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Research Article

THERMO-ACOUSTICS AND SOLVATION STUDY OF PROTEIN SALT SOLUTIONS USING ULTRASONIC TECHNIQUE

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ABSTRACT

Ultrasonic investigation finds extensive applications in probing in to the physico-chemical behavior of liquids leading to an understanding of the liquid state. This technique has been adequately employed to study the properties of any substance to understand the nature of molecular interactions in pure liquids and ionic interactions in protein salt solutions. The measurements of ultrasonic velocity of liquid play an important role in understanding the nature of molecular systems. The present work deals with the molecular interaction studies on some amino acids in non-aqueous solutions at various temperatures. From the measured parameters such as velocity, density, viscosity and some derived parameters like internal pressure, free volume, Rao's constant and Wada's constant are calculated. The study reveals the structural changes occurring in the solution through the FTIR study of L-Arginine derivatives in polar solvent. The diagnostic bonds observed at certain frequencies are assigned to the carbonyl stretch present is identified. The assignment of FTIR spectra has thrown light on the interaction between the solute and the solvent.

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INTRODUCTION

Amino acids (NH₂-CH-COOH-R, where R is a radical) are the basic units of proteins and peptides.¹ For an unknown reason nature has chosen 20 of these special molecules, differing in the R part, to form the impressive number of proteins found in our planet. Among other amino acids L-Arginine and its salts are known to protect protein. Due to the biological importance and large scale applications, L-Arginine and its derivatives are considered as samples for the study. Hence the study of these compounds in non-aqueous solutions is a new attempt, to understand the significant effects of salts on bio-molecules. N α -Benzoyl - L-Arginine (L-NBA) is an essential component of important biological molecules.² It is used in the study of peptide science and protein engineering which places significant demands. N α -Benzoyl L-Arginine was originally developed by Erlanger³ *et al.* as a trypsin substrate, is now widely used in the estimation of α_1 -antitrypsin. L-NBA is also necessary for inhibitory action.⁴ N α -p-Tosyl-L-Arginine methyl ester hydrochloride (L-TAME) increases nitric oxide production and acts as a vasodilator.⁵ It can be utilized as a substrate for the serine proteases trypsin, plasmin and thrombin. It has been used as a protease inhibitor,⁶ L-TAME has also been described to be a possible new cardiovascular risk factor among smokers.⁷

Experimental Technique

L-Arginine solutions and polar-protic solvent are used in the present study. With the high purity (99%) the samples are purchased from siscom research laboratories, Mumbai. The samples are measured using an electronic balance precise to 0.0001gm. Ultrasonic velocity (u) of L-Arginine salts in form amide is measured with a variable path interferometer (2MHz) with an accuracy of ± 2 m/s. The density (ρ) measurement is made with an accuracy of ± 0.001 gm. The following formulae are used for the computation of Internal Pressure (π_i), Free Volume (V_f), Rao's constant and Wada's constant, adiabatic compressibility, and solvation effect. All the solutions were prepared to the saturation molality by mass. FTIR spectrum of this solution was recorded in the region of 4000 – 400 cm⁻¹ using (PERKIN ELMER) model SPECTRUM RXI FTIR spectrometer. FTIR spectrum are taken from IIT Chennai.

1. Internal pressure

$$\pi_i = bRT \left(\frac{k\eta}{u} \right) \frac{1}{2} \times \left(\rho^{\frac{2}{3}} / M_{eff}^{\frac{7}{6}} \right) \text{atms.}$$

2. Free volume

$$V_f = [M_{eff} \times u / k\eta]^{\frac{3}{2}} \text{cc}$$

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3. Rao's constant
$$R = \frac{M_{eff}(U)^{\frac{1}{3}}}{\rho}$$
4. Wada's constant
$$W = \frac{M_{eff}}{(\rho)\beta^{-\frac{1}{7}}}$$
5. Adiabatic Compressibility
$$\beta = \left[\frac{1}{u^2 \rho} \right] \text{cm}^2 / \text{dyne}$$
6. Solvation Number
$$n_h = \left(\frac{n_s}{n_i} \right) \left[1 - \left(\frac{\beta}{\beta_0} \right) \right]$$

RESULTS AND DISCUSSION

Internal pressure & free volume

Internal pressure, free volume and temperature are the basic thermodynamic variables that describe the liquid systems of fixed composition.⁸ Internal pressure is closely related to the solubility parameters which determines the way in which the interactions occur in the system.⁹

The concept of free volume is an extension of the idea that each molecule is enclosed by its neighbors in a cell, the free volume is however not the whole cell volume but rather than average volume in which the center of the molecule can move inside the hypothetical cell due to the repulsion of the surrounding molecules.¹⁰

Internal pressure and free volume are the parameters, which are responsible for the structure of the liquids. At 5°C internal pressure of Nα-Benzoyl L-Arginine is high and at 15°C it has a rise as shown in Table 1.1 (a) and Figure 1.1 (a). From the study the cohesive energy of the solution is found to be increasing with respect to increase in Concentration and also structure making nature predominates in the solutions.¹¹

INTERNAL PRESSURE (atms)

Table 1.1(a) Nα - BENZOYL - L - ARGININE

Molality (m)	5° C	15° C	25° C	35° C	45° C	55° C
0.001	17527	15583	14076	12993	12021	10889
0.005	17780	16002	14562	13080	12151	10984
0.01	17944	16454	14672	13286	12334	11204
0.015	18668	17136	14773	13514	12569	11277
0.02	18960	17337	14928	13627	12651	11323

Table 1.1(b) Nα-p-TOSYL-L-ARGININE METHYL ESTER HYDROCHLORIDE

Molality (m)	5° C	15° C	25° C	35° C	45° C	55° C
0.001	19776	16566	14021	12816	11635	10566
0.005	20032	16598	14108	12901	11827	10919
0.01	20113	16740	14271	13024	11944	11142
0.015	20173	16781	14348	13287	12068	11201
0.02	20258	16886	14504	13285	12207	11256

These results are also confirms by FT- IR spectral studies.. But however the reduction in internal pressure shows the nature of dissociating tendency of the molecules in the solution.¹² But in the case of L-TAME the internal pressure value seems to increasing steadily with concentrations and temperatures as shown in Table 1.1(b) and Figure.1.1 (b).

INTERNAL PRESSURE (atms)

Figure 1.1(a) Nα - BENZOYL - L - ARGININE

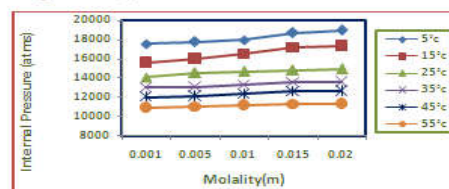
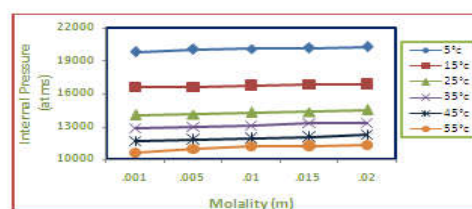


Figure 1.1(b) Nα-p-TOSYL-L-ARGININE METHYL ESTER HYDROCHLORIDE



These results supports that there may be strong solute solvent interaction occurring in the solutions.¹³ The increasing trend in internal pressure shows the orientation of the solvent molecules around the ions which may be due to the influence of electrostatic field of ions. This means that the solution become less compressible. It indicates the associating tendency of the molecules in the solutions. Free volume is one of the significant factors in explaining the variations in the physico-chemical properties of liquids.

FREE VOLUME (CC)

Table 2.1(a) Nα - BENZOYL - L - ARGININE

Molality (m)	5° C	15° C	25° C	35° C	45° C	55° C
0.001	0.0115	0.0180	0.0266	0.0367	0.0503	0.0733
0.005	0.0110	0.0165	0.0239	0.0357	0.0485	0.0710
0.01	0.0106	0.0151	0.0233	0.0341	0.0462	0.0670
0.015	0.0094	0.0134	0.0228	0.0323	0.0437	0.0654
0.02	0.0089	0.0129	0.0220	0.0315	0.0427	0.0645

Table 2.1(b) Nα-p-TOSYL-L-ARGININE METHYL ESTER HYDROCHLORIDE

Molality (m)	5° C	15° C	25° C	35° C	45° C	55° C
0.001	0.0080	0.0149	0.0269	0.0382	0.0556	0.0802
0.005	0.0076	0.0148	0.0263	0.0373	0.0528	0.0725
0.01	0.0075	0.0144	0.0254	0.0363	0.0511	0.0682
0.015	0.0074	0.0142	0.0249	0.0341	0.0494	0.0669
0.02	0.0073	0.0139	0.0241	0.0340	0.0475	0.0658

The molecules of the liquids are not closely packed and there is some free space between the molecules for movements which is called as free volume.¹⁴ The decreasing value of free volume

with molalities confirms the structure promoting nature in the systems, L-NBA, L-TAME Table 2.1(a, b) and Figure.2.1 (a, b). From the thermodynamical study of solutions of L-Arginine and its derivatives, the following results have been observed. In L-NBA, and L-TAME solutions, the structure of solvent is enhanced.

FREE VOLUME (CC)

Figure 2.1(a) Nα - BENZOYL - L - ARGinine

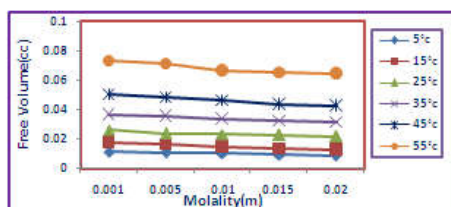
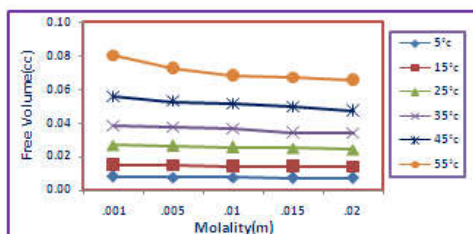


Figure 2.1(b) Nα-p-TOSYL-L-ARGININE METHYL ESTER HYDROCHLORIDE



Adiabatic compressibility

The velocity of propagation of ultrasonic waves in a solution is sensitive to the thermodynamic fluctuation of pressure, volume and temperature. The variations of acoustical parameters are due to the change in temperature and molality. The adiabatic compressibility values are given in Table 3.1(a) and Figure. 3.1(a). The adiabatic compressibility for L-NBA is found to be increases with increase in temperature. But at room temperature the β is found to be constant up to 0.01 molality and then increases with respect to concentration. It can be explained by the predominance of the associated molecules. This behavior shows that there may be association taking place between the molecules in the solution.

ADIABATIC COMPRESSIBILITY (10⁻¹¹ cm² / dyne)

Table 3.1(a) Nα - BENZOYL - L - ARGinine

Molality (m)	5° C	15° C	25° C	35° C	45° C	55° C
0.001	3.21	3.34	3.39	3.51	3.59	3.74
0.005	3.28	3.42	3.49	3.59	3.69	3.81
0.01	3.32	3.46	3.53	3.69	3.76	3.84
0.015	3.35	3.50	3.58	3.73	3.78	3.87
0.02	3.46	3.58	3.67	3.78	3.81	3.88

Table 3.1(b) Nα-p-TOSYL-L-ARGININE METHYL ESTER HYDROCHLORIDE

Molality (m)	5° C	15° C	25° C	35° C	45° C	55° C
0.001	3.20	3.26	3.31	3.37	3.43	3.48
0.005	3.23	3.29	3.35	3.41	3.48	3.52
0.01	3.27	3.32	3.37	3.44	3.51	3.58
0.015	3.30	3.34	3.39	3.49	3.54	3.62
0.02	3.34	3.38	3.45	3.53	3.58	3.66

This variation represents the existence of strong ionic bonding in the solutions due to the Zwitterions as a result of electrostatic forces.¹⁵ L-TAME the β value shows a linear increase with molality, in Table 3.1(b) and Figure.3.1(b). This behavior suggests that in those solutions, there is a strong association taking place between the molecules

ADIABATIC COMPRESSIBILITY (10⁻¹¹ cm² / dyne)

Figure 3.1(a) Nα - BENZOYL - L - ARGinine

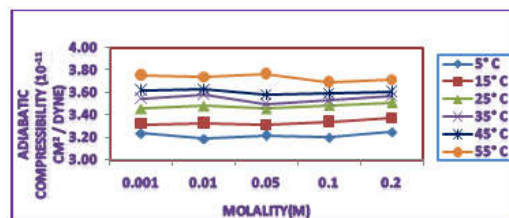
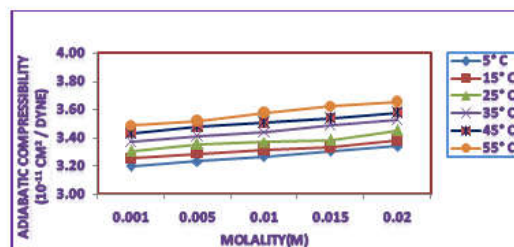


Figure 3.1(b) Nα-p-TOSYL-L-ARGININE METHYL ESTER HYDROCHLORIDE



Rao's constant & Wada's constant

Molar sound velocity or Rao's constant which is shown in the Tables 4.1(a, b) and Figure 4.1(a,b) is increasing with increasing concentration and also with temperature. The same trend is observed in molar compressibility or Wada's constant which is shown in the Tables 5.1(a,b) and Figs. 5.2(a,b). The non-linear variation of these constants indicates the existence of strong molecular association between the solute and solvent molecules.^{16, 17} This behavior is exhibited by the systems, L-NBA, L-TAME. Thus, the acoustical parameters, explains the nature and strength of the interactions taking place in the solutions.

RAO'S CONSTANT

Table 4.1(a) Nα - BENZOYL - L - ARGinine

Molality (m)	5° C	15° C	25° C	35° C	45° C	55° C
0.001	2149	2154	2161	2172	2176	2182
0.005	2145	2150	2160	2168	2177	2183
0.01	2143	2147	2155	2162	2173	2178
0.015	2140	2145	2150	2159	2171	2181
0.02	2135	2141	2148	2156	2172	2181

Table 4.1(b) Nα-p-TOSYL-L-ARGININE METHYL ESTER HYDROCHLORIDE

Molality (m)	5° C	15° C	25° C	35° C	45° C	55° C
0.001	2155	2164	2171	2188	2195	2209
0.005	2154	2162	2169	2185	2193	2208
0.01	2153	2162	2170	2183	2193	2203
0.015	2152	2160	2169	2181	2193	2201
0.02	2150	2158	2168	2180	2193	2200

RAO'S CONSTANT

Figure 4.1(a) Na - BENZOYL - L - ARGinine

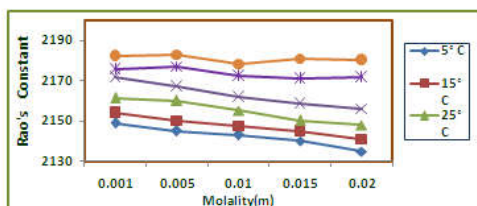
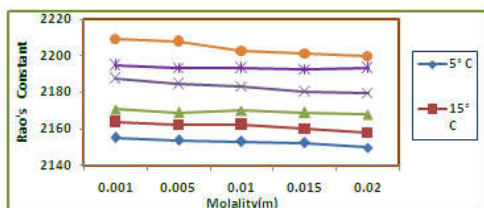


Figure 4.1(b) Na-p-TOSYL-L-ARGININE METHYL ESTER HYDROCHLORIDE



WADA'S CONSTANT

Table 5.1(a) Na - BENZOYL - L - ARGinine

Molality (m)	5° C	15° C	25° C	35° C	45° C	55° C
0.001	1235	1238	1243	1249	1253	1256
0.005	1233	1236	1243	1246	1251	1254
0.01	1235	1237	1241	1243	1249	1252
0.015	1231	1234	1238	1243	1249	1253
0.02	1232	1235	1239	1241	1249	1254

Table 5.1(b) Na-p-TOSYL-L-ARGININE METHYL ESTER HYDROCHLORIDE

Molality (m)	5° C	15° C	25° C	35° C	45° C	55° C
0.001	1240	1243	1248	1256	1260	1267
0.005	1240	1244	1249	1257	1259	1266
0.01	1240	1244	1248	1255	1260	1264
0.015	1240	1243	1248	1254	1260	1264
0.02	1241	1243	1247	1253	1260	1263

WADA'S constant

Figure 5.1(a) Na - BENZOYL - L - ARGinine

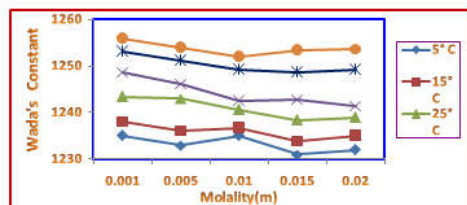
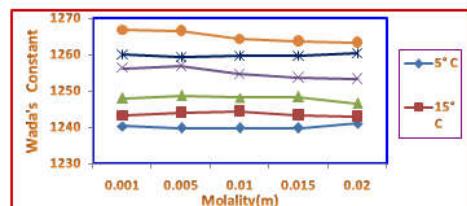


Figure 5.1 (b) Na-p-TOSYL-L-ARGININE METHYL ESTER HYDROCHLORIDE



Solvation number: The solvation approach is used to interpret ion-solvent interaction. The negative solvation number for various molalities is reported by many authors in literature. The decrease in solvation number with increasing molality is due to either not enough solvent molecules available for all the ions or preferentially ion-pairing occurred.¹⁸ From the study the solvation numbers of the samples reveal very high solvation number in formamide. The solvation number depends on the number of moles of the solute and solvent molecules. Solvation number increases with increase in temperature and decrease when concentration increases. This suggests that significant strong interaction is taking place in the systems. The increase in solvation number supports structure maker tendency of solute molecules.¹⁹ In L-NBA system positive solvation occurs at higher concentration for all the temperatures. But for other molalities it is negative at all temperatures except 0.005 m at 45°C and 55°C. This system exhibits very weak interactions in the solution.²⁰

The sample L-TAME exhibits positive solvation for all the temperatures at 0.001 m and also at higher temperatures 35°C, 45°C and 55°C. Their values and variations are given in the Table 6.1(a, b) and Figure.6.1 (a, b).

SOLVATION NUMBER

Table 6.1(a) Na - BENZOYL - L - ARGinine

Molality (m)	5° C	15° C	25° C	35° C	45° C	55° C
0.001	266	17	135	320	662	523
0.005	-38	-107	-89	-35	28	27
0.01	-46	-79	-70	-79	-31	-7
0.015	-42	-66	-70	-68	-27	-13
0.02	-70	-78	-80	-65	-28	-14

Table 6.1(b) Na-p-TOSYL-L-ARGININE METHYL ESTER HYDROCHLORIDE

Molality (m)	5° C	15° C	25° C	35° C	45° C	55° C
0.001	331.9	548.8	666.0	1134.3	1583.0	1930.4
0.005	19.3	66.0	75.7	178.3	267.0	345.8
0.01	-14.0	14.5	27.0	74.5	115.0	140.8
0.015	-24.8	2.4	12.0	28.8	64.7	77.0
0.02	-29.8	-14.0	-12.4	9.9	37.7	48.1

SOLVATION NUMBER

Figure 6.1(a) Na - BENZOYL - L - ARGinine

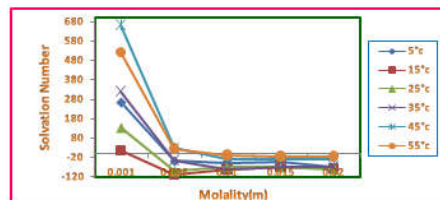
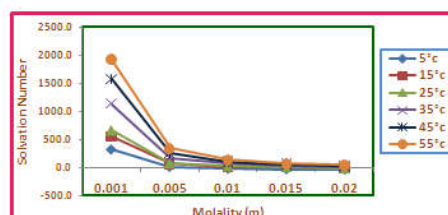


Figure 6.1(b) Na-p-TOSYL-L-ARGININE METHYL ESTER HYDROCHLORIDE



FTIR spectral study of *N*α-benzoyl- L-Arginine


L-Arginine is a basic amino acid, in which a benzoyl group is attached at the α-amino group; thereby reducing the basic nature²¹ This nature is reflected in the FT-IR solid spectrum Table 8.1 Figs.8.1(a,b,c) by the absence of primary NH stretching vibration at 3400 cm⁻¹. The secondary stretching NH vibration is found at 3208 cm⁻¹. The primary NH₂ group of solvent formamide is found at 3422 cm⁻¹. This band in the solution of different concentrations is found to be blue shifted up to 3409 cm⁻¹ due to the solute – solvent interactions.²²

Table 8.1(a) FT-IR observed spectral vibrational frequencies

Name of the Sample	Stretching Vibrations cm ⁻¹				CONH amide II band cm ⁻¹	ν _{C=O} cm ⁻¹	NH out of plane Bending δ _{CH} cm ⁻¹
	ν _{NH}	ν _{CH}	ν _{C=N}	Amide I Band ν _{C=O}			
No-Benzoyl-L-arginine Salt	3400						
	3208			1678	1373	1078	676
	3422	2868	-	1604	1359	1038	556
	3779			1651	1307		495
	3295			1557			
0.1 m	3175		2771	1682	1391	1091	605
	3412	2890	2198		1314	1052	470
	3184						
0.2 m	3418	2889	2771	1698	1392	1095	642
			2703		1312	1051	
Saturation m	3409	2890	2771	1672	1391	1087	607
			2197	1682	1314	1051	

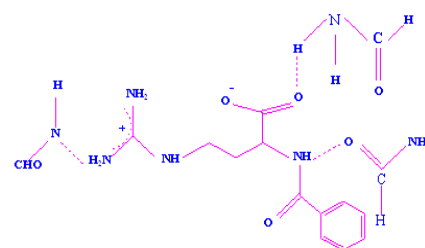
Table 8.1(b) FT-IR observed spectral vibrational frequencies

Name of the Sample	Stretching Vibrations cm ⁻¹				CONH amide II band cm ⁻¹	ν _{C=O} cm ⁻¹	NH out of plane Bending δ _{CH} cm ⁻¹
	ν _{NH}	ν _{CH}	ν _{C=N}	Amide I Band ν _{C=O}			
Na-p-Tosyl-L-arginine methyl ester hydrochloride Salt	3383			1723	1261		741
	3389	2979		1597	1160	1091	663
	3322	2931	2799	1659	1372	1050	548
	3249	2868	2733	1650	1340	1033	493
	3174			1621	1139		943
0.005 m	3758		2771	1685	1390	1092	619
	3407	2887	2699	1459	1314	1051	608
			2201				
0.010 m	3723		2774	1685	1380	1090	610
	3423	2890	2700		1315	1082	
			2403			1051	
0.015 m	3733		2770	1688	1390	1090	670
	3413	2889	2703	1458	1315	1075	623
			2398			1051	611
Saturation m	3437		2769	1634	1391	1082	614
	3381	2886	2700	1698	1315	1051	607
			2400	1681			601
		2206	1614				
			1694				

The guanidino group in L-Arginine is observed at 2394 cm⁻¹, 2290 cm⁻¹ and 2200 cm⁻¹ indicating the existence of NH₂⁺ group. This feature is absent in the benzoyl derivative of L-Arginine in the solid state. On dissolution in formamide a highly polar protic solvent, the *N*α-benzoyl L-Arginine in neutral form switches over to the Zwitterionic form evinced by the FT-IR spectrum at different dilutions in which stretching vibrations of resonating group  are observed in the

region 2200 cm⁻¹-2771 cm⁻¹. The binding of this end of the solute to the NH₂ of formamide is also predicted. Due to the blue-shift of the primary NH₂ stretching vibration of the solvent supporting intermolecular H-bonding, between the solvent and the protonated guanidino group. These predictions are further confirmed by the shift in bending vibrations.

It is found from the IR spectra that at (0.2m), maximum interaction occurs between the Zwitterionic form (Figure 7.1) of *N*α-benzoyl-L-Arginine and the solvent formamide. The C=O group of formamide is free at this concentration since a peak is found at 1721 cm⁻¹. At 3175 cm⁻¹ (0.1m) and 3184 cm⁻¹ further supports the presence of specific H-bonds. The red shift of the amide vibrations from 1604 cm⁻¹ in the (solid spectrum) to 1672 cm⁻¹ (saturation m), 1685 cm⁻¹ (formamide spectrum) is also a strong evidence of the intermolecular H-bonding interactions taking place between solute and solvent. Hence the solvated *N*-benzoyl-L-Arginine should have free CHO group and solvation occurs only at NH₂ group of formamide.⁸ The solvated structure L-NBA is shown in the (Figure 7.1)

Figure 7.1 Zwitterionic form of *N*α-benzoyl- L-Arginine solvation structure

FTIR spectral study *N*α-P-Tosyl-L-Arginine methyl ester hydrochloride

In the solid state FT-IR spectrum (Table 9.1 & Figures.9.1 (a, b, c)) stretching vibrations at 3389 cm⁻¹, 3383 cm⁻¹ and 3322 cm⁻¹ for primary amino group is found. The two secondary NH groups vibrate at 3249 cm⁻¹ and 3174 cm⁻¹. A series of vibrations in the region 2979 cm⁻¹ to 2733 cm⁻¹ is due to the CH₃ and CH₂ groups. The presence of non-interactive CO moiety in the ester part is confirmed by a sharp vibration⁸ observed at 1723 cm⁻¹.

The sulphanamide group (ν_{SO}) vibrates at 1659 cm⁻¹, 1654 cm⁻¹ and 1650 cm⁻¹ as coupled vibration there by indicating resonance between NH and SO₂ groups. The C-N and ring C=C vibrations overlap at 1621 cm⁻¹ and 1597 cm⁻¹. Three sharp vibrations observed at 1447 cm⁻¹, 1340 cm⁻¹ and 1261 cm⁻¹ are due to asymmetric and symmetric vibrations of C=N group. The C-N and C-O stretching vibrations occur at 1160 cm⁻¹, 1139 cm⁻¹ and 1091 cm⁻¹, 1050 cm⁻¹, 1033 cm⁻¹ respectively. δ_{S-O} vibrations occur at 943 cm⁻¹, 898 cm⁻¹ and 823 cm⁻¹. All other bending vibrations such as δ_{C-H}, δ_{C-C},

P-substituted ring vibrations occur in the region 663 cm⁻¹ to 548 cm⁻¹. On further dissolution in formamide the large numbers of stretching vibrations observed in the solid state entirely disappear, with the predominance of solvent peaks. However shift in these peaks are important in deciphering solute-solvent interactions. At saturation molality the NH₂ vibration (Table 9.1) of the solvent occurs at 3437 cm⁻¹. A red

shift of 26cm^{-1} indicates that the NH_2 group of formamide binds to the NH_2^+ group of p-Tosyl-L-Arginine methyl ester hydrochloride. On further dilution this peak undergoes blue shift to 3407cm^{-1} . Since dilution affects this stretching vibration, the NH_2^+ of arginine is bound to NH_2 of formamide by intermolecular H-bonding.

In the carbonyl region all the separate peaks in the solid state have coalesced. However very close coupled vibrations occur at 1698 cm^{-1} and 1694 cm^{-1} , 1634 cm^{-1} and 1614 cm^{-1} (saturation m). These vibrations are assigned to the SO_2 & CO group in Arginine while the 1681 cm^{-1} peak should be due to the solvent. On dilution only the broad band is observed at 1685cm^{-1} . It could be inferred that the solvent CO peak has shifted little ($\sim 3\text{cm}^{-1}$). On dilution the solvent may participate in H-bonding through the enol form.⁸ A survey of all other peaks indicates that they are not changed noticeably on dilution. Hence the solute-solvent interaction is depicted as taking place between neutral forms of the solute and solvent through H-bonding between the enol forms of formamide, NH , SO_2 and CO groups in the solute (Figure.7.2).

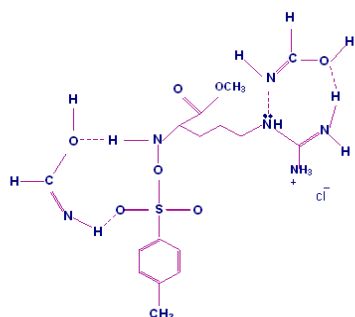


Figure 7.2 Enol form of Na-p Tosyl-L-arginine methyl ester hydrochloride

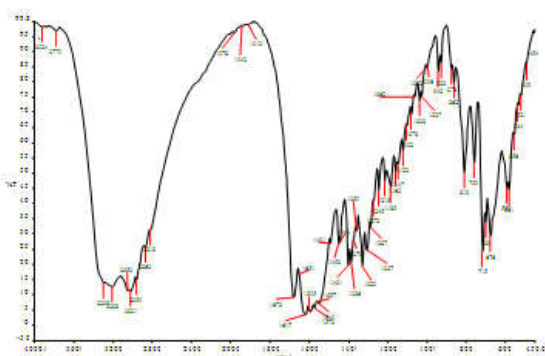


Figure 8.1 (a) - FT-IR Spectrum of Na-Benzoyl L-Arginine salt

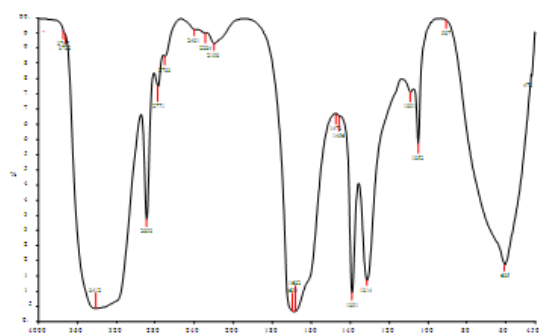


Figure 8.1 (b) - FT-IR Spectrum of Na-Benzoyl L-Arginine (0.1 m)

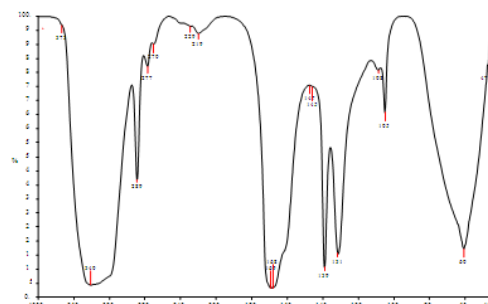


Figure 8.1 (c) - FT-IR Spectrum of Na-Benzoyl L-Arginine (saturation molality)

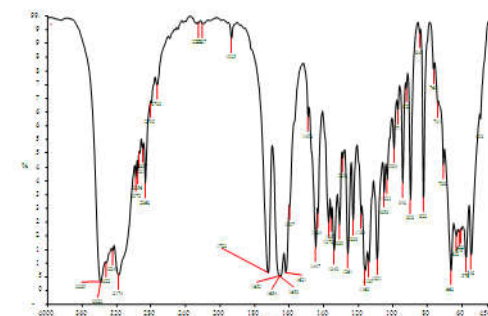


Figure 9.1 (a) - FT-IR Spectrum of Na-p-Tosyl-L-arginine methyl ester hydrochloride salt

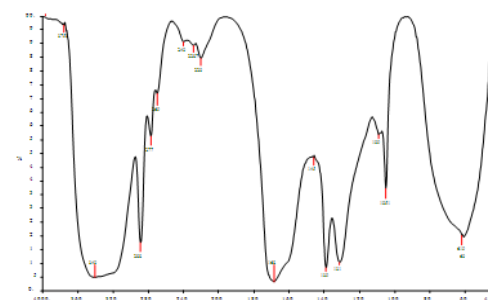


Figure 9.2 (b) - FT-IR Spectrum of Na-p-Tosyl-L-arginine methyl ester hydrochloride (0.005 m)

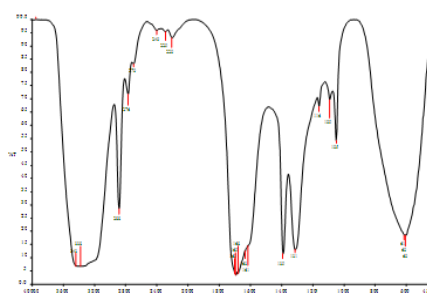


Figure 9.2 (c) - FT-IR Spectrum of Na-p-Tosyl-L-arginine methyl ester hydrochloride (saturation molality)

CONCLUSION

In the samples L-NBA, and L-TAME the β value shows a linear increase with molality. This behavior suggests that in those solutions, there is a strong association taking place between the molecules. The decreasing values of β is attributed to the formation of hydrogen bonds between solute and solvent molecules. The increasing trend in β with concentration supports that the solvent molecules are not much compressed due to electrical forces of solute molecules.²³

The solvation approach is used to interpret ion-solvent interaction. The negative solvation number for various molalities is reported by many authors in literature. The decrease in solvation number with increasing molality is due to either not enough solvent molecules available for all the ions or preferentially ion-pairing occurred.^{24,25} From the study the solvation numbers of the samples reveal very high solvation number in formamide. The solvation number depends on the number of moles of the solute and solvent molecules. Solvation number increases with increase in temperature and decrease when concentration increases. This suggests that significant strong interaction is taking place in the systems. The increase in solvation number supports structure maker tendency of solute molecules.²⁶

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