International Research Journal of Engineering and Technology (IRJET) e-ISSN: 2395-0056
Volume: 04 Special Issue: 09 | Sep -2017 www.irjet.net p-ISSN: 2395-0072
One Day International Seminar on Materials Science & Technology (ISMST 2017)
4th August 2017
Organized by
Department of Physics, Mother Teresa Women's University, Kodaikanal, Tamilnadu, India

ACOUSTICS THERMODYNAMIC AND SOLVATION STUDY OF PROTEIN SALT SOLUTIONS USING ULTRASONIC TECHNIQUE

Dr.Kannagi.K[1], Ilavarasi[2]

[1,2]*PG Department of Physics - Cauvery College for Women, Trichy, Tamil Nadu, India*

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

Keyword: Adiabatic compressibility, Solvation effect, FT-IR spectral analysis

1. Introduction

Amino acids (NH_2-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. Nα-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. Nα-Benzoyl L-Arginine was originally developed by Erlanger[3] 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] Nα-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]

2. 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 formamide is measured with a variable path interferometer ($2MH_Z$) with an accuracy of ±2m/s. The density (ρ) measurement is made with an accuracy of ±0.001gm.The(i) Internal pressure $\pi_i = bRT\left(\frac{k\eta}{u}\right)\frac{1}{2}\times\left(\rho^{\frac{2}{3}}/M_{eff}^{\frac{7}{6}}\right)atms.$

F (ii) Free volume $V_f = \left[M_{eff}\times u/k\eta\right]^{\frac{3}{2}}cc$

(iii) Rao's constant $R = \frac{M_{eff}(U)^{1/3}}{\rho}$

(iv) Wada's constant $W = \frac{M_{eff}}{(\rho)\beta^{-1/7}}$

(v) Adiabatic Compressibility $\beta = \left[\frac{1}{u^2\rho}\right]\mathrm{cm}^2/\mathrm{dyne}$

(vi) 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 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.

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). 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.
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 it derivatives, the following results have been observed. In L-NBA, and L-TAME solutions, the structure of solvent is enhanced.

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 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 0.01molality 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. 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

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]

P-substituted ring vibrations occur in the region 663 cm^{-1} to 548 $cm.^{-1}$ On further dissolution in formamide the large numbers of responsible for the structure of the liquids. At 5°Cinternal pressure of Nα-Benzoyl L-Arginine ishigh and at 15°C it has a rise as shown in Table1.1 (a) and Figure1.1 (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]

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).

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FTIR spectral study of L-NBA

L-Arginine is a basic amino acid, in which a benzoyl group is attached at the α-amino group; thereby reducing the basic nature[21] This nature is reflected in the FT-IR solid spectrum Table 8.1Figs.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 3208cm^{-1}. The primary NH_2 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]

The guanidino group in L-Arginine is observed at 2394 cm,$^{-1}$ 2290 cm^{-1} and 2200 cm^{-1} indicating the existence of NH_2^+ 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 Nα-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 2200cm^{-1}-2771 cm^{-1}.The binding of this end of the solute to the NH_2 of formamide is also predicted. Due to the blue-shift of the primary NH_2 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 Zwitteronic form (Figure7.1) of Nα-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 3175cm^{-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 NH_2 group of formamide.[8]. The solvated structure L-NBA is shown in the (Figure 7.1)

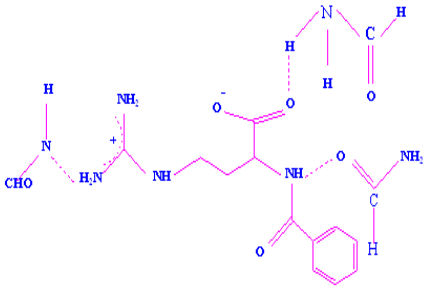

Figure 7.1 Zwitterionic form of Nα-benzoyl- L-Arginine solvation structure

FTIR spectral study Nα-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}$3383 cm^{-1} and 3322cm^{-1} for primary amino group is found. The two secondary NH groups vibrate at 3249cm^{-1} and 3174 cm.$^{-1}$ A series of vibrations in the region 2979 cm^{-1} to 2733 cm^{-1} is due to the CH_3 and CH_2 groups. The presence of non-interactive CO moiety in the ester part is confirmed by a sharp vibration[8] observed at 1723 cm.$^{-1}$

The sulphanamide group (ν_{SO}) vibrates at 1659 cm,$^{-1}$ 1654 cm^{-1}and1650 cm^{-1} as coupled vibration there by indicating resonance between NH and SO_2 groups. The C-N and ring C=C vibrations 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 NH_2 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 (~3cm^{-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, SO2 and CO groups in the solute (Figure.7.2).

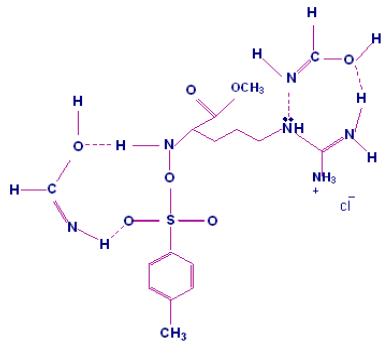

Figure 7.2 Enol form of Nα-p Tosyl-L-arginine methyl ester hydrochloride

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 modalities 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 form amide. 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] overlap at 1621cm^{-1} and 1597cm.$^{-1}$ Three sharp vibrations observed at 1447cm,$^{-1}$ 1340cm^{-1} and 1261cm^{-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},

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

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

ADIABATIC COMPRESSIBILITY (10^{-11} cm^2 / 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

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

WADA'S CONSTANT

Table 5.1(a) Nα - 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) Nα-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

SOLVATION NUMBER

Table 6.1(a) Nα - 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) Nα-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

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INTERNAL PRESSURE (atms)

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

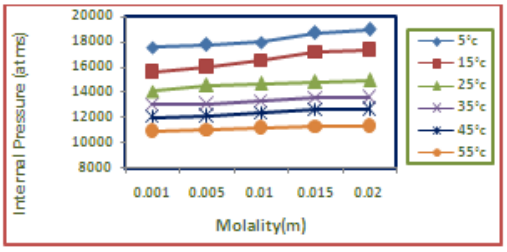
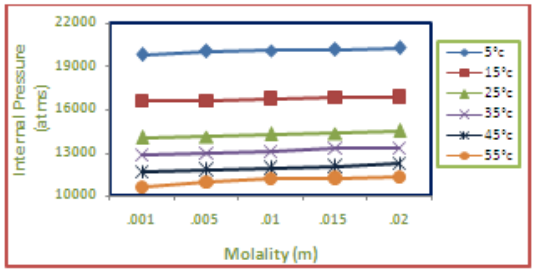


Figure 1.1(b) Nα-p-TOSYL-L-ARGININE METHYL ESTER HYDROCHLORIDE



FREE VOLUME (CC)

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

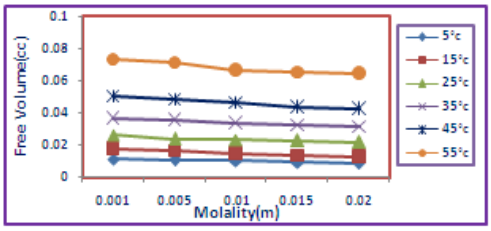
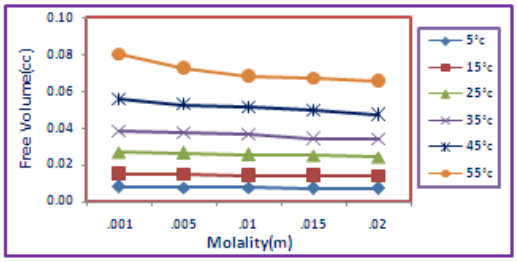


Figure 2.1(b) Nα-p-TOSYL-L-ARGININE METHYL ESTER HYDROCHLORIDE



ADIABATIC COMPRESSIBILITY (10^{-11} cm^2 / dyne)

Figure 3.1(a) Nα - BENZOYL - L – ARGININE

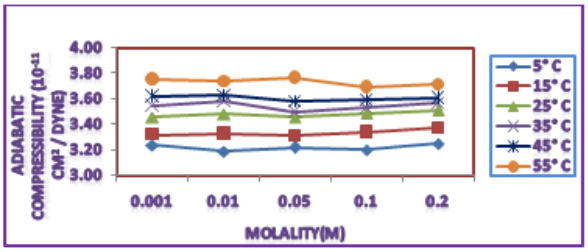
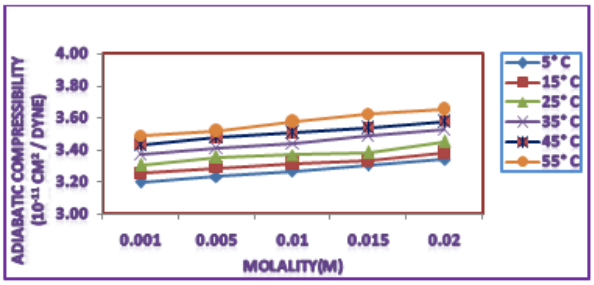


Figure 3.1(b) Nα-p-TOSYL-L-ARGININE METHYL ESTER HYDROCHLORIDE



RAO'S CONSTANT

Figure 4.1(a) Nα - BENZOYL - L – ARGININE

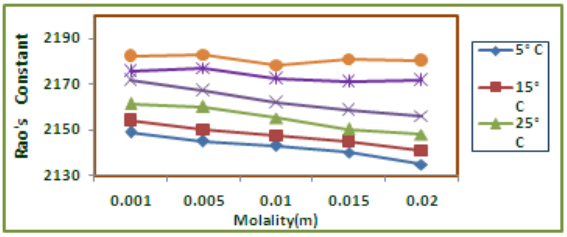
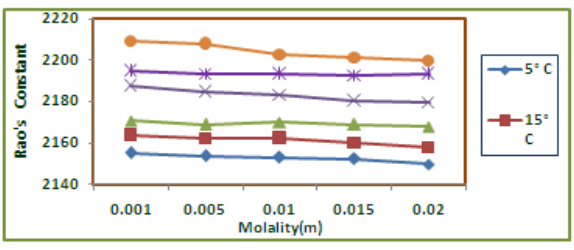


Figure 4.1(b) Nα-p-TOSYL-L-ARGININE METHYL ESTER HYDROCHLORIDE



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WADA'S constant

Figure 5.1(a) Nα - BENZOYL - L - ARGININE

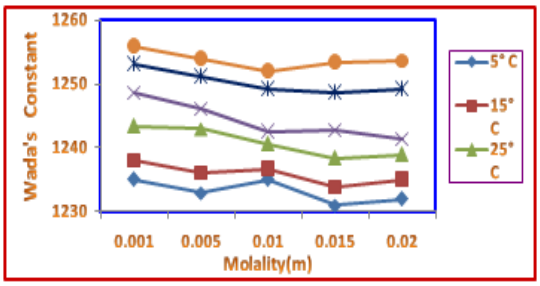
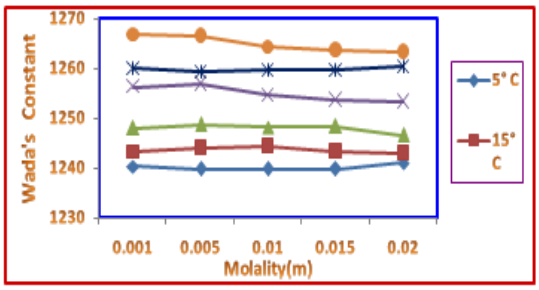


Figure5.1 (b) Nα-p-TOSYL-L-ARGININE METHYL ESTER HYDROCHLORIDE



SOLVATION NUMBER

Figure 6.1(a) Nα - BENZOYL - L - ARGININE

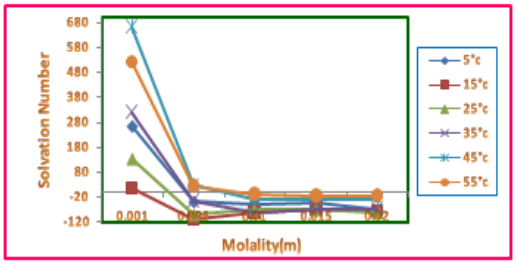
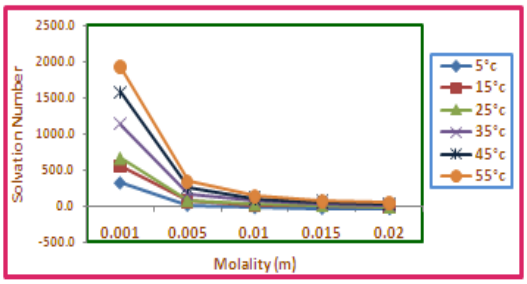


Figure 6.1(b) Nα-p-TOSYL-L-ARGININE METHYL ESTER HYDROCHLORIDE



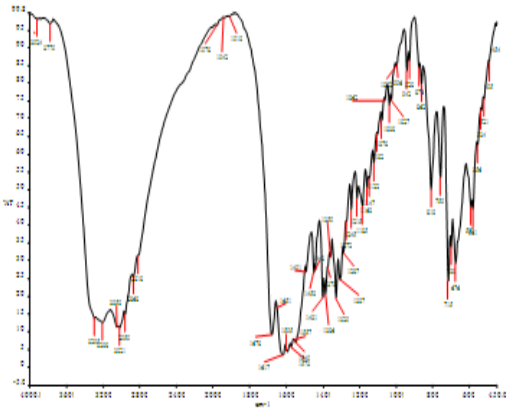


Figure 8.1 (a) - FT-IR Spectrum of Nα-Benzoyl L-Arginine salt

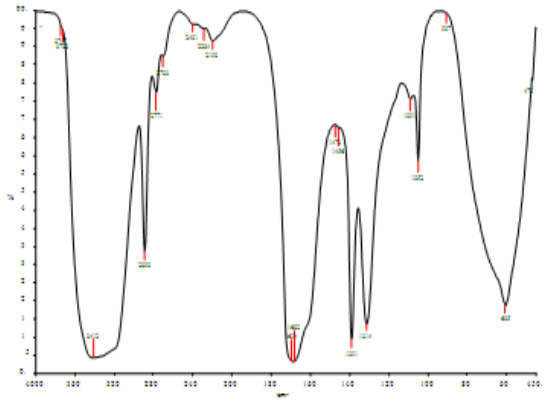


Figure 8.1 (b) - FT-IR Spectrum of Nα- Benzoyl L-Arginine (0.1 m)

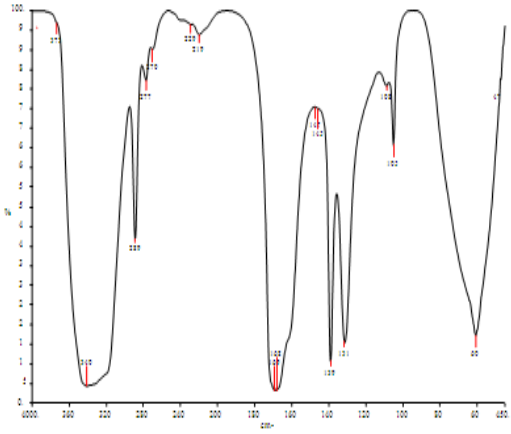


Figure 8.1 (c) - FT-IR Spectrum of Nα- Benzoyl L-Arginine (saturation molality)

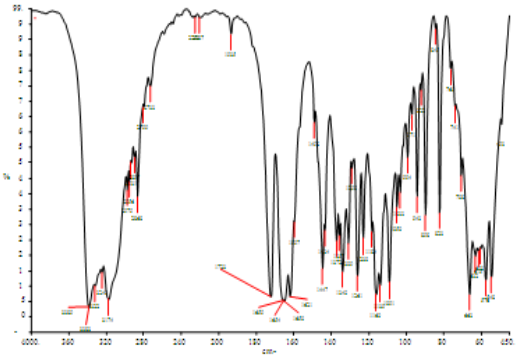


Figure 9.1 (a) - FT-IR Spectrum of Nα-p Tosyl-L- arginine methyl ester hydrochloride salt

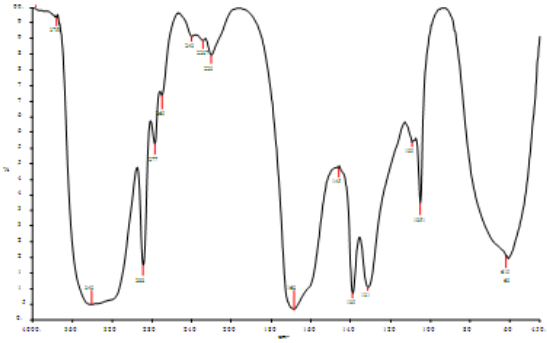


Figure 9.2 (b) - FT-IR Spectrum of Nα-p Tosyl-L- arginine methyl ester hydrochloride (0.005 m)

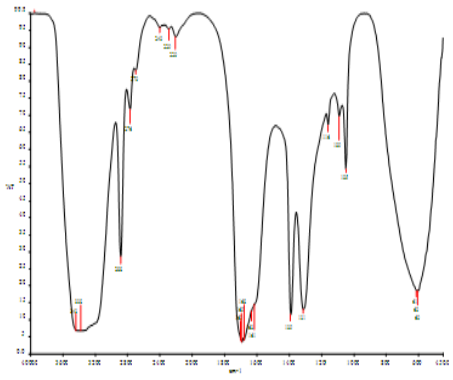


Figure 9.2 (c) - FT-IR Spectrum of Nα-p Tosyl-L- arginine methyl ester hydrochloride (saturation molality)

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}	$\nu_{C=N}$	Amide I Band $\nu_{C=O}$			
Nα-Benzoyl-L-arginine Salt	3400 3208 3422 3779 3295 3050	2868	-	1678 1604 1651 1557	1373 1359 1307	1078 1038	676 556 495
0.1 m	3175 3412 3184	2890	2771 2198	1682	1391 1314	1091 1052	605 470
0.2 m	3418	2889	2771 2703	1698	1392 1312	1095 1051	642
Saturation m	3409	2890	2771 2197	1672 1682	1391 1314	1087 1051	607

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}	$\nu_{C=N}$	Amide I Band ν_{C-O}			
Nα-p-Tosyl-L-arginine methyl ester hydrochloride Salt	3383 3389 3322 3249 3174	2979 2931 2868	2799 2733	1723 1597 1659 1650 1621 1654	1261 1160 1372 1340 1139	1091 1050 1033	741 663 548 493 943 898 823
0.005 m	3758 3407	2887	2771 2699 2201	1685 1459	1390 1314	1092 1051	619 608
0.010 m	3723 3423	2890	2774 2700 2403 2198	1685	1380 1315	1090 1082 1051	610
0.015 m	3733 3413	2889	2770 2703 2398 2199	1688 1458	1390 1315	1090 1075 1051	670 623 611
Saturation m	3437 3381	2886	2769 2700 2400 2206	1634 1698 1681 1614 1694	1391 1315	1082 1051	614 607 601

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