FT-IR, Acoustic, Thermodynamic and Docking Study of a Drug in a Peptide

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ABSTRACT

Peptides have a wide range of application in medicine and biotechnology. They regulate most physiological process, acting at some sites as endocrine or paracrine signals and at others as neurotransmitter or growth factors. Ultrasonic studies of solutions yield valuable information about the molecular interactions, the nature and strength of interactions since the ultrasonic velocity is highly sensitive to molecular structure. The ultrasonic velocity (U), density (ρ) and viscosity (η) measurements have been carried out for peptide with fixed concentration of the sulfa drug in non – aqueous solution of ternary mixture at different temperatures. Experimental data have been used to estimate the internal pressure (π_i) and free volume (V_f). The results are discussed in terms of interionic/ intermolecular interaction. To study the biological activity of the investigation molecule, molecular docking was done to identify the hydrogen bond lengths and binding energy with different antimicrobial protein. In the present investigation, ternary system of peptide solutions are carried out and an attempt is made to corroborate the ultrasonic, antimicrobial and docking study.

Key Words: Ultrasonic velocity, Acoustical, Transport properties, Docking

INTRODUCTION

Ultrasonic study is very much useful to distinguish the physico-chemical behaviour of ternary mixtures and it helps to identify the molecular interactions in non-aqueous solution [1-4]. Benzene sulphonamide have been recognized as a very good photo sensitizer in clinical test and in cell culture [5]. Glycyl-L- glycine is the simplest dipeptide made from the scraps of two glycine molecules. The transition of dipeptide from the molecular form to the zwitterions form does not depend on the acidity of the medium [6]. Therefore it is important to study the characterization, antibiotic testing and drug patent of the title compound for forthcoming studies. Moreover, researches are also very beneficial for the theoretical calculation of the experimental indication since enumerative methods are reliable to characterize the molecule because of their efficiency and exactness with regard to the estimation of molecular properties.

Herole et al., [7] reported Synthesis, characterization and antimicrobial screening of Benzene sulphonamide, 4-Amino-N-(5-Methyl-3-Isoxazolyl) Monosilver salt and related compounds. Mrinalini et.al [8] reported Mixed ligand cobalt (III) complexes with 1- Amidino-O-Methylurea and Amino

Acids. Umesh et.al [9] Synthesis and Biological evaluation of some sulphonamide schiff's bases. Anamika Gupta et.al [10] reported Photochemistry of photodynamic biological action of photosensitizing drug Tolamide. Aastha Pareek et.al [11] reported A short review on: Sulphonamides. Parveen Srivastwa et.al [12] reported Quantitative structure-activity relationship study of benzene sulphonamides as inhibitor of carbonic anhydrase based on quantum chemical descriptor.

Literature survey exhibits that there is no spectroscopic, ultrasonic and molecular docking studies for title compound peptide with drug in non-aqueous medium. In the present study, the polar protic solvent is taken along with a peptide and drug, the solutions were prepared at different molalities with peptide and a fixed concentration of drug. The thermodynamic parameters such as internal pressure, free volume and adiabatic compressibility were evaluated. The results accomplished from these parameters were associated with spectroscopic, ultrasonic and docking studies. Docking detection has been performed to discriminate the Bonded residues, hydrogen bond, Bond distance ($\text{\AA}$), Estimated Inhibition constant (μm) and Binding energy (kcal/mol) of the molecule.

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MATERIALS AND METHODS

Experimental Details

The density of the non-aqueous peptide with drug solutions were measured using Anton Paar DMA 4100 Digital densitometer with an accuracy of ± 0.0001 gm/cc. The viscosities of the solutions were measured using Cannon Fenske viscometer (±0.1 %) error with the experimental solution was immersed in a temperature controlled water bath. The time of flow was measured using a stop watch with an accuracy of 0.1 sec. Ultrasonic velocity was measured using

digital ultrasonic interferometer of fixed frequency 2MHz (Model F-81 Mittal enterprises, New Delhi) with an accuracy of ± 0.2 m/s.

Computation

The measured data were used to calculate the thermodynamic and acoustic parameter using the standard relations,

Internal Pressure $\Pi_i = b RT (K \eta / U)^{1/2} \rho^{2/3} / M_{eff}^{7/6} (10^9) \text{ Pa} \text{ -- } 1$

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

Adiabatic compressibility $\beta = (1/u^2 \rho) (10^{-12}) \text{ TPa}^{-1} \text{ ----- } 3$

RESULTS AND DISCUSSION

Table 1: FT-IR spectral values of the samples

Samples Name	(ν_{NH})	($\nu_{NH,CH}$)	$\nu_{C=O,NH}$ amide I	$\nu_{C=O}$ amide II	δ_{C-N}
Solvent (Formamide)	3410	2887, 2771 2692, 2290 2200	1687.84	1391 1053	602
Solute-I (Glycyl-L-glycine)	3289 3058	2930, 2762 2636, 2412 2316, 2056	1659, 1629 1604, 1577	1483, 1443 1403, 1337 1311, 1252 1157, 1133 1097, 1001	964, 918 710, 662 589, 530 444, 407
Solute-II (Benzene Sul- fonamide)	3351 3259 3071 3059	2926, 2889 2784, 2627 2488, 2434 2346, 2252 2188, 2308 2003	1980, 1909 1853, 1819 1781, 1688 1581, 1556	1448, 1480 1404, 1335 1312, 1289 1160, 1091 1072, 1026	998, 905 757, 688 592, 537 460, 434
Glycyl-L-glycine+Benzene Sulfonamide solution	3411 3396 3371 3324	2882, 2763	1783, 1685 1600	1392, 1309 1098, 1050	808, 674 602, 415

Figure 1: FT-IR spectrum of Formamide.

Figure 2: FT-IR spectrum of Glycyl-L-glycine.

Figure 3: FT-IR spectrum of Benzene Sulfonamide IR spectral analysis Formamide (Solvent).

Figure 4: FT-IR spectrum of Glycyl-L-glycine+ Benzene Sulfonamide+Formamide solution.

Formamide consists of only three important functional groups. NH, CO and CH. The structure of formamide fig (7.5) is $\text{H}-\text{C}(=\text{O})-\text{NH}_2$. The amide CONH_2 comprising both CO and NH_2 group, exhibit stretching vibration at 3410 cm^{-1} due to NH_2 group. At 1688 cm^{-1} amide I due to CO stretching mode is observed as a broad peak. The CH stretching vibration occurs at 2887 cm^{-1} as a sharp band.

Formamide can also exist in enolic form as $\text{HO}-\text{C}(\text{NH})=\text{NH}$ due to which $-\text{OH}$, CH and $\text{C}=\text{NH}$ vibration can arise. The asymmetric stretching vibrations of CH groups are found at 2771 and 2692 cm^{-1} and at 2339 cm^{-1} , 2290 cm^{-1} and 2220 cm^{-1} for $\text{C}=\text{NH}$ groups. The broadened peak at 3410 cm^{-1} encompasses the OH stretching vibration. The broad peaks at 1391 cm^{-1} and 1310 cm^{-1} are due to $\delta_{\text{C-N}}$ and $\delta_{\text{C-H}}$ vibrations. The stretching vibrational mode C-O function is found at 1053 cm^{-1} . The $\delta_{\text{C-H}}$ and $\delta_{\text{O-H}}$ bending vibrations are observed as a broad peak at 602 cm^{-1} .

Glycyl-L-glycine

The NH_2 and NH stretching vibrations are found at 3250 and 3259 cm^{-1} respectively. The CH stretching vibrations are present at 3071 and 3059 cm^{-1} as merged shoulder peaks on the broad NH_2 stretching band. Several peaks in the region 2627 to 1781 cm^{-1} arises due to $\text{C}=\text{N}^+$ and CONH stretching modes. The C-C^+ , COO^- stretching vibrations are found

at 1581 , 1556 , 1448 , 1404 cm^{-1} . At 1334 and 1312 cm^{-1} the bending vibrations i.e., $\delta_{\text{C-N}}$ and $\delta_{\text{C-C}}$ of O-N and C-C groups are deciphered. The C-O stretching modes are observed at 1091 , 1072 , 998 and 905 cm^{-1} . The bending vibrations of groups such as C-H, NH, COO^- are found at 757 , 688 , 592 and 537 cm^{-1} .

Benzene Sulfonamide

The NH_2 stretching vibration occurs at 3411 , 3396 , 3370 and 3324 cm^{-1} . The CH stretching vibration is found at 2882 and 2763 cm^{-1} . At 1783 cm^{-1} S = O stretching mode is present as a broad shoulder peak. The C-C stretching vibration occurs at 1685 cm^{-1} as sharp peak. At 1392 and 1309 cm^{-1} the stretching vibration of S-NH stretching and C-C bending modes are found. The S-O asymmetric and symmetric stretching vibrations are very broad at 1098 and 1050 cm^{-1} respectively. The bending vibrations are at 808 , 673 and 602 cm^{-1} along with S-O stretching vibration and C-S stretching vibrations merged with bending modes.

Glycyl-L-glycine+Benzene Sulfonamide+ Formamide Solution

Twin peaks at 3351 and 3259 cm^{-1} are due to NH stretching modes in Glycyl-L-glycine, benzene sulfonamide and formamide. At 3071 and 3059 cm^{-1} are due to CH stretching vibrations. Several sharp but low intensity peaks in the region 2627 to 2780 cm^{-1} are due to $\text{C}^+=\text{N}$, and CONH stretching modes in Glycyl-L-glycine. The shoulder peak at 1688 cm^{-1} is due to SO_2NH group in benzene sulfonamide. The stretching vibrations at 1581 and 1556 cm^{-1} are due to C-C stretching vibrations. At 1480 , 1448 and 1404 cm^{-1} are assigned to stretching mode. At 1334 , 1312 and 1289 cm^{-1} are assigned to C-C, C-N, CN^+ stretching and C-C bending modes. The S=O and C-O stretching modes are found at 1091 , 1071 , 998 and 905 cm^{-1} . The S-O stretching, C-S stretching and bending vibrations are found at 756 , 688 , 592 and 537 cm^{-1} as sharp peaks.

Figure 5: Solvated structure of Glycyl-L-glycine+Benzene Sulfonamide+Formamide.

Ultrasonic study

Internal pressure and Free volume

Internal pressure is a single factor that appears vary due to all the intermolecular interactions. In the present investigation the internal pressure increases with increasing concentration, at all temperatures. A dip is observed at 0.01m which dip may be due to the weak peptide-drug-amide interactions that exist among the non-aqueous solutions. Hence the cohesion between the solutes and the solvent is reduced whereas the interaction between the Glycyl-L-glycine + Benzene sulphonamide+Formamide i.e, solute-solute interaction is dominating than the solute-solvent interaction. Thus the solute-solute interaction favours the dipole-dipole, dipole induced- dipole and electrostrictive forces hence the structure breaking nature of the peptide in the non-aqueous drug system (Glycyl-L-Glycine + Benzene sulphonamide + Formamide). The free space and its dependent properties have close connection with molecular structure. The increase in the free volume at 0.01m indicates that the dipeptide + drug loosens the close-packed structure of the solvent molecules around it.

Adiabatic compressibility

The adiabatic compressibility increases with decrease in concentration and rise in temperature as shown in figure 7. When the salt is added to the solvent, the compressibility is lowered. This lowering is attributed to the influence of the electrostatic field of the ions, on the surrounding solvent molecules. Such a decrease may be due to (i) an increase in number of incompressible molecule, (ii) structural changes occurring in the solution. This may be due to the association taking place between the molecules when the temperature increases. The associated groups of molecules breakdown increasingly and the forces of attraction between the molecules decrease. This leads to an increase in the adiabatic compressibility of the system.

Figure 6: Internal pressure.

Figure 7: Free volume.

Figure 8: Adiabatic compressibility.

Docking study

The Glycyl – L –glycine + Benzene Sulfonamide + formamide entity is selected to be docked into the active site of 4HOE, 3EQA protein. The ligand is docked into the functional site of respective protein and docking energy is examined to achieve a minimum value. The Auto Dock results indicate the binding position of the peptide together with a rough estimate of interaction. The number of hydrogen bond formation in this molecular docking study is 6 with 4HOE, 2 with 3EQA (Table 2). Peptides are structurally related to proteins. Glycyl-L-glycine is a dipeptide with NH₂ group at one terminal and COOH group at another end. This dipeptide is considered as a ligand and used for docking analysis on antimicrobial protein targets. The binding sites on the protein surface is recognized by the ligand.

Table 2: The computed values of internal pressure, free volume and adiabatic compressibility of non- aqueous ternary solutions, at different concentrations are shown in the table

Molality (m)	Internal pressure (10 ⁹) Pa					
	278.15 K	288.15 K	298.15 K	308.15 K	318.15 K	328.15 K
0.001	1.8819	1.6694	1.4172	1.3182	1.2051	1.0876
0.005	1.9054	1.6805	1.4292	1.3317	1.2176	1.1014
0.01	1.8414	1.6283	1.3885	1.2948	1.1834	1.0813
0.025	1.9392	1.6710	1.4207	1.3306	1.2172	1.0971
0.05	1.9810	1.6773	1.4317	1.3345	1.2258	1.1115
Free volume (10⁻⁹)m³						
0.001	9.5232	14.9842	26.8351	36.3212	51.5460	75.5663
0.005	9.2134	14.7270	26.2292	35.2783	50.6225	72.8787
0.01	10.2083	16.1894	28.5914	38.3581	54.4486	77.0178
0.025	8.7212	14.9536	26.6291	35.2640	49.9117	73.5823
0.05	8.1507	14.7343	25.9110	34.8162	48.7050	70.5067
Adiabatic Compressibility 10⁻¹². TPa⁻¹						
0.001	32.7704	33.7936	34.8493	35.9498	37.1597	38.5108
0.005	32.5039	33.5432	34.6160	35.7416	36.9743	38.2780
0.01	32.4434	33.4434	34.4631	35.5825	36.7730	38.0374
0.025	32.4239	33.2100	34.3666	35.4150	36.6682	37.8456
0.05	32.3527	33.0533	34.2810	35.2096	36.3586	37.6399

Table 3: Hydrogen bonding and molecular docking with antimicrobial protein targets

Protein (PDB ID)	Bonded residues	No. of hydrogen bond	Bond distance (Å)	Estimated Inhibition Constant (μm)	Binding energy (kcal/mol)	Reference RMSD (Å)
4HOE	HIS 129	6	4.7	498.37	-4.51	27.02
	ILE 143		3.8			
	VAL 6		5.2			
	GLN 64		29.9			
	LIGA 1		2.2			
	GLU 144		3.7			
3EQA	AEG 329	2	2.3	13.16	-6.17	25.892
	GLU 203		1.9			

The autodock results indicate the binding position of the Peptide together with Benzene sulfonamide (Figure 3-5). The molecular docking binding energies and inhibition constants are also listed in the table (4). Among them 4HOE is found to exhibit the lowest free energy of -4.51kcal/mol. Hence it can be considered most docked inhibitors interacted well with the ligands within the 4HOE. The term active sites in 4HOE protein clearly visible in Figure 3. In Figure 4 the two H- bonding sites are shown for the protein 3EQA.

Figure 9: Docking and Hydrogen bonding interaction of Glycyl – L –glycine+ Sulfadiazine +Formamide with 4HOE Protein structure.

Figure 10: Docking and Hydrogen bonding interaction of Glycyl – L – glycine+ Benzene sulphonamide +Formamide with 3EQA Protein structure.

CONCLUSION

Ultrasonic measurements are very beneficial in chemical and food processing, material testing and under water range cleaning, wide range of applications in the fields of physics, chemistry, biology and medicine. Benzene sulfonamide are essential clinical agents, mainly they are used in the treatment of gastro-intestinal-duodenal ulcers, neurological disorders, glaucoma, altitude sickness and for some forms of tumors. Internal pressure is a vital factor which varies due to all the interactions present between a peptide-drug and solvent. Investigation of the acoustic, thermodynamic properties, suggest that there is a strong peptide - drug - amide interaction in the non-aqueous solutions. The above results are determined by spectroscopic, ultrasonic and docking studies. The molecular dock output implies the lowest binding energy for 4HOE there six hydrogen bonding interaction and most docked inhibitors interacted with the ligand within the 4HOE binding site.

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