ISSN 1996-3343

Asian Journal of Applied Sciences

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Asian Journal of Applied Sciences 3 (1): 13-24, 2010
ISSN 1996-3343

Viscometric Studies on the Molecular Association of Biomolecules Based on Thermodynamic Parameters Supported by UV Spectra

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Abstract: Density and Viscosity for ternary systems (amino acid + I_2 + water) containing amino acid viz; alanine, valine, leucine, isoleucine in aqueous solutions have been measured for several concentrations of amino acids as donors at different temperatures (303, 313, 323 K). Using above data internal pressure, absorbance, relative viscosity, Falkenhagen coefficient A, Jones-Doles coefficient B and thermodynamic parameters have been computed, Viscosities of the ternary mixtures have been correlated successfully using Jones-Doles and Vand's equation. Kinetic and thermodynamic parameters have been calculated to interpret 1:1 molecular association. The UV spectra have been used to support the mechanism of association in correlation with evaluated, observed and derived parameters.

Key words: Density, viscosity, molecular association, thermodynamic parameters, UV spectra, ternary mixtures

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INTRODUCTION

Viscometric property (De Ruiz-Holgado *et al*., 2001) has been regarded as a sensitive tool for understanding interactions in solution. The study of viscous behavior of macromolecules in solution is important in understanding the mechanism of transport processes. Viscosity and its derived parameters provide the valuable informations (Dasgupta and Hazra, 1989) regarding molecules.

Thermodynamic studies of systems (amino acids + iodine + water) are useful to understand several biochemical processes such as, protein dehydration, denaturation, aggregation etc. amino acids are very important from structural point of view as they are main building blocks of proteins and essential for the development of cells and tissues in human body. In some recent publications (Vasantharani *et al*., 2009; Pandey *et al*., 2000; Akhtar, 2007; Ali *et al*., 2007; Oswal *et al*., 2005; Riyazuddeen and Bansal, 2006; Thirumaran and Sahu, 2009) efforts have been made to correlate biological activity with calculated physical parameters with the help of densities and viscosities for binary/ternary/quaternary mixtures at different temperatures.

Molecular association of amino acids (Liner, 1964) with iodine in water proceeds in two stage Jones and Dole (1929) and Vand (1948) equations have been tested to study the extent of molecular association. Where, $(‰_0 - 1)/C^{0.5} = A + BC^{0.5}$ and $‰_0 = 2.5 \smile C$, are Jonnes and Dole (1929) and Vand (1948) equations.

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The absorption band of iodine I_2 reduces in intensity with increase in tri iodide ion (I_3^-) absorption band in the first stage. In the second stage with gradual loss of I_2 and I_3^- absorption is compensated by the appearance of iodide ion (I^-) absorption band leading to the formation of amino acid iodide. Where, $‰ = A/(C\ l)$, $l = 1$ cm and $\pi = b\,RT\,(K\,‰/u)^{1/2}\,(d^{2/3}/M_{eff}^{7/6})$, (‰) is molar extinction coefficient, (π) is internal pressure, K is a temperature dependent function equal to 4.25×10^9 for all liquids, R is gas constant, T is absolute temperature and b is cubic packing factor assumed to be 2 for all liquids and solutions.

The physicochemical and thermodynamic properties of amino acids are of considerable interest as these biomolecules are building blocks of all living organisms. According to Gibb's Helmholthz equation, (Glasstone *et al*., 1941) $\square G^* = \square H^*\text{-}T\ \square S^*$ and $\square G = RT\ \ln\ (‰V_m/hN)$, where, $\square G^*$, $\square H^*$, $\square S^*$ are standard change in free energy, enthalpy and entropy, respectively.

They are found to provide valuable information (Nikam *et al*., 2007; Banipal and Kapoor, 1999) leads to better understanding of proteins, which are large complex molecules. Therefore, an useful approach is to investigate interactions of model compounds of proteins e.g., amino acids in aqueous and mixed aqueous solutions. Some of these interactions are found implicated in several biological and physiological processes in living cells.

Recently, there has been increased interest to understand the role of water solvated to soluble organics in the living cells, because of the fact that most biological macromolecules are physiologically active in aqueous solutions. The choice of water for preparing mixed solvents systems is (Palani and Geetha, 2007) due to its important and unique role in determining the structure and stability of proteins since, its presence is known to give rise hydrophobic forces, which are of prime importance in stabilizing the native globular structure of proteins.

Ultrasonic studies made by other workers are confined to electrolyte systems in aqueous or non aqueous phase but studies of amino acids with non metals in aqueous medium have not been made widely. These are very important because the interactions of drugs in bio phase belong to this category of system. Iodine is a good acceptor and also present in traces in human body. The deficiency of iodine or its excess leads to disorder in the body. Density and viscosity measurements have been made at 303, 313 and 323 K to achieve a capable mechanism and model of drug action in bio phase.

MATERIALS AND METHODS

The amino acids viz; alanine, valine, leucine, isoleucine of high grade purity (> 99%) used in the present studies, were purchased from BDH (India) in 2008. The amino acids were recrystallized twice in (ethanol + water) mixture, dried at 383 K and kept in vacuum desiccator over P_2O_5 for 72 h before use. Triple distilled water is used for the preparation of amino acid solutions. The purity of amino acids has been checked by the determination of melting points.

A double stem calibrated pyknometer and Ostwald viscometer purchased from M/S Science Corporation, Allahabad, has been used to determine the density and viscosity of (solvent and solutions), respectively.

The procedure of calibration (Saksena and Aradhana, 1985) of pyknometer and viscometer has already been described in previous studies. Uncertainty in density and viscosity is nearly 0.01 and 0.02%, respectively. A properly insulated NBE type ultrathermostat has been used to circulate water at a precision of ±0.1°C. Perkin Elmer UV spectrophotometer (Lambda 25 UV/VIS systems) of Nano center, Physics Department, Allahabad University, Allahabad, has been used to record spectra. All measurements are carried out at 303 K. Fresh donor-acceptor solutions in water have been prepared by variation of solutions keeping total volume constant.

RESULTS AND DISCUSSION

The values of density and viscosity for all systems containing alanine, valine, leucine and isoleucine at different temperatures 303, 313 and 323 are given in Table 1. It is seen that density and viscosity increases with concentration of amino acids and decreases with increase in temperature, The increasing value of density and viscosity value shows (Roy *et al*., 2009) that there is moderate attraction with solute and solvent molecules. The decrease of values with temperature shows a decrease in intermolecular forces due to increasing thermal energy of system. Internal pressure (π) is fundamental property of liquid state and is measure of resultant forces of attraction and repulsion in liquid mixture Table 2.

An attempt has been made to correlate the results obtained thermodynamically and from spectroscopic parameters. Absorbance (A) decreases upto certain concentration. Decrease in A values are indicative of higher degree of interaction and spectral band is of less intensity. In the study of UV spectra, systems have been prepared from solutions of amino acids, KI and Iodine in a concentration range so that Beer-Lambert's law follows. Molar extinction coefficient (‰) has been calculated with the help of UV spectra for alanine, valine, leucine and isoleucine given in Fig. 1-4 and Table 2, respectively. With the help of viscosity data by using Jones-Dole (Banerjee and Kishore, 2005) equation graph is plotted in between (‰-‰$_0$ ‰)/‰$_0$C$^{0.5}$ vs. C$^{0.5}$, slope is B and intercept is A given in Fig. 5, Table 3 with their

Table 1: Density (d) and viscosity (‰) values for systems (amino acid + KI + I_2)

		d (kg m^{-3})			‰ (Kg/m/sec)		
		Temperature (K)			Temperature (K)		
Amino acid	C (mole L^{-1})×10^{-3}	303	303	313	303	313	323
A_1	5.57	1028.29	1027.06	1013.86	0.8467	0.7611	0.6762
A_2	6.58	1030.31	1027.23	1015.53	0.8622	0.7841	0.6976
A_3	7.59	1036.16	1027.39	1017.21	0.8814	0.8077	0.7198
A_4	8.60	1039.45	1027.56	1018.89	0.9025	0.8321	0.7426
A_5	9.61	1043.38	1027.73	1020.56	0.9261	0.8572	0.7661
A_6	10.63	1047.32	1027.89	1022.24	0.9522	0.8831	0.7904
V_1	5.49	1038.46	1037.22	1023.89	0.8633	0.7760	0.6894
V_2	6.49	1040.51	1037.39	1025.58	0.8773	0.7971	0.7113
V_3	7.49	1046.41	1037.56	1027.27	0.8951	0.8187	0.7338
V_4	8.49	1049.73	1037.73	1028.97	0.9147	0.8410	0.7571
V_5	9.49	1053.71	1037.90	1030.66	0.9366	0.8638	0.7811
V_6	10.49	1057.68	1038.06	1032.35	0.9612	0.8873	0.8058
L_1	5.69	1097.24	1041.13	1027.74	0.9126	0.8203	0.7288
L_2	6.73	1099.39	1041.30	1029.44	0.9274	0.8426	0.7519
L_3	7.76	1105.63	1041.46	1031.14	0.9462	0.8655	0.7758
L_4	8.80	1109.14	1041.63	1032.84	0.9669	0.8890	0.8004
L_5	9.83	1113.34	1041.80	1034.54	0.9902	0.9132	0.8257
L_6	10.87	1117.54	1041.97	1036.24	1.0161	0.9380	0.8519
I_1	5.49	1103.24	991.72	881.08	0.9142	0.7972	0.7153
I_2	6.49	1121.14	1018.67	909.01	0.9530	0.8409	0.7601
I_3	7.48	1143.91	1046.34	937.83	0.9824	0.8661	0.7842
I_4	8.48	1168.92	1074.77	967.55	1.0426	0.8897	0.8010
I_5	9.48	1197.02	1103.96	998.22	1.0577	0.9163	0.8347
I_6	10.48	1228.40	1133.95	1029.85	1.0803	0.9412	0.8869

Note: A: Alanine (A_1-A_6) are sets having different concentrations of Alanine, V: Valine (V_1-V_6) are sets having different concentrations of Valine, L: Leucine (L_1-L_6) are sets having different concentrations of Leucine, I: Isoleucine(I_1-I_6) are sets having different concentrations of Isoleucine

Table 2: Values of internal pressure (π_i) and molar extinction (‰) for systems (aminoacid + KI+I_2)

	‰		$\pi\times10^{-10}$	
	Temperature (K)		Temperature (K)	
Amino acids	303	303	313	323
A_1	90.0400	1.3158	1.2842	1.2352
A_2	8.2000	1.3275	1.2929	1.2375
A_3	-8.9030	1.3407	1.3087	1.2573
A_4	25.7090	1.3578	1.3168	1.2582
A_5	-12.6670	1.3777	1.3300	1.2680
A_6	12.5620	1.3966	1.3476	1.2882
V_1	63.7850	0.9538	0.9309	0.8954
V_2	97.5600	0.9613	0.9359	0.8971
V_3	38.8780	0.9699	0.9459	0.9114
V_4	36.3700	0.9813	0.9504	0.9121
V_5	19.2260	0.9947	0.9585	0.9192
V_6	35.7700	1.0073	0.9697	0.9338
L_1	281.1850	0.8884	0.8380	0.8060
L_2	228.0240	0.8954	0.8424	0.8075
L_3	71.5040	0.9035	0.8515	0.8204
L_4	53.9900	0.9140	0.8555	0.8210
L_5	56.0780	0.9265	0.8628	0.8274
L_6	32.0880	0.9383	0.8729	0.8406
I1	0.0113	0.8731	0.7823	0.7049
I_2	0.0165	0.8996	0.8113	0.7310
I_3	0.0339	0.9212	0.8359	0.7575
I_4	0.0467	0.9616	0.8549	0.7693
I_5	0.0294	0.9832	0.8788	0.7946
I_6	0.0275	1.0081	0.9050	0.8356

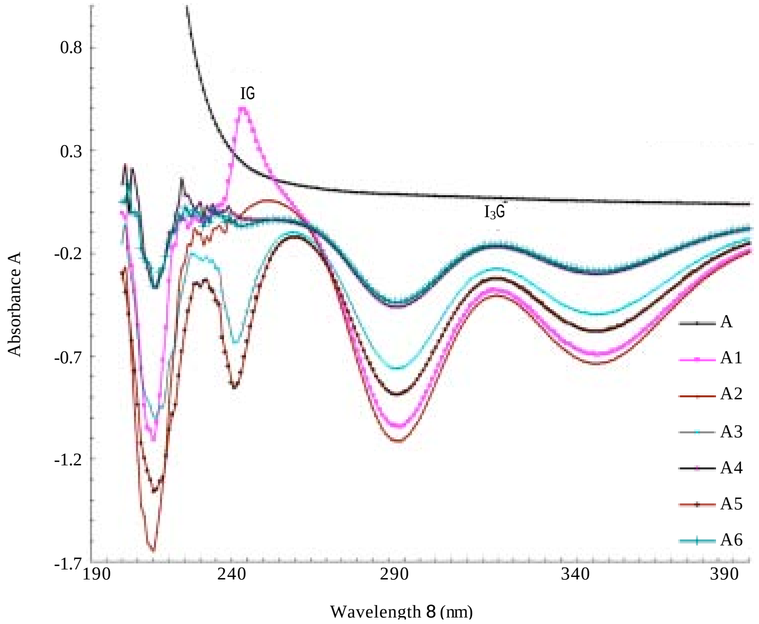

Fig. 1: Spectra of systems containing alanine at different concentrations, A: 5.06×10^{-2}, M; A_1-A_6 (Table 1)

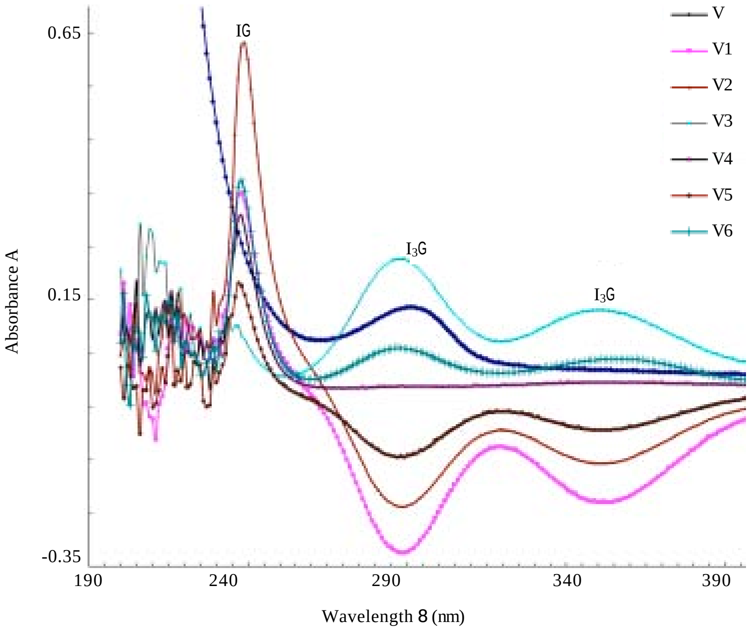

Fig. 2: Spectra of systems containing valine at different concentrations, V: 4.99×10^{-2}, M; V_1-V_6 (Table 1)

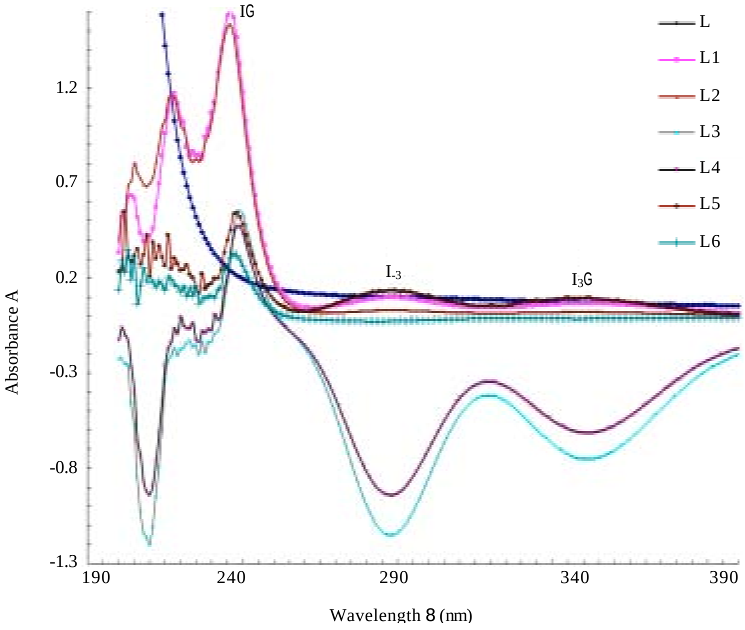

Fig. 3: Spectra of systems containing leucine at different temperatures, L: 5.176×10^{-2}, M; L_1-L_6 (Table 1)

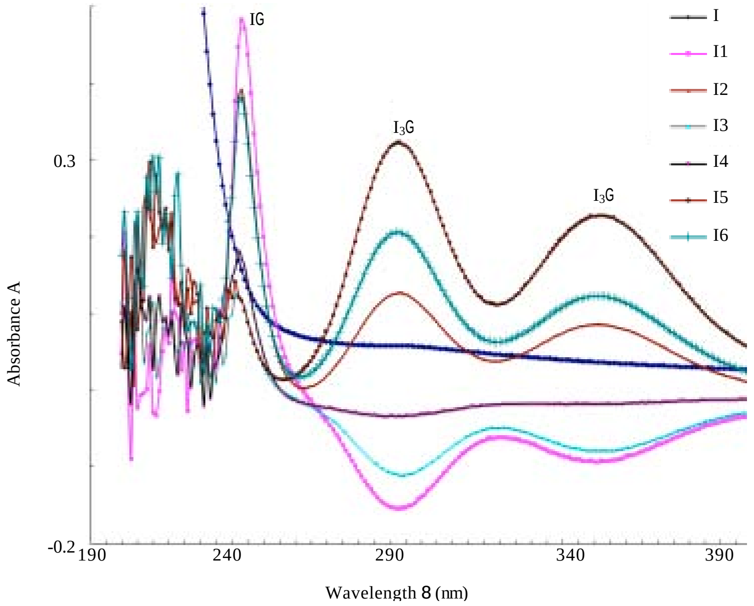

Fig. 4: Spectra of systems containing isoleucine at different concentrations, I: 4.98×10^{-2}, M; I_1-I_6 (Table 1)

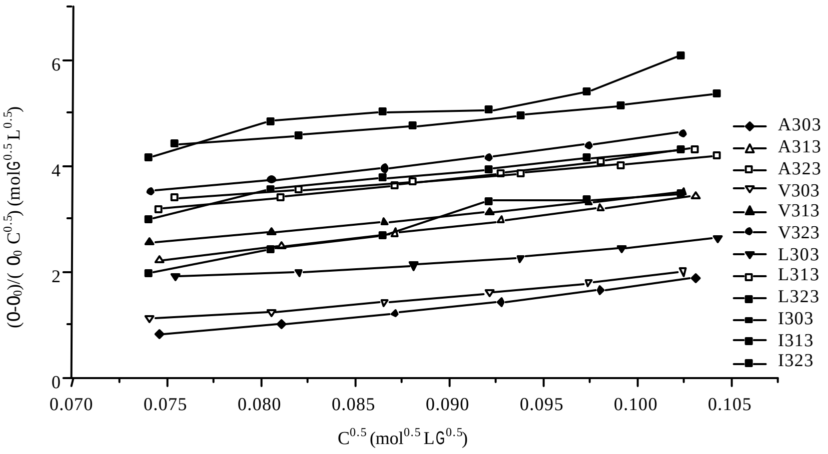

Fig. 5: Plot between $C^{0.5}$ and $(\eta-\eta_0)/(\eta_0C^{0.5})$ for amino acids systems (A: Alanine, V: Valine, L: Leucine, I: Isoleucine) at different temperatures

linear least square fit coefficients. In case of Vand's equation graph is plotted in between C/log‰$_r$ vs. C. Fig. 6 and values of regression coefficients are given in Table 4. At different temperatures (Sadeghi *et al*., 2009) various thermodynamic parameters □H*, □G* and □S* have been calculated for all amino acids given in Table 5 with the help of graph plotted in between ln (‰V$_m$/hN) and T shown in Fig. 7 along with their linear least square fit coefficients given in Table 6.

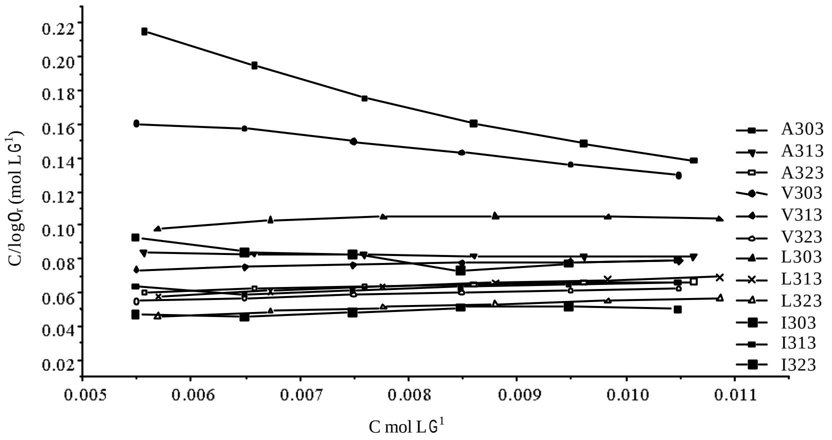

Fig. 6: Plot between C vs. C/logη$_r$ for amino acids systems (A: Alamime, V: Valine, L: Leucine, I: Isoleucine) at different temperatures

Table 3: Linear least square fit coefficients A and B for Jones - Dole equation for systems (amino acid + KI + I$_2$) at different temperatures (K)

Amino acids	A	B	R	S
A$_{303}$	-1.99808	37.17243	0.99543	0.04242
A$_{313}$	-0.93851	42.07965	0.99966	0.01313
A$_{323}$	0.12754	40.39197	0.99884	0.02314
V$_{303}$	-1.29204	31.67254	0.99243	0.04632
V$_{313}$	-0.00340	34.03063	0.99923	0.01579
V$_{323}$	0.58100	39.15684	0.99835	0.02661
L$_{303}$	-0.04885	25.08689	0.98302	0.05634
L$_{313}$	1.28940	27.49255	0.99724	0.02462
L$_{323}$	1.89630	32.72218	0.99588	0.03586
I$_{303}$	-2.07118	55.61065	0.96806	0.17014
I$_{313}$	-0.08780	43.55951	0.97241	0.12345
I$_{323}$	0.86486	47.47231	0.85718	0.33693

Table 4: Coefficients and intercepts of equation, C/log ‰$_r$ = mC + K by linear regression analysis for the systems (amino acid + KI + I$_2$) at different temperatures (K)

Amino acids	K	m	R	S
A$_{303}$	0.29495	-15.18950	-0.99069	0.00442
A$_{313}$	0.08641	-0.51553	-0.98055	0.00022
A$_{323}$	0.05437	1.17582	0.98718	0.00040
V$_{303}$	0.19706	-6.39373	-0.99536	0.00129
V$_{313}$	0.06802	1.06763	0.98598	0.00038
V$_{323}$	0.04691	1.51635	0.98878	0.00048
L$_{303}$	0.09479	1.00129	0.66521	0.00243
L$_{313}$	0.04565	2.22327	0.99091	0.00065
L$_{323}$	0.0346	2.07069	0.99201	0.00057
I$_{303}$	0.10329	-2.72628	-0.75606	0.00493
I$_{313}$	0.05573	0.89711	0.67788	0.00203
I$_{323}$	0.04056	1.03754	0.78265	0.00172

In the present study, there is only possibility of charge transfer interactions or colloisional complex formation between donor and acceptor molecule. Increase in $\square S^*$ with temperature indicates increase in repulsion energy between donor and acceptor molecule. In alanine, one methyl group is attached to aliphatic side chain in valine two methyl groups are connected to carbon in leucine methyl group is attached to C$_{‰}$. Leucine and isoleucine are

Table 5: Coefficients and intercepts of equation, ln (‰V_m)/hN = mT +K by linear regression analysis for the systems (amino acid + KI + I_2) at different concentrations

Amino acids	K	m	R	S
A_1	22.50602	-10.53610	-1.00000	4.08E-09
A_2	22.31417	-9.86474	-0.99923	0.00548
A_3	22.12443	-9.20913	-0.99656	0.01083
A_4	21.99875	-8.75378	-0.99316	0.01455
A_5	21.89654	-8.37493	-0.98908	0.01765
A_6	21.81314	-8.10272	-0.98530	0.01987
V_1	22.21429	-10.53610	-1.00000	4.08E-09
V_2	21.97162	-9.76484	-0.99960	0.00392
V_3	21.71095	-9.00933	-0.99818	0.0077
V_4	21.53863	-8.45408	-0.99662	0.00985
V_5	21.37718	-7.97533	-0.99495	0.01138
V_6	21.26245	-7.60322	-0.99379	0.01204
L1	21.55187	-7.97139	-0.98318	0.02094
L_2	21.22686	-7.20018	-0.97148	0.02486
L_3	20.94292	-6.44466	-0.95400	0.02864
L_4	20.78993	-5.88942	-0.93794	0.03079
L_5	20.65141	-5.41066	-0.92119	0.03232
L_6	20.51381	-5.03855	-0.90752	0.03298
I_1	19.78249	-1.02544	-0.66023	0.0165
I_2	19.74541	-0.81893	-0.55869	0.01719
I_3	19.90574	-1.33149	-0.70064	0.01918
I_4	20.66314	-3.72822	-0.86533	0.03054
I_5	20.36005	-2.76140	-0.80710	0.02857
I_6	19.82883	-1.04811	-0.35756	0.03871

Table 6: Various Thermodynamic parameters □G*, □H* and □S* for the systems (amino acid + KI + I_2) at different concentrations

	□S*			□G*			□H*		
	Temperature (K)			Temperature (K)			Temperature (K)		
Amino acids	0.303	0.313	0.323	0.303	0.313	0.323	0.303	0.313	0.323
A_1	-26383	-27259	-28136	48.66	49.988	51.302	-7945.2	-8482.2	-9036.7
A_2	-24691	-25512	-26333	48.69	50.035	51.369	-7432.7	-7935.3	-8454.3
A_3	-23039	-23806	-24572	48.71	50.075	51.428	-6932.3	-7401.2	-7885.4
A_4	-21892	-22621	-23349	48.74	50.119	51.485	-6584.6	-7030.2	-7490.3
A_5	-20938	-21635	-22332	48.77	50.162	51.540	-6295.4	-6721.5	-7161.6
A_6	-20252	-20926	-21601	48.77	50.167	51.552	-6087.6	-6499.8	-6925.5
V_1	-26385	-27262	-28139	47.92	49.228	50.518	-7946.7	-8483.7	-9038.3
V_2	-24442	-25255	-26068	47.90	49.225	50.536	-7358.1	-7855.5	-8369.3
V_3	-22539	-23289	-24039	47.82	49.162	50.491	-6781.5	-7240.3	-7714.0
V_4	-21140	-21844	-22547	47.81	49.166	50.510	-6357.7	-6788.0	-7232.3
V_5	-19934	-20598	-21262	47.77	49.136	50.491	-5992.3	-6398.1	-6817.1
V_6	-18997	-19630	-20262	47.76	49.140	50.506	-5708.3	-6094.9	-6494.3
L_1	-19923	-20586	-21250	48.21	49.594	50.964	-5988.4	-6393.9	-6812.7
L_2	-17981	-18580	-19179	47.98	49.376	50.760	-5400.2	-5766.2	-6144.2
L_3	-16078	-16614	-17151	47.84	49.252	50.653	-4823.8	-5151.0	-5489.0
L_4	-14679	-15169	-15659	47.88	49.307	50.724	-4399.9	-4698.6	-5007.2
L_5	-13473	-13923	-14373	47.90	49.336	50.767	-4034.4	-4308.6	-4591.9
L_6	-12536	-12955	-13374	47.83	49.281	50.720	-3750.4	-4005.6	-4269.2
I_1	-2421	-2507	-2592	49.05	50.647	52.237	-684.6	-734.0	-785.0
I_2	-1901	-1969	-2037	49.12	50.719	52.317	-526.9	-565.6	-605.7
I_3	-3192	-3303	-3414	49.13	50.718	52.303	-918.1	-983.1	-1050.4
I_4	-9230	-9540	-9851	49.21	50.737	52.258	-2747.5	-2935.4	-3129.5
I_5	-6794	-7024	-7254	49.18	50.736	52.283	-2009.5	-2147.8	-2290.8
I_6	-2478	-2565	-2653	49.15	50.749	52.342	-701.8	-752.2	-804.5

isomers with difference in positions of methyl group, it is branched amino acid and has asymmetric center at carbon. The interactions in these systems follow the sequence, may be due to hydrophilic character of side chain of amino acids. The observed thermo-acoustic

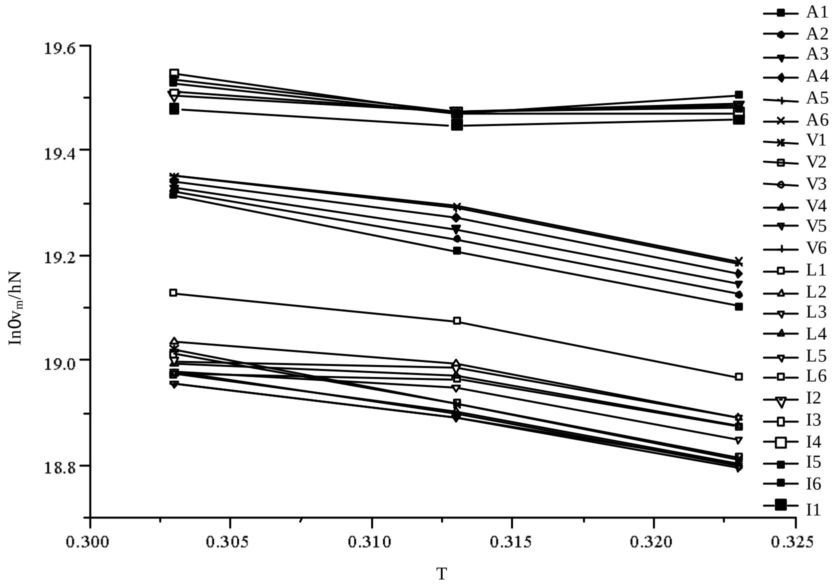

Fig. 7: Thermodynamic plot for various amino acid systems (A: Alamime, V: Valine, L: Leucine, I: Isoleucine) at different concentrations

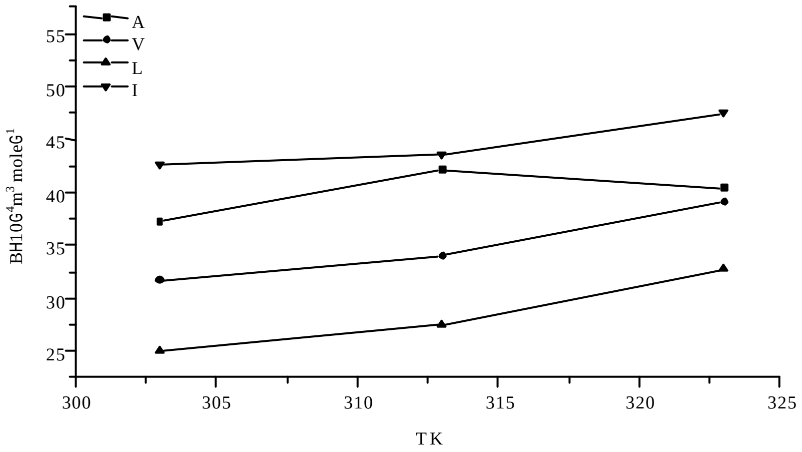

Fig. 8: Plot between B and T for various amino acid systems (A: Alamime, V: Valine, L: Leucine, I =Isoleucine)

parameters are explained on the basis (Ogawa *et al*., 1984; Wadi and Goyal, 1992) of inter-molecular interactions present in the mixture. The results are discussed in terms of structure making or structure breaking effects of amino acids in mixture can be explained by studying B with T shown in Fig. 8. The free energy of activation □G^* is almost constant for amino acids (Gupta and Singh, 2005) indicating charge transfer complexes in other systems. In the determination of viscosity A is Falkenhagen coefficient, a measure of solute-solute interactions and B the Jones-Dole coefficient an empirical constant and is indicative of solute-solvent (Taniewska and Nouica, 1993) interactions. The values of A and B and regression coefficients are summarized in Table 3 show that B coefficients are positive and larger than those of A coefficients suggesting the presence of strong solute-solvent interactions as compared to solute-solute interactions. The sign dB/dT is an indicative of structure making/breaking ability of solute (Ali *et al*., 2005) rather than size or sign of B-coefficient. The negative value of dB/dT implies a structure making, whereas positive value reflects structure breaking ability (Ojha *et al*., 2009) of solute.. The variation of B with T is depicted graphically in Fig. 8 shows it is positive for all amino acid studied revealing the structure breaking nature of amino acid in presence of I_2 and water.

CONCLUSIONS

The conclusions drawn from viscosity measurements are in agreement with those obtained from transition state treatment to obtain contribution of solutes to free energy of activation for viscous flow of solvent. The variation of B with T is depicted graphically in Fig. 8 shows it is positive for all amino acid studied revealing the structure breaking nature of amino acid in presence of I_2 and water. These parameters are found to be sensitive in exploring the interactions between the component molecules, which enable us to have a better understanding of liquid mixtures.

Interpretation of spectra although supports the conclusions drawn from viscosity measurements and thermodynamic parameters yet there are some variations depending on temperature and range of concentration taken in preparing ternary systems. With the limitations of recording the spectral bands after 400 nm, I_2 spectral band due to solvated iodine generally appears at 465 nm has not been recorded. However, due to interaction of Iodine on initially mixing with amino acids shows absorption bands nearly at 353 and 287 nm on account of I_3^- formation have been recorded with some spectral shift.

In systems containing alanine Fig. 1, there is shift in position of I_3^- (287 nm) absorption band at 317 nm may be due to increased molecular collisions and disappearance of I_3^- secondary band nearly at 353 nm. Figure 2, represents I_3^- spectral band at 287 nm and I_3^- secondary spectral band at 350 nm while in Fig. 3, I_3^- spectral band appears at 287 and 346 nm but shows low absorbance. In Fig. 4, I_3^- absorption bands are marked and distinct at 287 and 352 nm. In Fig. 1 the spectral band of I^- appears at higher wave length (288 nm) than the expected band at 240 nm, perhaps this may be due to increased molecular interactions between the donor and acceptor and other species while in Fig. 2-4 the I^- absorption band appears at 238, 236 and 238 nm, respectively. This is in accordance with the spectra of isoleucine and Iodine in previous studies.

Spectral changes occurring in similar systems with n- donors and I_2 as an acceptor have been interpreted as due to Charge Transfer interaction (Slifkin, 1971) between donor and acceptor. The mechanism of interaction can be proposed as follows:

$$\text{Amino acid}+2I_2 \rightleftarrows \underset{\text{CTC}}{[\text{Amino acid:I }]^+}+\text{I3}^-$$

The first stage is faster leading to CTC and I_3^- and the second stage is a slower reaction between amino acid and iodine in the CTC leading to a different form of amino acid iodide and recovery of I^- ions, as KI has been mixed initially with I_2 to increase solubility of Iodine in water. Possibility of ion pair formation (low concentration) or collisional complex formation between donor and acceptor (weak in nature) i.e., formation of NO BOND complex due to Van Der Waal's forces cannot be totally ignored.

ACKNOWLEDGMENT

The author is indebted to Dr. Avinash Chand Pandey for his help in providing UV spectrophotometer and thankful to department of Chemistry of the college for providing the necessary facilities.

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