larger internal pressure facilitating stronger hydrophobic interaction. Strategically such intermolecular engineering of the proteins is of biotechnological and biophysical use in regards to medium and the physical conditions. The 0.65916 and 0.35135, 0.00191 and 0.00913, and 0.00781 and $0.01851/10^{-1}kg^2.m^{-1}mol^{-1}$ variations in S_d values for Gp, SBp and MTX, respectively, for each 5°C elucidate mild effect of concentration on interactions with temperature. The lower S_d values for MTX prove weaker effect of compositions on MTX-MTX interactions at each K, con-

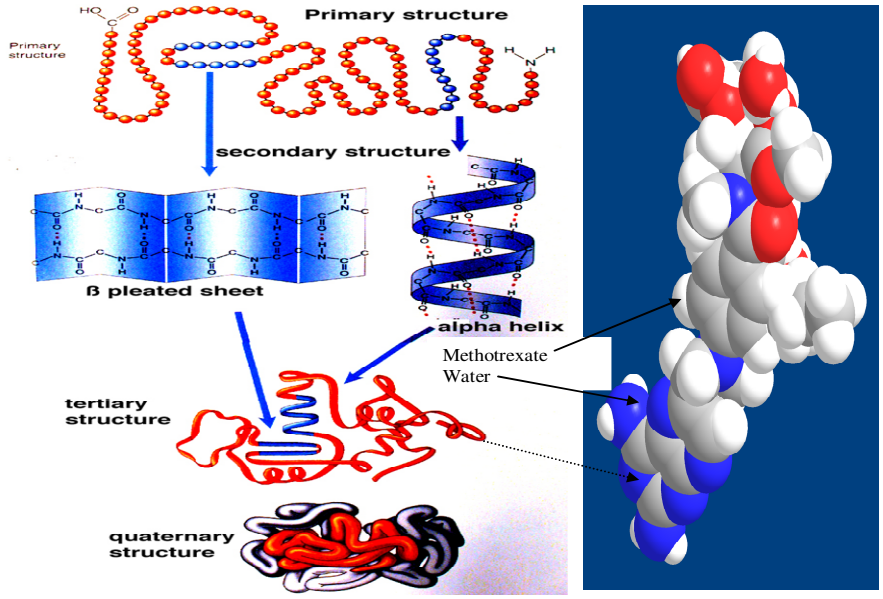

Figure 1b. Protein-water-Methotrexate interaction.

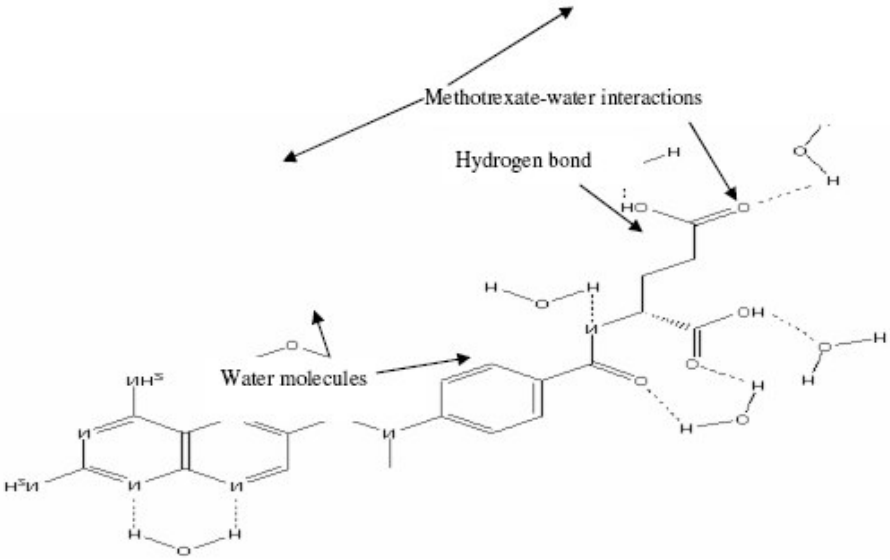

Figure 1c. Methotrexate-Water interaction

cluding that the hydrated MTX molecules may not further destabilize the water inferring stabilization of water structure breaking action. The V_ϕ^0 values for binary aqueous MTX are negative and for Gp and SBp the positive. Its SBp>Gp>MTX order is reverse to ρ^0 values supporting the stronger MTX intermolecular interactions with water. The MTX>Gp>SBp order of S_v is noted with values in a range of $2x10^7$ and $4x10^7/10^{-6}$ kg m^3 mol^{-2} resolving a larger expansion in size of the conformatory states but the ρ^0 failed to resolve such changes. This motivated us to determine the V_ϕ^0 values and mark a cage formation of larger size around protein molecules. Although it is contradictory in relation to the ρ^0 values, due to intermolecular force between MTX and water, and water itself, but such changes in size of the molecules are attributed to change in dipole moment. Larger expansion in SBp volume with composition at each K confirms slightly stronger volumetric interactions with weakening of hydrophobic forces as compared to other systems. An expansion in V_ϕ^0 predicts unfolding of tertiary structure with larger entropy. The V_ϕ values are fitted in polynomial against concentration predicting different intermolecular interactions around lower, mediocre and higher concentrations. The MTX>Gp>SBp and SBp>Gp>MTX

Table 5. Parameters in the equation $\rho = \rho^0 + S_d m$ and $V_\phi^0 = V_\phi^0 + S_v m + S_v m^2$ of aqueous Methotrexate, Gram and Soya Bean Proteins at 298.15, 303.15 and 308.15K.

Aqueous Methotrexate					
T/K	**$\rho/10^3$kg.m^{-3}**	**$S_d/10^{-1}$kg^{2}m^{-1}mol^{-1}**	**$V_\phi^0/10^6$m^3 mol^{-1}**	**S_vm/10^3kgm^{3}mol^{-2}**	**S_v*m2/kg^{2}m^{3}mol^{-3}**
298.15	0.99775	0.03982	-17970	1×10^8	-2×10^{11}
303.15	0.99613	0.04763	-12353	8×10^7	-1×10^{11}
308.15	0.99429	0.06614	-6322.2	4×10^7	-7×10^{11}
Aqueous Gram protein					
298.15	1.00051	0.77928	-25912	6×10^8	-9×10^{11}
303.15	0.99592	0.12012	58910	5×10^7	-7×10^{10}
308.15	0.99417	0.47147	62584	2×10^7	-3×10^{10}
Aqueous Soya Bean protein					
298.15	0.99741	0.13712	71064	6×10^7	-1×10^{11}
303.15	0.99584	0.13521	75275	3×10^7	-5×10^{10}
308.15	0.99404	0.14434	159324	-5×10^8	8×10^{11}

Table 6: Regression constants of $(\eta_r - 1)/m$ of aqueous Methotrexate, Gram and Soya Bean proteins at 298.15, 303.15 and 308.15K.

Aqueous Methotrexate			
T/K	**B/10^6 kg mol^{-1}**	**D/(10^6 kg mol^{-1})^2**	**C/(10^6 kg mol^{-1})^3**
298.15	472.8750	-3093040.32260	4976612903.22580
303.15	351.7500	-2319435.48390	3727419354.83880
308.15	239.8333	-1607774.19350	2637634408.60210
Aqueous Gram protein			
298.15	3174.0833	-20324338.70970	32910215053.76330
303.15	2268.3333	-14405741.93550	23176344086.02140
308.15	512.8750	-3357685.48390	5402419354.83870
Aqueous Soya bean protein			
298.15	4693.5833	-29685209.67740	47765053763.44070
303.15	2531.7917	-15972508.06450	25698655913.97830
308.15	617.1667	-4053258.06450	6523655913.97850

orders of S_v and S_V^* values are reverse of each other denoting stronger intermolecular interactions for higher compositions. The latter predicts more opening of tertiary structures. The S_V^* of SBp for higher concentration show larger expansion and the MTX the least with values ranging from 2×10^7 to $55\times10^7/10^{-6}$ kg^2 m^3 mol^{-3}.

The SBp>Gp>MTX sequence for B values show larger decrease with K with the values between 111.9167 and $2161.7916/10^{-3}$ kg mol^{-1}. From 2161.7916 to $1914.6250/10^{-3}$ kg mol^{-1} decrease in B values with K is noted for SBp proving a larger hydrodynamic volume than of others due to an effective conformational structure. It shows larger dependence of the stability of hydrodynamic size of their hydrated complex on thermal energy. The B values for MTX are lower by 2701.2083, 1916.5833, 273.0417 and 1519.5000, 263.4584, $104.2917/10^{-3}$ kg mol^{-1} than those of SBp and Gp at three temperatures. It proves weaker Newtonian force on viscous flow due to weakly stable water-MTX hydrogen bonding. But the B values of SBp are higher than of Gp by 1519.5000, 263.4584 and $104.2917/10^{-3}$ kg mol^{-1} at each K (Table 6) with reverse behavior of MTX.

Ternary systems

The ρ^0 values of proteins for aqueous MTX are found higher than those of the aqueous (Table 13) by 0.00079 to 0.00255 and 0.00090 to $0.00182/10^3$ kg m^{-3} at each K. The MTX asserts an additional internal pressure in solutions, credited to stronger protein-MTX interaction rather than water-proteins (Figure 1a). Here, the MTX with polar parts, foster the water structure breaking action of proteins weakening hydrogen bonds of water facilitating a cage formation of it and MTX around the proteins (Figure 1b). The hydrated MTX may be fitted into the interstitiary spaces of the β pleated sheets and α helix of the protein to channe-

Table 7. Densities (ρ) of Soya Bean protein in aqueous Methotrexate system with uncertainty in each data given ±σ sign at 298.15, 303.15 and 308.15K.

0.0004m						
298.15K			303.15K		308.15K	
m/μ.mol.kg^{-1}	ρ/10^{3}kg.m^3	± σ	ρ/10^{3}kg.m^3	± σ	ρ/10^{3}kg.m^3	± σ
50	0.99832	±0.0002	0.99712	±0.0002	0.99569	±0.0002
100	0.99833	±0.0002	0.99736	±0.0002	0.99594	±0.0002
200	0.99834	±0.0002	0.99785	±0.0002	0.99645	±0.0002
400	0.99837	±0.0002	0.99882	±0.0002	0.99747	±0.0002
0.0002m						
50	0.99786	±0.0002	0.99711	±0.0002	0.99567	±0.0002
100	0.99801	±0.0002	0.99735	±0.0002	0.99593	±0.0002
200	0.99836	±0.0002	0.99783	±0.0002	0.99643	±0.0002
400	0.99936	±0.0002	0.9988	±0.0002	0.99745	±0.0002
0.0001m						
50	0.99789	±0.0002	0.99663	±0.0002	0.99431	±0.0002
100	0.99797	±0.0002	0.99687	±0.0002	0.99456	±0.0002
200	0.99813	±0.0002	0.99736	±0.0002	0.99507	±0.0002
400	0.99844	±0.0002	0.99833	±0.0002	0.99609	±0.0002
0.00005m						
50	0.99777	±0.0002	0.99662	±0.0002	0.99237	±0.0002
100	0.99786	±0.0002	0.99686	±0.0002	0.99263	±0.0002
200	0.99812	±0.0002	0.99735	±0.0002	0.99314	±0.0002
400	0.99842	±0.0002	0.99832	±0.0002	0.99415	±0.0002

Table 8. Apparent molal volumes (V_ϕ) of Soya Bean protein in aqueous Methotrexate system with uncertainty in each data given ±σ sign at 298.15, 303.15 and 308.15K.

298.15K(0.0004m)			303.15K		308.15K	
m/μ.mol.kg^{-1}	**V_ϕ/10^{6}m^{3}mol^{-1}**	**± σ**	**V_ϕ/10^{6}m^{3}mol^{-1}**	**± σ**	**V_ϕ/10^{6}m^{3}mol^{-1}**	**± σ**
50	69467.23	±0.00003	61193.10	±0.00005	53175.17	±0.00007
100	74959.04	±0.00006	68495.60	±0.00012	64421.99	±0.00016
200	77720.15	±0.00012	72119.12	±0.00034	70016.37	±0.00043
400	79111.90	±0.00024	73875.50	±0.00107	72755.63	±0.00126
0.0002m						
50	78721.13	±0.00001	61179.72	±0.00005	53281.59	±0.00007
100	78156.03	±0.00002	68526.27	±0.00012	64512.82	±0.00016
200	77622.75	±0.00012	72196.67	±0.00034	70070.62	±0.00043
400	76547.72	±0.00064	73915.76	±0.00106	72783.47	±0.00126
0.0001m						
50	77832.10	±0.00001	70979.45	±0.00002	80827.13	±0.00002
100	78451.95	±0.00002	73406.34	±0.00007	78296.99	±0.00003
200	78752.49	±0.00008	74592.02	±0.00024	77002.83	±0.00015
400	78884.01	±0.00028	75129.44	±0.00088	76297.65	±0.00072
0.00005m						
50	80239.01	±0.00001	70924.74	±0.00002	120216.60	±0.0001
100	79522.31	±0.00001	73379.26	±0.00007	98060.54	±0.00017
200	78803.34	±0.00007	74578.76	±0.00024	86953.30	±0.00023
400	78952.34	±0.00027	75123.08	±0.00088	81341.36	±0.00006

Table 9. Viscosity (η) of Soya Bean protein in aqueous Methotrexate system with uncertainty in each data given ±σ sign at 298.15, 303.15 and 308.15K.

298.15K	0.0004m		0.0002m		0.0001m		0.00005m	
m/μ.mol.kg^{-1}	η/cp	±σ/cp	η/ cp	±σ/cp	η/ cp	±σ/cp	η/ cp	±σ/cp
50	1.0424	0.0046	1.0384	0.0038	1.0366	0.0035	1.0357	0.0033
100	1.0409	0.0021	1.0369	0.0018	1.0352	0.0016	1.0343	0.0015
200	1.0378	0.0009	1.0343	0.0007	1.0323	0.0006	1.0314	0.0006
400	1.0317	0.0003	1.0291	0.0002	1.0265	0.0002	1.0257	0.0001
303.15K								
50	1.0381	0.0047	1.0346	0.0040	1.0323	0.0036	1.0315	0.0034
100	1.0372	0.0022	1.0337	0.0019	1.0314	0.0017	1.0306	0.0016
200	1.0353	0.0010	1.0317	0.0008	1.0295	0.0007	1.0287	0.0007
400	1.0313	0.0004	1.0277	0.0003	1.0255	0.0002	1.0247	0.0002
308.15K								
50	1.0301	0.0040	1.0298	0.0039	1.0276	0.0035	1.0247	0.0029
100	1.0291	0.0019	1.0288	0.0018	1.0265	0.0016	1.0238	0.0013
200	1.0280	0.0008	1.0276	0.0008	1.0255	0.0007	1.0227	0.0006
400	1.0272	0.0003	1.0269	0.0003	1.0247	0.0002	1.0219	0.0002

Table 10. Densities (ρ) of Gram protein in aqueous Methotrexate system with uncertainty in each data given ±σ sign at 298.15, 303.15 and 308.15K.

0.0004m						
298.15K			303.15K		308.15K	
m/μ.mol.kg^{-1}	ρ/10^{3}kg.m^{-3}	±σ/10^{3}kg.m^{-3}	ρ/ 10^{3}kg.m^{-3}	± σ/10^{3}kg.m^{-3}	ρ/ 10^{3}kg.m^{-3}	± σ/10^{3}kg.m^{-3}
50	0.99855	± 0.0002	0.99768	± 0.0002	0.99660	± 0.0002
100	0.99855	± 0.0002	0.99768	± 0.0002	0.99660	± 0.0002
200	0.99857	± 0.0002	0.99770	± 0.0002	0.99662	± 0.0002
400	0.99859	± 0.0002	0.99772	± 0.0002	0.99664	± 0.0002
0.0002m						
50	0.99855	± 0.0002	0.99767	± 0.0002	0.99660	± 0.0002
100	0.99856	± 0.0002	0.99768	± 0.0002	0.99660	± 0.0002
200	0.99857	± 0.0002	0.99769	± 0.0002	0.99662	± 0.0002
400	0.99860	± 0.0002	0.99772	± 0.0002	0.99664	± 0.0002
0.0001m						
50	0.99854	± 0.0002	0.99768	± 0.0002	0.99661	± 0.0002
100	0.99855	± 0.0002	0.99768	± 0.0002	0.99662	± 0.0002
200	0.99856	± 0.0002	0.99770	± 0.0002	0.99663	± 0.0002
400	0.99859	± 0.0002	0.99772	± 0.0002	0.99666	± 0.0002
0.00005m						
50	0.99854	± 0.0002	0.99767	± 0.0002	0.99660	± 0.0002
100	0.99855	± 0.0002	0.99768	± 0.0002	0.99660	± 0.0002
200	0.99857	± 0.0002	0.99769	± 0.0002	0.99662	± 0.0002
400	0.99859	± 0.0002	0.99772	± 0.0002	0.99665	± 0.0002

lize the residual force that shrinks the molecules. Secondly MTX might be causing stronger hydrophilic interaction and hydrophobic with proteins making sandwiched between them or their hydrated complex. In either way it is reorienting and modifying structures and activity of the proteins hampering the hydrophobic force.

Table 11. Apparent molal volumes (V_ϕ) of Gram protein in aqueous Methotrexate system with uncertainty in each data given ±σ sign at 298.15, 303.15 and 308.15K.

0.0004m						
298.15K			303.15K		308.15K	
m/μ.mol.kg^{-1}	$V_\phi/10^6 m^3 mol^{-1}$	± σ	$V_\phi/10^6 m^3 mol^{-1}$	± σ	$V_\phi/10^6 m^3 mol^{-1}$	± σ
50	64840.81	± 0.00021	49984.15	± 0.00017	34855.88	± 0.00012
100	72657.40	± 0.00043	65264.16	± 0.00034	57743.69	± 0.00023
200	76566.00	± 0.00085	72904.47	± 0.00068	69187.91	± 0.00046
400	78519.20	± 0.00172	76723.54	± 0.00137	74908.92	± 0.00094
0.0002m						
50	64748.66	± 0.00021	49864.63	± 0.00017	34634.27	± 0.00012
100	72611.14	± 0.00043	65204.56	± 0.00034	57632.85	± 0.00023
200	76542.68	± 0.00086	72874.84	± 0.00068	69132.44	± 0.00046
400	78507.36	± 0.00172	76708.88	± 0.00137	74881.15	± 0.00094
0.0001m						
50	64731.96	± 0.00021	49795.35	± 0.00017	34177.78	± 0.00012
100	72603.15	± 0.00043	65169.79	± 0.00034	57404.10	± 0.00023
200	76539.06	± 0.00085	72857.31	± 0.00068	69017.56	± 0.00047
400	78505.92	± 0.00172	76699.98	± 0.00137	74823.20	± 0.00095
0.00005m						
50	64670.95	± 0.00021	49717.46	± 0.00017	34412.81	± 0.00012
100	72572.53	± 0.00043	65131.09	± 0.00034	57522.08	± 0.00023
200	76523.62	± 0.00085	72838.21	± 0.00068	69077.02	± 0.00047
400	78498.08	± 0.00172	76690.68	± 0.00137	74853.40	0.00094

Table 12. Viscosity (η) of Gram protein in aqueous Methotrexate system with uncertainty in each data given ±σ sign at 298.15, 303.15 and 308.15K.

m/μ.molkg^{-1}	0.0004m		0.0002m		0.0001m		0.00005m	
298.15K	η/cp	±σ/cp	η/cp	±σ/cp	η/cp	±σ/cp	η/cp	±σ/cp
50	1.0421	0.0035	1.0386	0.0033	1.0369	0.0046	1.0357	0.0039
100	1.0406	0.0016	1.0370	0.0015	1.0354	0.0021	1.0342	0.0018
200	1.0375	0.0006	1.0340	0.0006	1.0324	0.0009	1.0311	0.0007
400	1.0313	0.0002	1.0279	0.0001	1.0262	0.0003	1.0251	0.0002
303.15K								
50	1.0379	0.0036	1.0345	0.0034	1.0329	0.0047	1.0316	0.0040
100	1.0365	0.0017	1.0331	0.0016	1.0314	0.0022	1.0302	0.0018
200	1.0335	0.0007	1.0301	0.0007	1.0285	0.0009	1.0273	0.0007
400	1.0274	0.0002	1.0241	0.0002	1.0225	0.0003	1.0213	0.0002
308.15K								
50	1.0300	0.0035	1.0298	0.0029	1.0290	0.0040	1.0277	0.0039
100	1.0285	0.0016	1.0283	0.0013	1.0275	0.0018	1.0261	0.0018
200	1.0263	0.0007	1.0261	0.0006	1.0253	0.0007	1.0240	0.0007
400	1.0235	0.0002	1.0233	0.0002	1.0225	0.0002	1.0212	0.0002

It could affect normal growth based processes of the life; thus it ruptures the cancerous cells of the body curing the cancer patient. Gp > SBp sequence of ρ^0 values in MTX at each K with 0.00086 to 3.29044/10^3 kg m^{-3} lowering in values (Table 13), reveals a comparatively stronger Gp-MTX interactions with larger activity of the Gp along with stronger concentration effect. Thus water with MTX favors cage formation around Gp with stronger molecular forces due to hydrophobic interactions of Gp with MTX and weaker with SBp. The Gp causes stronger cohesive forces that increase with composition with higher ρ^0 values in MTX. The ρ^0 values of Gp and SBp systems decrease with composition and K. It elucidates an increase in residual forces attributed to solute-solute interac-

Table 13. The regression constants of ρ of proteins in aqueous Methotrexate system. The limiting density, ρ^0/g cm^{-3}, slope constants $S_d/10^3 g^2 cm^{-3} mol^{-1}$ at 298.15, 303.15 and 308.15K.

0.0004m				
Regression constants of Gram protein			Regression constants of Soya Bean protein	
T/K	$\rho^0/10^3 kg.m^{-3}$	$S_d/10^{-1} kg^2 m^{-1} mol^{-1}$	$\rho^0/ 10^3 kg.m^{-3}$	$S_d/10^{-1} kg^2 m^{-1} mol^{-1}$
298.15	0.99854	0.12174	0.99831	0.13913
303.15	0.99767	0.12174	0.99688	4.86087
308.15	0.99659	0.12174	0.99543	5.09043
0.0002m				
298.15	0.99854	0.13913	0.99758	4.33913
303.15	0.99766	0.13913	0.99687	4.82957
308.15	0.99659	0.12174	0.99542	5.07826
0.0001m				
298.15	0.99853	0.13913	0.99781	1.57043
303.15	0.99767	0.12174	0.99639	4.86087
308.15	0.99660	0.13913	0.99405	5.09043
0.00005m				
298.15	0.99854	0.14087	0.99769	1.87304
303.15	0.99766	0.13913	0.99638	4.86087
308.15	0.99659	0.15130	0.99212	5.08000

Table 14. The regression constants of V_ϕ of proteins in aqueous Methotrexate system.

0.0004m						
Regression constants of Gram protein				Regression constants of Soya protein		
T/K	$V_\phi^0/10^6 m^3$ mol^{-1}	$S_v m/10^3 kg m^3 mol^{-2}$	$S_v^* m2/kg^2 m^3 mol^{-3}$	$V_\phi^0/10^6 m^3$ mol^{-1}	$S_v m/10^3 kg m^3 mol^{-2}$	$S_v^* m2/kg^2 m^3 mol^{-3}$
298.15	59955	1E+08	-2E+11	66028	90000000	-1E+11
303.15	40434	3E+08	-4E+11	56636	10000000	-2E+11
308.15	20551	4E+08	-6E+11	46153	20000000	-3E+11
0.0002m						
298.15	59834	1E+08	-2E+11	79086	-9000000	-6000000
303.15	40277	3E+08	-4E+11	56570	1E+08	-2E+11
308.15	20260	4E+08	-6E+11	46285	2E+08	-3E+11
0.0001m						
298.15	59812	1E+08	-2E+11	77447	10000000	-2E+10
303.15	40186	3E+08	-4E+11	69470	40000000	-7E+10
308.15	19661	4E+08	-6E+11	82416	40000000	-7E+10
0.00005m						
298.15	59732	1E+08	-2E+11	80982	-2E+07	3E+10
303.15	40084	3E+08	-4E+11	69398	40000000	-7E+10
308.15	19969	4E+08	-6E+11	134071	4E+08	6E+10

tions but decrease with thermal energy indicating MTX, Gp and SBp stronger van der Waals forces (Papanagopoulos and Dondos, 1996; Tewari and Srivastav, 1992) with broken water. The higher densities illustrate stronger heteromolecular forces among MTX, protein and water increasing with concentrations. Probably the side effects of MTX in body could be rationalized due to the MTX-MTX interactions reported by Monahan et al. (1996, 1997). The possible sites of MTX molecule due to MTX-MTX interaction may change the pharmacokinetics including (1) gastrointestinal absorption, (2) Gp and SBp binding, (3) stronger MTX-MTX, Gp-Gp and SBp-SBp interactions. It could be due to the stronger electrostatic forces of pep-tide bonds due to weaker hydrogen bond-

Table 15. The regression constants of $(\eta_r - 1)/m$ of proteins in aqueous Methotrexate system.

Soya Bean protein in aqueous Methotrexate			
T/K	**0.0004**		
	B/kg mol^{-1}	**D/(kg mol^{-1})^2**	**C/(kg mol^{-1})^3**
298.15	639.00000	-4102000.00000	6600000000.00000
303.15	646.37500	-4085459.67740	6573387096.77410
308.15	540.87500	3467040.32260	5576612903.22580
0.0002			
298.15	532.83330	-3415387.09680	5502150537.63440
303.15	554.50000	-3506540.30710	5641935483.87090
308.15	541.41670	-3424725.00650	5512365591.39780
0.0001			
298.15	489.08330	-3148209.67740	5065053763.44080
303.15	496.75000	-3142661.29030	5056451612.90320
308.15	483.25000	-3053532.25810	4911290322.58060
0.00005			
298.15	465.08330	-2992919.35480	4813440860.21510
303.15	475.75000	-3010338.70970	4843548387.09670
308.15	410.08330	-2589629.03230	4161827956.98920
Gram protein in aqueous Methotrexate			
T/K	**0.0004**		
298.15	631.08330	-4051500.00000	6516666666.66660
303.15	644.20830	-4134201.61290	6649731182.79570
308.15	549.75000	-3539629.03230	5695161290.32250
0.0002			
298.15	539.25000	-3473467.74190	5588709677.41930
303.15	555.00000	-3572709.67740	5748387096.77410
308.15	544.50000	-3506548.38710	5641935483.87090
0.0001			
298.15	497.20830	-3207943.54840	5159408602.15050
303.15	513.00000	-3308064.51610	5322580645.16130
308.15	523.50000	-3374225.80650	5429032258.06450
0.00005			
298.15	465.75000	-3010338.70970	48435483387.09680
303.15	481.50000	-3109580.64520	5003225806.45160
308.15	489.37500	-3159201.61290	5083064516.12900

bonding with water against water-water molecules. Such interac-tions play key role in chemical denaturation and conform-ational state of proteins and contribution of MTX as is estimated with the present set of thermodynamic and transport properties. The SBp>Gp order of V_ϕ^0 values in MTX at each K indicates that the SBp is comparatively less packed. Thus its packing might be associated with the number of amino group and nature of amino acids of proteins. The ρ^0 values in MTX could be rationalized to strengthening of the protein-water interactions by MTX due to their stronger binding with each other exerting higher internal pressure. With MTX concentration, the ρ^0 of proteins decreases (Tables 7 and 10) weakening electrostatic forces between water and proteins. For higher MTX concentrations the ρ^0 values remains almost same depicting stabilizing effect on protein conformational states that overcomes the influence of protein concentrations. Thus stronger water-MTX-protein interactions exist facilitating their solvation, where MTX concentrations cause unrest increasing entropy leading to the unfolding of proteins. For SBp, an increase in MTX composition seems to weaken the intermolecular forces between protein and water as MTX decreases the electrorestriction of water by increasing MTX- water interactions (Figure 1c). The van der Waals forces operating among MTX, protein and water (Figure 1b) does monitor hydrophilic as well as hydrophobic interactions. The action of MTX for concentrations and temperature

remains similar for Gp (Table 13). Their V_ϕ^0 (Marshall, 1993; Pandey et al., 1987) in MTX with concentrations and temperature decrease while the S_v and S_v^* increase. It infers generation of slightly stronger intermolecular forces with stabilization of hydrophobic forces amounting to their restriction of rearrangement of bonds around higher concentrations. Their V_ϕ^0 values with MTX concentration decrease (Table 14) with a close agreement with the observations of (Marsh et al., 1995). Thus MTX develops higher internal pressure by strengthening heteromolecular forces shrinking the volume. Such action mechanism of MTX matches the exertion effect caused by nonsteroidal anti-inflammatory drugs reported (Dewan et al., 1998). Molecular ion of MTX causes stronger dipole-dipole and dipole-induced-dipole with polyions (proteins) interactions due to hydrogen bonding. Due to the larger sizes of the molecular and polyions, a weak London or dispersive molecular forces operate in solutions. The compositions enhance the magnitude of their contributions due to various types of interactions and temperatures. Critically these effects derived from the S_v and S_v^* values are positive and negative, respectively and show similarity with organic molecules in ethanol systems (Parmar et al., 2004; Fort, 1966).

The SBp>Gp sequence of B values in MTX (Table 15) with 7.9167 to 79.2917/kg.mol^{-1} decreases at each K is lower than of aqueous proteins by 37.0000 to 4024.5833/kg.mol^{-1} and predicts stronger influence of MTX on their hydrodynamic sphere. In this context the Gp is seen to be mild water structure breaker while SBp slightly strengthen the intermolecular forces. Such behaviors of them could be attributed to the torsional forces in complexes due to their geometry. Notably the η values for SBp and Gp are higher in MTX than in water proving protein-protein interaction as hindrance to the laminar flow causing stronger tortional forces (Tables 4, 9 and 12). The stronger intermolecular force on viscous flow causes stronger protein interactions with larger cohesive forces. This depicts that the cage model remains very effective and polyvalent polyions of proteins develop the hydration sphere of larger size. The B values are found higher (Table 15). The B values of SBp and Gp are lower in aqueous MTX than those of in water, it prove disruption of the cage model developing smaller sized hydration sphere due to the weakening of solute-cosolute-solvent interactions by Newtonian flow. Due to a large hydrophobic part in proteins and the possible dominance of the hydrophobic and hydrohpilic groups, their interactions in MTX are expected to produce lower B values as compared to those in water. The positive B values denote a net structure-promoting tendency of MTX, also the MTX, SBp and Gp produce positive dB/dT values which are reported to be structure breakers in MTX, while in aqueous behave as structure maker. For aqueous proteins a slight decrease in the B values with K, denotes the thermal denaturation and a negligible decrease in B values in MTX, shows less denaturation. Thus the MTX bears with the thermal changes proteins structure at higher temperatures as is reported elsewhere (Nishida et al., 2002, 2001; Ramesh et al., 1992; Shitara et al., 2003).

Conclusion

The studies substantiate the naturally occuring proteins-anticancer drug interactions focusing a drug phamacokinetics and dynamics. Also the impact of composition and thermal changes on the solute-solvent and solute-cosolute-solvent interactions denoted with ρ^0, V_ϕ^0 and B functions. The V_ϕ^0 and slope values of proteins demonstrate that Gp casts stronger intra and intermolecular forces shrinking the molecule to a lower volume. The higher and lower S_v and S_v^* values for SBp in MTX prove that SBp-SBp interaction becomes stronger than that of Gp-Gp and higher value of slope proves stronger pairwise interaction.

ACKNOWLEDGEMENT

Authors are thankful to UGC, Govt. of India, New Delhi, for financial support and Mr. Ashok Kumar Yadav (Indian Army), for moral support.

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