Solvation, Thermodynamic and UV-Visible Studies of Non-Aqueous Peptide Solutions

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***Abstract* - Peptides are most important molecules due to their wide application in drug production and their role as signal transmitters in the cell communications. The systematic study of peptides can prove valuable information about their behaviour in solution and insight into the conformational stability of proteins. Amino acids and peptides are the fundamental structural units of protein. The properties of protein such as their structure, solubility, denaturation activity of enzymes, etc. are generally influenced by electrolytes. The study of intermolecular interaction plays an important role in the development of molecular sciences. Large number of studies have been made on the molecular interaction in liquid systems by various physical methods like ultra violet and ultrasonic technique. The present investigation deals with the study of molecular interaction of non-aqueous dipeptide solution of various concentrations and at different temperatures. Using the experimental data, solvation number (S_n) and internal pressure (π_i) are computed by using the standard formulae. These parameters have been interpreted in terms of solute-solvent interaction in the solutions. The results obtained from thermodynamic, UV - visible spectroscopic and antimicrobial study reveals the entry of solute into the peptide solution.**

Keywords: Ultrasonic velocity, internal pressure, UV – Visible spectra, E.Coli

I.INTRODUCTION

Ultrasonic is a versatile non-destructive technique and highly useful for the investigation of various physical properties such as residual stress, hardness, grain size micro structure, elastic constant etc. Recent developments have found use of ultrasonic energy in medicine, engineering and agriculture. Ultrasonic study on the amino acids with aqueous solution of electrolytes and non-electrolytes provides useful information in understanding the behavior of liquid systems[1]. The ultrasonic study of liquid is very important in understanding the nature and strength of molecular interactions. The biological activity of drug molecules and the activation energy of the metabolic process [2] basically depend on the type and strength of the intermolecular interactions. Ultrasonic velocity studies of amino acids [3-4], peptides [5-9] and proteins [10] in aqueous media, aqueous urea solution, mixed aqueous solutions and organic solvents have been carried out by number of researchers for investigation of solute- solute, solute-solvent intermolecular/ interionic interactions. However few authors have studied the behaviour of amino acids and peptides in aqueous electrolyte solutions. The Glycyl-L-glycine+ sodium nitrite+ formamide system into gives the interesting information about various interactions operative in solution. The Spectroscopic techniques are used to determine the structure and function of bio-molecules. The results arrived from the internal pressure and solvation number parameters were correlated with UV-visible and antimicrobial study.

II. MATERIALS AND METHODS

Ultrasonic velocity was measured using Digital ultrasonic interferometer of fixed frequency 2MHz (Model F-81 Mittal Enterprises, New Delhi) with an accuracy of ± 0.2m/s. The density of the non – aqueous solutions of peptide with electrolyte are measured using Anton Paar DMA 4100 Digital Densitometer with an accuracy of ± 0.0001 gm/cc. The viscosity of solutions are measured using Cannon Fenske Viscometer (± 0.1 %) with the experimental solutions immersed in a temperature controlled water bath. The time of flow was measured using a stop watch with an accuracy of 0.1 sec. The derived parameters such as solvation number (S_n) and internal pressure (π_i) are calculated by the formulae given below

Solvation number $S_n = n_i/n_f\ [1- \beta_{soln}/ \beta_{solv}]$

Internal pressure

$\pi_i = bRT\ [K\eta/u]^{1/2}\ [\rho^{2/3}/M_{eff}^{7/6}]\ (10^9\ Pa)$

III.RESULTS AND DISCUSSION

A. *Solvation number*

The solvation number is computed from compressibility measurements. The solvation number is positive at all temperatures and at higher molalities. The solvation approach is used to interpret ion-solvent interaction. The decrease in solvation number with increasing molality is due to either not enough solvent molecules available for the ions or preferentially ion-pairing occurred. In very dilute solution, there is a basic structural changes happens in the first co-ordination sphere. Hence the filling of cation and anion influence spheres occurred in a set of sequence in relation to other solvation. The solvation number of an ion depends on the solvent.

B. *Internal pressure*

Internal pressure represents the resultant of the forces of attraction and repulsion between the molecules. In the present investigation, the Π_i increases with increasing concentration. When a non-aqueous electrolyte solution is added to Glycyl-L-glycine dipeptide, it attracts the more solvent molecules towards itself by wrenching the molecules

When the temperature is increased, there is a tendency for molecules to move away from each other, reducing the cohesion between them. Thus Π_i decreases as it is a measure of cohesive energy. The dipeptide tightens the formamide molecules around it, hence the molecules comes closer together. In the present dipeptide systems, this may be taking place as solvophilic group interactions between the ions of sodium nitrite (Na^+, NO_2^-) and (NH_3^+, COO^-), (-OH) group of peptides . (i.e) Solvophilic group interaction between the ions of sodium nitrite and non-polar parts of peptides.

Table: 1 The computed values of solvation number and Internal pressure of non – aqueous ternary solutions at different temperatures are zone in the table

Molality (m)	Solvation Number			Internal Pressure (10^9Pa)		
	308.15 K	318.15 K	328.15 K	308.15 K	318.15 K	328.15 K
0.001	9.34	22.81	17.85	1.3135	1.1912	1.1028
0.005	14.56	18.82	21.21	1.3295	1.2196	1.1107
0.1	13.96	14.78	18.84	1.3539	1.2236	1.1148
0.025	10.55	9.41	13.55	1.3570	1.2362	1.1312
0.05	7.55	6.14	9.08	1.3749	1.2462	1.1407

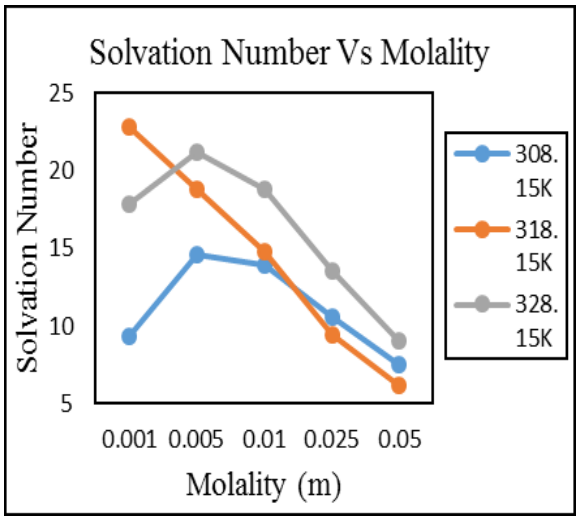


Fig 1: Solvation Number

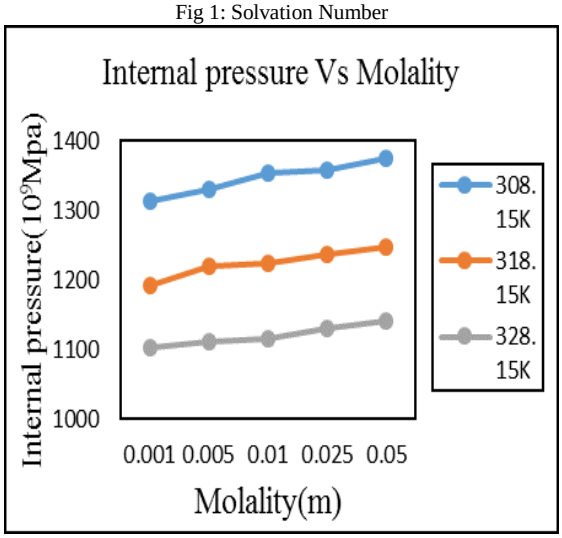


Fig 2: Internal Pressure

Table: 2 UV VISIBLE SPECYTRA OF THE SAMPLES

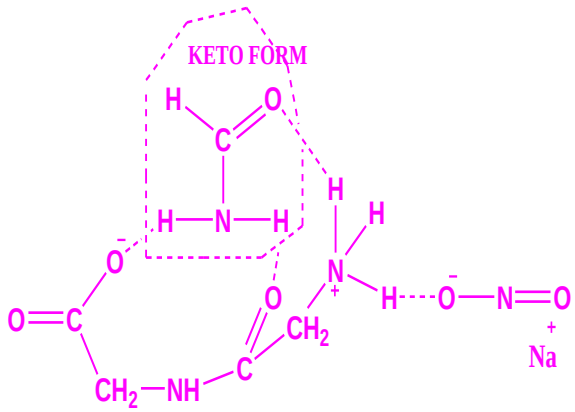
SAMPLES	**200-400 nm**
Formamide (Solvent)	233.03
Glycyl-L-glycine (solute-I)	293.22 280.93
Sodium-Nitrite (solute-II)	234.62
0.001m solution of Glycyl-L-Glycine with 0.01m of Sodium nitrite in Formamide	334.34
0.01m solution of Glycyl-L-Glycine with 0.01m of Sodium nitrite in Formamide	323.01 339.83
Saturated Solution of Glycyl-L-Glycine with Sodium nitrite in Formamide	317.43 350.04

IV. UV-VISIBLE SPECTRUM ANALYSIS

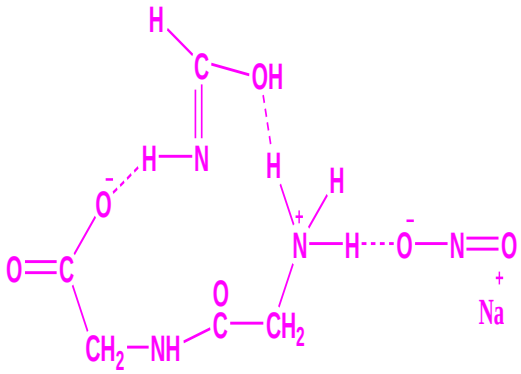
Glycyl-L-glycine exhibit two peaks at 293 and 280 nm. The UV-Visible spectra of sodium nitrite exhibits a sharp peak at 334.6nm which may be due to intermolecular interactions in the solid state of sodium nitrite. At 0.001m molality a single peak is found at 334.3nm.At 0.01m molality two peaks are observed at 323nm and 339.8nm.On dissolution of Glycyl-L-glycine in sodium nitrite formamide mixture at the UV-Visible spectral peaks are found at 317.4nm and 350nm of high intensity (~3.18-3.24). The interaction between solute and solvent is represented as Figure 3 respectively. The sodium nitrate peak at 334.6nm has shifted 17.6nm hypsochromically and 5.4nm bathochromically and solute-solvent interaction is represented as in I_a+I_b. The peak at 317nm has bathochromically shifted from 317-323nm by 6nm, but the peak at 350nm has shifted hypsochromically from 350-339.8nm by 11.2nm and the solute-solvent interaction.

The peaks at 823nm + 339.8nm have merged at 334.3nm. There is a bathochromic shift of 11.3nm + 5.5nm of both peaks and correspond to III. The above results indicate compact structure up to 0.01m and good solute-solvent interaction at 0.001m.

Fig 3: Solvated Structure of Glycyl-L-Glycine + Sodium Nitrite + Formamide



Keto form of formamide



Enol form of formamide

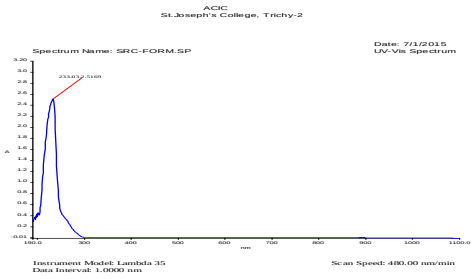


Fig 4:UV-Vis Spectrum of Formamide

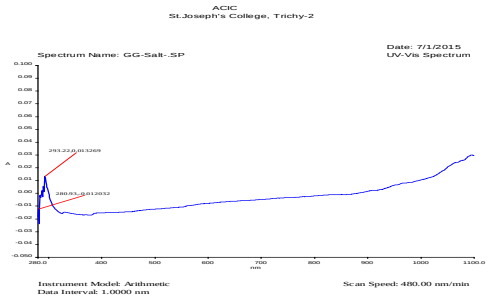


Fig 5:UV-Vis Spectrum of Glycyl-L- glycine

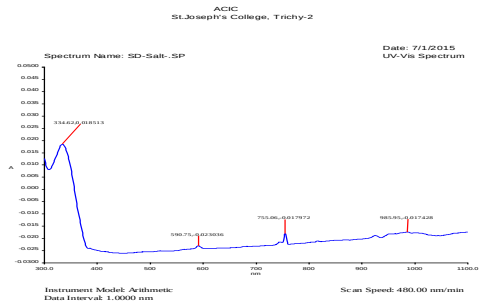


Fig 6:UV-Vis Spectrum of Sodium Nitrite

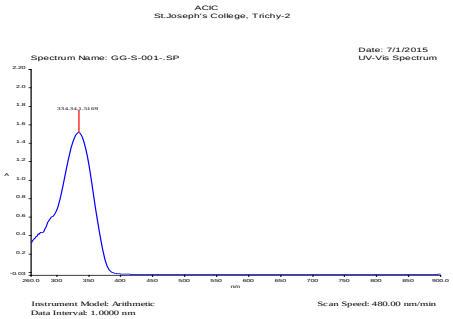


Fig 7: UV-Vis Spectrum of of 0.001m

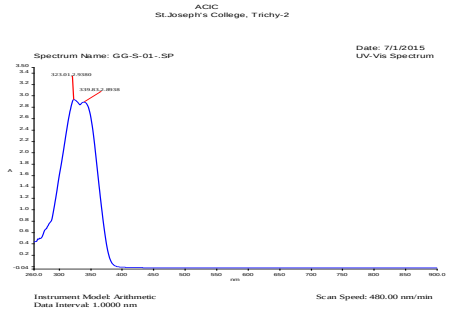


Fig 8:UV-Vis Spectrum of 0.01m

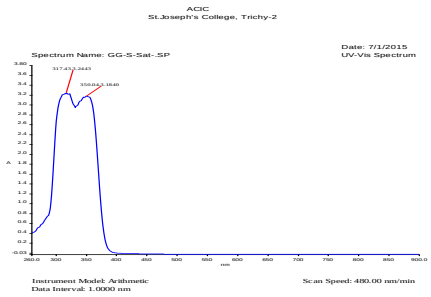


Fig 9:UV-Vis Spectrum of Saturated Solution

V. SALIENT FEATURES OF THE SAMPLES TEST ORGANISMS:

The test microorganisms of E.Coli and Fungus Aspergillusniger, are obtained from National Chemical Laboratory (NCL) Pune. Antibacterial and antifungal properties of the ligand and its complexes were tested *in vitro* against the bacterial species *Escherichia coli;* fungal species *Aspergillusniger*, by the disc diffusion method.

A.Antibacterial activity

The NCIM numbered strains bought from National Chemical Laboratory (NCL) Pune was periodically Sub cultured in Nutrient agar and maintained in the laboratory. The Strains namely Proteus vulgaris and Klebsiellaaerogenes were brought to the active phase by sub culturing in Nutrient broth and incubated at 37°C for 18 hours.The Standardized inoculam about 0.1 ml was inoculated on Muller hinton agar (Hi media) Uniformly. The sterile disc (watt man No.2 of 6mm diameter was placed at equal interval on uniformly inoculated plate and a standard disc Ciprofloxacin 5 mcg/disc was also placed by aseptic technique). The plates were incubated at 37°C for 24 hours. During this period the drug diffuse through the agar and inhibit the growth if the drug is potent. The diameter of inhibiting zone around the disc was measured by using the ruler and Interpretation has to be assessed based on the following table.

B. Antifungal activity

The ATCC numbered strains bought from Madras Medical College was periodically sub cultured in Sabouraud dextrose agar and maintained in the laboratory. The strains namely Aspergillusniger, Aspergillusflavus, Aspergillusfumigatus, Mucor& candida albicans were brought to the active phase by sub culturing in Sabouraud dextrose broth (table 2) and incubated at room temperature for 4 days. The standardized inoculam about 0.1 ml was inoculated on Sabouraud dextrose agar uniformly. The sterile disc watt man No.2 of 6 mm diameter was placed at equal interval on uniformly inoculated plate and a standard disc Nystain 100 units/disc was also placed by aseptic technique. The test sample about 100 µl was loaded to the sterile disc by using aseptic precautions. The plates were incubated at room temperature for 2 to 4 days. During this period the drug diffuse through the agar and inhibit the growth if the drug is potent. The diameter of inhibiting zone around the disc was measured by using the ruler and Interpretation has to be assessed based on the following table.

S.No	Zone of inhibition in mm	Report
1.	Zone of inhibition between 6mm to 12mm	Intermediate
2.	Zone of inhibition below 6mm	Resistent
3.	Zone of inhibition more 12mm	Sensitive

Report on Antimicrobial activity of the given samples

The anti-microbial activity for the given Sample was carried out by disc diffusion Technique (Indian Pharmacopoeia 1996, Vol II a-105). The test microorganisms 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).

S. No	Name of the Microorganisms	Zone of inhibition in mm				
		GG	Sod.Nit	GG+ Sod.Nit	FMA (Solvent)	Standard
1.	E.coli (NCIM 2065)	16	18	20	08	38
2.	Aspergillus niger (NCIM 105)	12	17	16	10	30

In the present investigation, it is identified that the non-aqueous solutions of Glycyl-L-glycine with Sodium Nitrite act as an intermediate with respect to E.coli whereas this combination is sensitive in the case of Aspergillus niger, ie this combination may act as an disinfectant.

Antibacterial activity (E.Coli)

Antifungal activity (Aspergillus niger)

Fig 10: Antimicrobial activity of Glycyl –L – glycine + Sodium Nitrite + Formamide solutions

VI. CONCLUSION

Analysis of the transport properties suggest that there is a strong solute-solvent interaction exist in the ternary solution. The above results are confirmed by solvation studies. UV-Visible spectra are reveals that there is a strong interaction takes place in ternary solutions. The (N-C-O) peptide linkage is dependent on the structure flexibility of the molecule involved and is of importance in the synthesis of protein. An attempt is made to corroborate with solvation, thermodynamic and spectroscopic analysis.

VII. ACKNOWLEDGEMENT

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