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Thermodynamic, Vibrational Spectroscopic and Antimicrobial Study of a Peptide with an Electrolyte in Non-Aqueous Medium

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Abstract. A peptide can be used as a functional building block to create artificial structures when there is sufficient displacement and functional ability in terms of its assigned function. The nature and the relative strength of the intermolecular interactions between the components of the liquid mixtures/solutions have been investigated by ultrasonic method. In the present study density (ρ), ultrasonic velocity (u) and viscosity (η) of the ternary solutions of peptide–amide with electrolyte have been measured at 298.15, 308.15 and 318.15 K respectively, over the entire composition range using digital density meter, ultrasonic interferometer and Cannon Fenske viscometer respectively. The measured data have been used to compute the thermodynamic parameters such as internal pressure and free volume. The results have been analyzed on the basis of variation in concentrations and temperatures. The results obtained from the ultrasonic methods using the basic parameters have been correlated with FT-IR and Antimicrobial studies and they have been interpreted in terms of peptide–amide interactions. An attempt is made to corroborate the thermodynamic, experimental FT-IR and antimicrobial studies of non-aqueous peptide solutions.

KEY WORDS: Amino acids, internal pressure, free volume, FT-IR spectroscopy, antimicrobial study.

1 Introduction

Amino acids act as structural subgroups of peptides and proteins but play miscellaneous roles in metabolism, neurotransmission and interstitial signalling. Peptides serve as autocrine and endocrine signalling molecules that regulate appetite, vascular tone and electrolyte homeostasis, as well as carbohydrate and

mineral metabolism [1]. The properties of proteins such as their structure, solubility, denaturation and activity of enzymes are greatly affected by electrolytes [2]. Ultrasonic investigations of ternary solutions with polar and non-polar compounds are of significant importance in understanding the relation between component molecules and they find several applications in industry and technological processes [3]. Many researchers use ultrasonic technique to compute the thermodynamic properties of amino acids, [4–10] peptides [11–13] and proteins [14, 15]. Formamide is a simple amide with a peptide link (-NH-CO-) and a study of the hydrogen bonding results in the nature of the protein structure. Glycyl-glycine is the dipeptide of glycine and a potentially useful zwitter ionic buffer in the physiological pH range. Potassium acetate can be used as a de-icer to remove ice and prevent its formation. It is a substantial of macromineral with numerous physiological abilities and is basis for nerve conduction, heart, skeletal and smooth muscle constriction, energy generation, nucleic acid synthesis as well as maintaining the blood pressure and normal renal function [16]. The present study provides the measured values of density, viscosity and ultrasonic velocity of Potassium acetate in non-aqueous solutions as a function of different temperatures and concentrations. Thermodynamic parameters such as internal pressure and free volume were calculated using the basic parameters.

An attempt is made to corroborate the thermodynamic, spectroscopic and antimicrobial study of the sample.

2 Materials and Methods

In the present study, formamide is taken as polar protic solvent, glycyl-glycine as solute and Potassium Acetate as co-solute. The solutions were prepared at different molalities (0.001 M, 0.005M, 0.01M, 0.025M and 0.05M) of solute (glycyl-glycine) and a fixed molality (0.01M) of co-solute (Potassium acetate). Weights of the salts were measured by a Kern electronic balance with an accuracy of ± 0.0001 g and the non-aqueous solutions were prepared. Ultrasonic velocity was determined using digital ultrasonic interferometer of fixed frequency 2 MHz (Model F-81 Mittal enterprises, New Delhi). The density of the non-aqueous peptide with electrolyte solutions are computed using Anton Paar DMA 4100 digital densitymeter with an accuracy of ± 0.0001 g/cc. The viscosity of the solutions are measured using Cannon Fenske viscometer ($\pm 0.1\%$) with the experimental solution immersed in a temperature controlled water bath. The time of flow was measured using a stop watch with an accuracy of 0.1 s. The experiment was carried out at different temperatures and concentrations to study the thermodynamical parameters. The structure making/breaking tendency of the samples are identified.

3 Computation

Using the measured data, the following parameters have been calculated using the standard relations:

3.1 Internal pressure

$$\pi_i = bRT\Big(\frac{K\eta}{U}\Big)^{2/3}\Big(\frac{\rho^{2/3}}{M_{\text{eff}}^{7/6}}\Big)\ [10^9\text{Pa}]\,, \tag{1}$$

where b represents for cubic pressing, which is thought to be 2 for all fluids, k is a dimensionless consistent free of temperature and nature of fluids. Its value is 4.281×10^9. T is the absolute temperature in Kelvin, M_{eff} is the effective molecular weight, R is the universal gas constant, η is the viscosity of solution in Ns/m^2, U is the ultrasonic speed in m/s, and ρ is the density in kg/m^3 of the solution.

3.2 Free volume

$$V_f = \Big(\frac{M_{\text{eff}}U}{K\eta}\Big)^{3/2}\ [10^{-9}\text{m}^3]\,, \tag{2}$$

where M_{eff} is the effective molecular weight of the mixture ($M_{\text{eff}} = \sum_i m_i X_i$, where m_i and X_i are the molecular weight and mole fraction of individual constituents respectively), K is the temperature independent constant which is equal to 4.281×10^9 for all fluids.

4 Results and Discussion

The internal pressure and free volume are easily measurable and fundamentally responsible for the various interactions occurring in the solutions. Cohesive energy is the measure of internal pressure and it is a single factor that varies due to all the intermolecular interactions. When internal pressure increases cohesion is created among the molecules hence internal pressure increases. Free volume is the effective volume in which the centre of a molecule can move when all other molecules are held fixed at their mean positions. The variation of internal pressure (π_i) and free volume (V_f) with different concentrations of non-aqueous peptide solutions at various temperatures are shown in Tables 1 and 2 and Figures 1 and 2. As the concentration increases, the free volume (V_f) decreases whereas the internal pressure (π_i) increases. The increase in internal pressure generally indicates association through hydrogen bonding [17]. Hence cohesive energy increases due to the addition of solute and co-solute present in the solvent. The decrease of V_f (increase of π_i) indicates the formation of hard or tight solvation layer around the ion. Thus, free volume is an inverse function of internal pressure.

Table 1. Internal pressure [10^9Pa]

Molality [M]	Temperature [K] 298.15	308.15	318.15
0.001	1.4186	1.3152	1.2042
0.005	1.4262	1.3302	1.2245
0.01	1.4416	1.3417	1.2287
0.025	1.4486	1.3618	1.2408
0.05	1.4566	1.3670	1.2499

Table 2. Free volume [10^{-9}m^3]

Molality [M]	Temperature [K] 298.15	308.15	318.15
0.001	26.8202	36.5922	51.6897
0.005	26.4051	35.4410	49.2835
0.01	25.5645	34.5692	48.7871
0.025	25.1592	33.0103	47.3533
0.05	24.6583	32.5444	46.3252

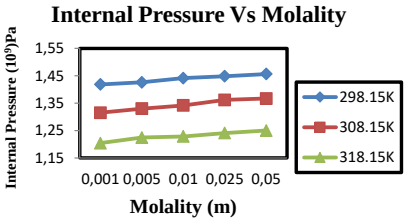

Figure 1. Internal pressure vs molality

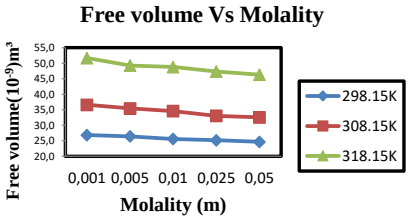

Figure 2. Free volume vs molality

5 FT-IR Spectroscopy

Fourier transform infrared spectroscopy is an excellent method used for identifying the types of chemical bonds present in a molecule by producing an infrared absorption spectrum which is known as molecular fingerprint. Table 3 shows the recorded spectral values of glycyl-glycine + Potassium acetate + formamide (solutions) and the solvated structure is shown in Figure 3.

Table 3. FT-IR spectral values of the samples

Samples	ν_{NH} [cm^{-1}]	$\nu_{NH,CH}$ [cm^{-1}]	AMIDE I $\nu_{C=O,NH}$ [cm^{-1}]	AMIDE II ν_{C-O} [cm^{-1}]	δ_{C-N} [cm^{-1}]
Formamide (solvent)	3410	2887, 2771, 2692, 2396, 2290, 2200	1687	1391, 1309, 1053	602
Glycyl-glycine + Potassium acetate + Formamide (solution)	3409, 3326	2887, 2770, 2691, 2396, 2196	1705, 1694, 1683, 1606	1392, 1309, 1050	601

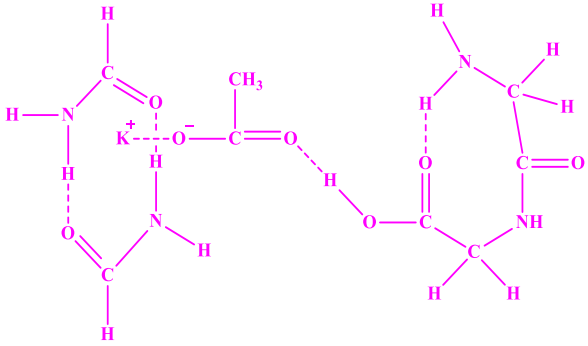

Figure 3. Solvated structure of glycyl-glycine + Potassium acetate + formamide (solution).

Formamide

Formamide molecule contains NH_2 and CHO groups as H_2-N-CHO. In the FT-IR spectrum of formamide (Figure 4), NH_2 group stretching is found at 3410 cm^{-1}. The CH stretching vibration occurs at 2887 cm^{-1}. There are two other stretching bands at 2771 cm^{-1} and 2692 cm^{-1} which may be due to NH^+ and CH groups in dimer and enolic forms. The se-

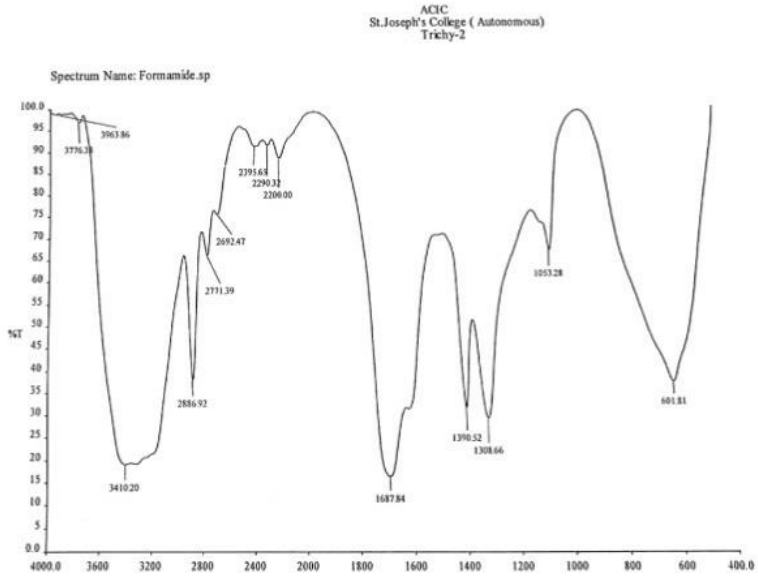

Figure 4. FT-IR Spectrum of formamide (solvent).

ries of bands at 2396 cm^{-1}, 2290 cm^{-1} and 2200 cm^{-1} are attributed to the >C=NH group in the enolic form of formamide. The $\nu_{C=O}$ vibration in formamide is found at 1687 cm^{-1}. Two sharp bands at 1391 cm^{-1} and 1309 cm^{-1} are attributed to $\nu_{C\text{-}N}$ vibration. The $\nu_{C\text{-}O}$ vibration is found at 1053 cm^{-1}. All bending modes overlap as a broad band at 602 cm^{-1}.

Glycyl-glycine + Potassium acetate + formamide (solution)

In the FT-IR spectrum of the solutions of glycyl glycine, Potassium acetate and formamide (Figure 5), the presence of a broad band from 3500–3000 cm^{-1} are indicative of strong solute–solvent interactions. The important bands at 3409 cm^{-1} and 3326 cm^{-1} are due to H-bonded OH, NH and CH groups. A sharp peak at 2887 cm^{-1} is attributed to CH group stretching vibration in formamide. The presence of shoulder peaks at 2770 cm^{-1} and 2691 cm^{-1} indicates that the peptide and formamide CH groups overlap in this region. The carbonyl stretching vibration at 1705 cm^{-1} is spiked by vibrations at 1694 cm^{-1}, 1683 cm^{-1} and 1606 cm^{-1} indicating the presence of several types of groups such as CO, CONH and COO^{-}. Twin bands at 1392 cm^{-1} and 1309 cm^{-1} are similar to $\nu_{C\text{-}C}$, $\nu_{C\text{-}N}$ of formamide. The $\nu_{C\text{-}O}$ stretching vibration in formamide at 1053 cm^{-1} is found at 1050 cm^{-1} in the solutions. At 601 cm^{-1}, a merged band encompasses all bending modes in solution.

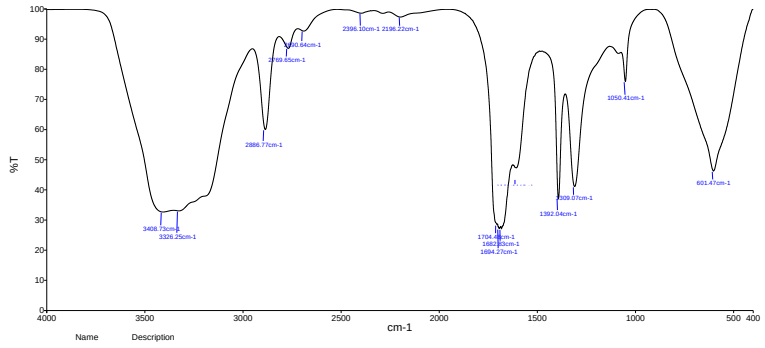

Figure 5. FT-IR spectrum of glycyl-glycine + Potassium acetate + formamide (solutions)

6 Antimicrobial Activity

The anti-microbial activity for the chosen sample was carried out by Disc Diffusion Technique (Indian Pharmacopoeia 1996, Vol II A-105). The test microorganisms of bacteria *E.coli* and fungi *Aspergillus niger* were obtained from National Chemical Laboratory (NCL) Pune and maintained by periodical sub culturing on Nutrient Agar and Sabouraud dextrose Agar medium for bacteria

and fungi respectively. The effect produced by the sample was compared with the effect produced by the positive control (Reference standard Ciprofloxacin 5 μg/disc for bacteria; Nystatin 100 μg/disc for fungi). The obtained results are tabulated as follows:

Table 4. Report on antimicrobial activity of glycyl glycine + Potassium acetate + formamide

	Zone of inhibition in mm				
Name of the Microorganism	FMA	GG	Pot.Ace	GG + Pot.Ace	Standard
E.coli (NICM 2065)	14	18	30	32	38
Aspergillus niger (NICM 105)	15	18	20	26	30

Antibacterial and antifungal activity of Schiff base and its complexes have been tested by disc diffusion technique. The various gram positive and gram negative bacterial organisms such as bacteria (*E.coli*) and fungi (*Aspergillus niger*) are used to find out the antimicrobial activity [18]. The antibacterial activity of non-aqueous solution of glycyl-glycine + Potassium acetate + formamide (Figure 6) showed zone of inhibition against *Escherichia coli* as 32 mm which is greater than individual zones of inhibition. The antifungal activity of glycyl-glycine + Potassium acetate + formamide (Figure 7) showed maximum zone of inhibition against *Aspergillus niger* as 26 mm which is more than individual zone of inhibition. *E.coli* response is much higher than that of *Aspergillus niger*.

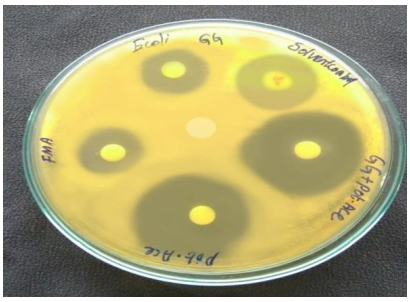

Figure 6. Antibacterial activity (*E.Coli*) of formamide + glycyl-glycine + Potassium acetate.

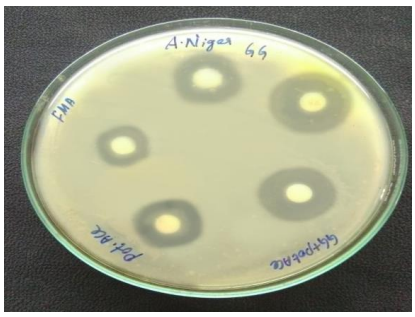

Figure 7. Antifungal activity (*Aspergillus niger*) of formamide + glycyl-glycine + Potassium acetate.

7 Conclusion

The present investigation indicates that thermodynamic parameters are sensitive to molecular interactions of ternary solutions/mixtures at different concentrations and at varying temperatures. Thus it is to be concluded that in the title

compound, both solute and co-solutes interactions are predominant. Thus the presence of peptide–electrolyte enhances the structure of the solvent. The FT-IR spectra reveals that there is a strong solute, co-solute and solvent interactions taking place in the ternary mixture of glycyl-glycine + Potassium acetate + Formamide. The present investigation exhibits that zone of inhibition is highly appreciable for glycyl-glycine + Potassium acetate + Formamide in *Escherichia coli* and *Aspergillus niger* studies. The present analysis reveals that the chosen sample may act as a promising disinfectant.

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