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A STUDY ON TERNARY MIXTURE OF PEPTIDE USING ULTRASONIC, *IN-VITRO* AND *IN-SILICO* PROCESSES

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Keywords:

Internal pressure, Free volume, Antimicrobial, *in-silico* studies

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ABSTRACT: Penetrating biomolecules by ultrasonic techniques is an innovative and influential tool for characterizing their physicochemical properties. Peptides have been used as extensive applications in drug construction and as an ingredient in nutritional complements. An investigation based on the behavior of promulgation of ultrasonic waves in solids and the liquid system is now rather well recognized as an effective means for scrutinizing certain physical properties of the materials. The data attained from ultrasonic proliferation parameters such as ultrasonic velocity, density, viscosity, etc., and additional variation with concentration of one of the medium modules are useful in consideration of the nature of molecular interaction in terms of physical parameters. Owing to the sensitivity to very low populace densities at high energy circumstances, ultrasonic methods have been preferred and are testified to be corresponding to other techniques. An exhaustive study of literature exposes that the ternary solutions and liquid mixtures were premeditated and reported by different inventors. They testified on the nature of forces elaborate in the formation of the hydrogen bonds. The research work on peptides with a fixed concentration of drug at 298.15 K to 318.15 K system is scanty. Further, to exhibit the biological activity of the non-aqueous solutions, molecular docking (ligand-protein) inspirations are performed by consuming Auto Dock 4.2.6 tools, and their binding energy, hydrogen bond lengths values are determined. The increasing trend in thermodynamical parameters indicates strong peptide-drug-amide molecular interactions captivating in the non-aqueous solutions. The attained ultrasonic results are correlated with antimicrobial and molecular docking studies.

INTRODUCTION: In recent years, the dimension of ultrasonic velocity has been fruitfully employed to consider the molecular interactions in aqueous and non-aqueous solutions. Ultrasonic velocity measurements are very delicate and accurate in determining the molecular interactions and can be used to deliver quantitative information about the physical environment and strength of molecular interaction in ternary solutions [1-3].

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The ultrasonic velocity of a solution is essentially associated with the binding forces between the particles or the molecules and has been sufficiently employed in considering the nature of molecular interactions in pure liquids/solutions, binary and ternary combinations [4-5].

Glycylglycine (Gly-Gly) and dipeptides have helped as imperative model systems for theoretical and experimental calculations of the amide linkages in proteins and peptides. The study of these species has been inspired by the expectation that an important understanding of protein structural constriction and their role in determining biological functions, demanding a deep understanding of the structure and dynamics of the peptide amide linkages [6].

Sulfonamides have been identified as the first chemotherapeutic mediators administered clinically in the human biological systems as antibacterial drugs [7]. The antibacterial drug compound of sulfanilamide is used as an active agent to breakdown the prontosil in the human body.

Muhamed Hassan Mohammed *et al*., [8] have reported as design, synthesis, and pharmacological evaluation of sulfanilamide-ciprofloxacin conjugates utilizing hybridization approach as new antibacterial agents. Hira Saleem *et al*., [9] have reported as design, synthesis, characterization, and computational docking studies of novel sulfonamide derivatives. Prajapat *et al*., [10] have stated as thermal, spectroscopic, and antimicrobial properties of novel nickel (II) complexes with sulfanilamide and sulfamerazine drugs. Ana Tack *et al*., [11] have also reported as antimicrobial sulfonamide drugs. Muthuselvi *et al*., [12] have reported growth, characterization and antibacterial study of sulfanilamide doped by 4-aminobenzoic acid, respectively.

Detailed scrutiny of the literature review has revealed that detailed ultrasonics, anti-microbial, and docking study has not been carried out for the title compound so far. Hence, the study is undertaken. These experimental measurements are associated with antimicrobial and molecular docking studies using Autodock 4.2.6.

In the present work, the ultrasonic, antimicrobial, and molecular docking exploration of ternary solutions have been analyzed using AutoDock 4.2.6. The molecular docking properties of bonded residues, a number of hydrogen bonds, bond distance, estimated inhibition constant, and binding energy has been computed. Ligand-Protein analysis is also performed to elucidate hydrogen bonding interaction in the solutions.

MATERIALS AND METHODS:

Experimental Details: Ultrasonic velocity was measured using a digital ultrasonic interferometer of fixed frequency 2MHz (Model F-81 Mittal enterprises, New Delhi) with an accuracy of ± 0.2 m/s. The density of the non-aqueous peptide (solute) with drug (cosolute) solutions were measured using Anton Paar DMA 4100M Digital density meter with an accuracy of ± 0.0001 gm/cc. The viscosities of the solutions were measured using Cannon Fenske viscometer (±0.1%) with the experimental solution was immersed in a temperature-controlled water bath. The time of flow was measured using a stopwatch with an accuracy of ± 0.1 sec.

Computation: The measured data were used to calculate the thermodynamic parameters using standard relations.

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

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

RESULTS AND DISCUSSION:

Ultrasonic Study:

Internal Pressure and Free Volume: Internal pressure and free volume are calculated from equations (1) and (2). The properties are fundamentally responsible for various interactions occurring in the solution. Internal pressure is a single factor that represents several internal interactions. In the non-aqueous solutions of sulphanilamide and glycyl-L-glycine, the internal pressure increases with increasing concentrations **Table 1** at all the temperatures **Fig. 1**. The increase in cohesive energy designates due to strong solute interactions. Hence, the increase may be attributed to the structure making nature of the peptide and drug in the solvent.

TABLE 1: THE COMPUTED VALUES OF INTERNAL PRESSURE AND FREE VOLUME OF NON- AQUEOUS TERNARY SOLUTIONS, AT DIFFERENT CONCENTRATIONS ARE SHOWN IN THE TABLE

Molality (m)	Internal Pressure (10^9Pa)		
	Glycyl-L-glycine + 0.01m of drug		
	298.15K	308.15K	318.15K
0.001	1.3912	1.3257	1.2220
0.005	1.4026	1.3387	1.2303
0.01	1.4181	1.3490	1.2380
0.025	1.4260	1.3588	1.2528
0.05	1.4424	1.3688	1.2591
	Free Volume (10^{-9}) m³		
	Glycyl-L-glycine + 0.01m of drug		
0.001	28.3334	35.7582	49.4517
0.005	27.6791	34.7244	48.4619
0.01	26.7748	33.9294	47.5438
0.025	26.2786	33.1249	45.7811
0.05	25.4160	32.3262	44.9160

Free volume is the free space available for the molecules to move freely. The variations in the free volume (V_f) for molalities and temperatures of

sulphanilamide and glycyl-L-glycine in formamide behave contrary to internal pressure **Table 1**. The variations of V_f with molalities **Fig. 2** confirm the structure making nature of the solute and cosolute in the solvent.

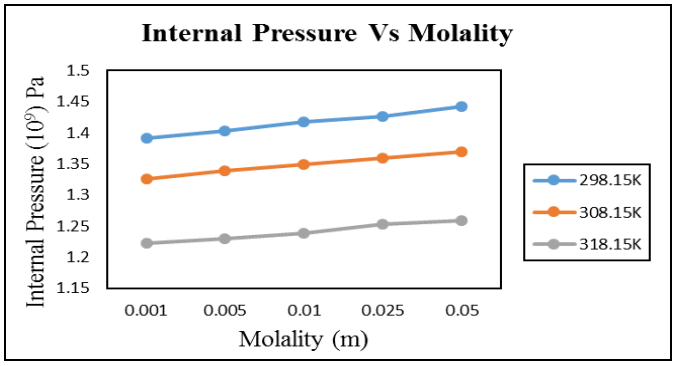

FIG. 1: INTERNAL PRESSURE

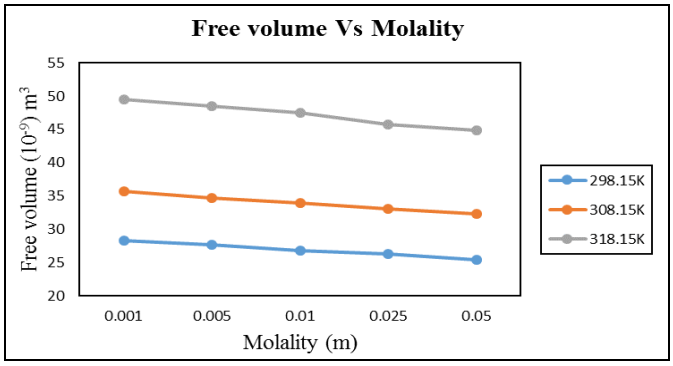

FIG. 2: FREE VOLUME

Antimirobial Study: An antimicrobial is a mediator that destroys microorganisms or inhibits their growth. Antimicrobial drugs can be grouped based on the microorganisms they act primarily against. The core classes of antimicrobial agents are antiseptics, which kill a wide range of microbes on non-living surfaces to prevent the feast of infection, sanitizers, and antibiotics.

In the present exploration, antibacterial and antifungal activities of the ternary solutions of peptide-drug-amide have been tested by the disc diffusion method. The various bacterial organisms, such as gram-negative bacteria (*E. coli*) and fungi (*Aspergillus niger*) are used to treasure out the antimicrobial activity. The solutions of glycyl-L-glycine + sulphanilamide + formamide exhibited a minimum zone of inhibition for *Escherichia coli* as 24 mm and *Aspergillus niger* as 30 mm. The antimicrobial activity of sulphanilamide exhibited a minimum zone of inhibition against *Escherichia coli* of only 20 mm. Since the mixture of glycyl-L-glycine + sulphanilamide + formamide solutions used in the current study exhibited an inhibitory zone of 24 mm, it may be a tremendously effective disinfectant.

The ternary mixture also showed a minimum inhibitory zone of 30 mm with *Aspergillus niger*. The ternary mixture should also be an effective antifungal agent. The obtained results are tabulated as follows in **Table 2**.

TABLE 2: ZONE OF INHIBITIONS BY DISC DIFFUSION METHOD

S. no.	Name of the Microorganism	Zone of inhibition in mm				
		FMA	GG	SFA	GG+SFA	Standard
1	*E. coli* (NCIM 2065)	15	16	20	24	38
2	*Aspergillus niger* (NCIM 105)	18	20	28	30	30

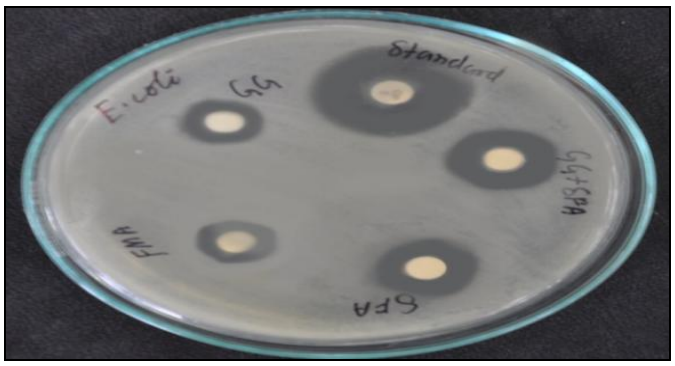

FIG. 3: ANTIBACTERIAL (*E. COLI*)

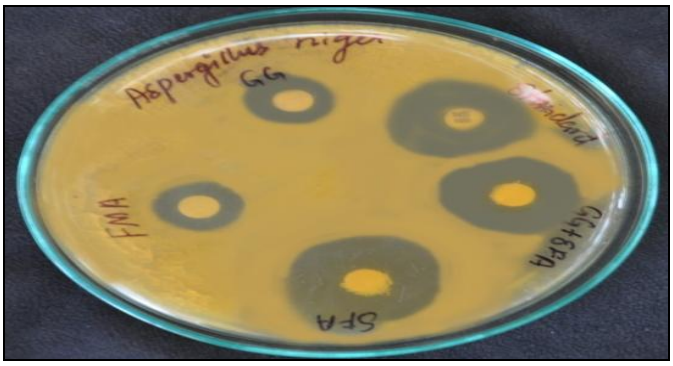

FIG. 4: ANTIFUNGAL (*ASPERGILLUS NIGER*)

Molecular Docking: AutoDock suite 4.2.6 is that has been used as an expedient tool to get insights into the molecular mechanism of protein-ligand interactions that binds to a receptor of known three-dimensional structure. With the aim to explore the binding mode, a molecular modeling study was

performed using Auto Dock Tools for docking. Glycyl-L-glycine + sulphanilamide + formamide were selected as samples, antimicrobial proteins were downloaded from RCSB protein data bank to be docked into the active site of five receptors 4HOE, 3EQA, 4HBT, 4HOF and 3K4P.

The Glycyl - L - glycine + Sulphanilamide + formamide were selected to be docked into the active site of 4HOE, 3EQA, 4HBT, 4HOF, and 3K4P proteins. The ligand is docked into the useful site of a respective protein, and docking energy **Table 3** is examined to accomplish a minimum value. The Auto Dock results designate the binding position of the peptide composed with a rough estimate of interactions. The number of hydrogen bond formation in this molecular docking study is 7 with 4HOE and 6 with 4HOF **Table 3**. Peptides are architecturally related to proteins. This dipeptide is considered as a ligand and used for docking analysis on antimicrobial protein targets. The binding sites on the protein surface are documented by the ligand.

TABLE 3: HYDROGEN BONDING AND MOLECULAR DOCKING WITH ANTIMICROBIAL PROTEIN TARGETS OF GLYCYL–L-GLYCINE + SULPHANILAMIDE + FORMAMIDE

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	7	2.7	5.94	-7.13	27.12
	VAL 109		2.8			
	ILE 112		2.7			
	ALA 11		3.6			
	ILE 19		2.1			
	ASN 5		1.7			
	LEU 69		13.9			
3EQA	TRP 202	4	2.4	1.05	-8.16	24.74
	TYR 335		4.5			
	ASP 79		1.7			
	ARG 329		2.0			
4HBT	GLU 271	6	2.6	8.32	-6.93	6.07
	ARG 274		1.7			
	ARG 274		2.6			
	ASP 275		1.8			
	ALA 219		2.2			
	ASP 275		2.0			
4HOF	ILE 19	6	2.5	17.04	-6.51	27.41
	ILE 112		2.5			
	ALA 11		2.8			
	GLU 32		2.2			
	GLU 32		2.2			
	ALA 11		4.0			
3K4P	SER 198	3	2.4	13.89	-6.63	43.39
	PHE 195		2.0			
	ASP 188		1.7			

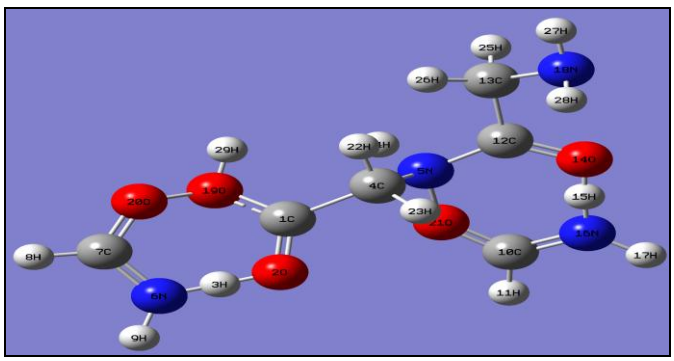

FIG. 5: SOLVATED STRUCTURE OF GLYCYL – L – GLYCINE + SULFANILAMIDE+ FORMAMIDE SOLUTIONS

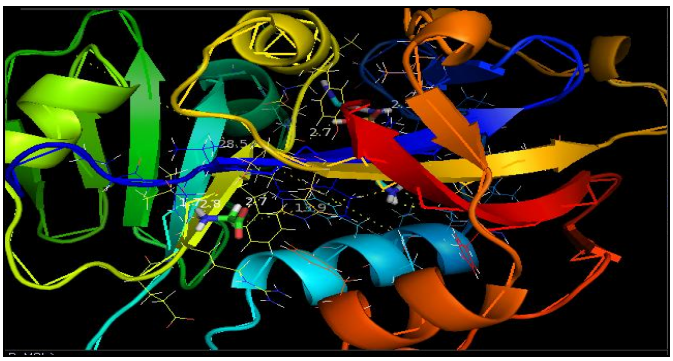

FIG. 6: DOCKING AND HYDROGEN BONDING INTERAC-TION OF GLYCYL – L –GLYCINE + SULFANILAMIDE+ FORMAMIDE WITH 4HOE PROTEIN STRUCTURE

The autodock results designate the binding position of the peptide together with sulphanilamide **Fig. 6-10**. The molecular docking binding energies and inhibition constants are also listed in **Table 3**. Among them 4HOE is found to exhibit the lowest free energy of -8.16 kcal/mol. Hence, it can be inferred that the most docked inhibitors interacted well with the ligands within the 4HOE. The term active sites in 4HOE is evidently noticeable in **Fig. 6**. The seven H- bonding sites are shown for the protein.

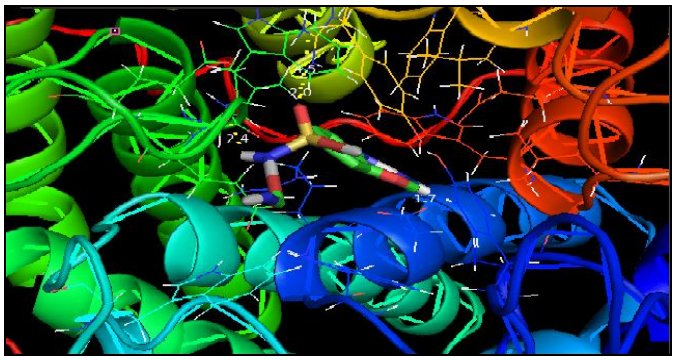

FIG. 7: DOCKING AND HYDROGEN BONDING INTERACTION OF GLYCYL – L –GLYCINE + SULFANILAMIDE+ FORMAMIDE WITH 3EQA PROTEIN STRUCTURE

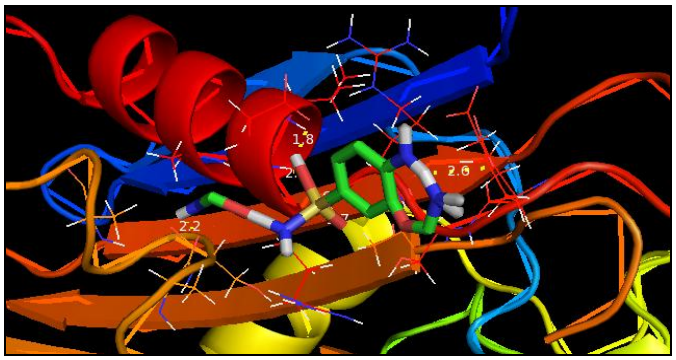

FIG. 8: DOCKING AND HYDROGEN BONDING INTERACTION OF GLYCYL – L –GLYCINE + SULFANILAMIDE+ FORMAMIDE WITH 4HBT PROTEIN STRUCTURE

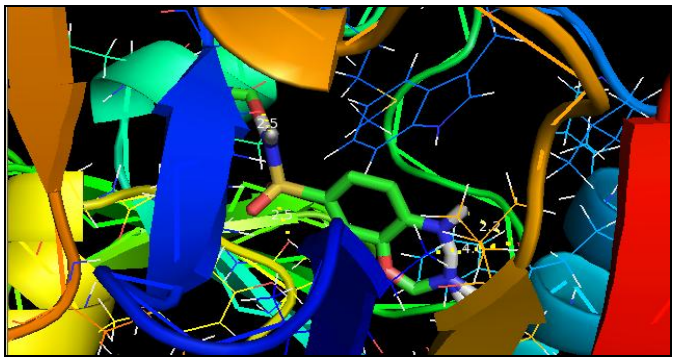

FIG. 9: DOCKING AND HYDROGEN BONDING INTERACTION OF GLYCYL – L –GLYCINE + SULFANILAMIDE+ FORMAMIDE WITH 4HOF PROTEIN STRUCTURE

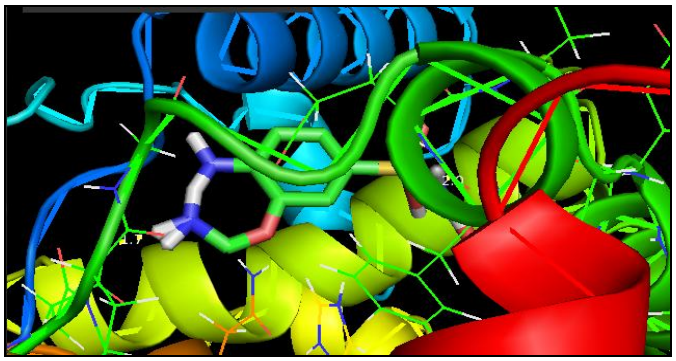

FIG. 10: DOCKING AND HYDROGEN BONDING INTERACTION OF GLYCYL – L –GLYCINE + SULFANILAMIDE+ FORMAMIDE WITH 3K4P PROTEIN STRUCTURE

CONCLUSION: In the current exertion, experimental and theoretical inspection of the peptide with drug molecule using ultrasonics, antimicrobial, and molecular docking consequences from the AutoDock are studied. In general, good interactions between the peptide-drug-amide interactions has been inspected. Bonded residues, number of hydrogen bonds, bond distance, estimated inhibition constant, binding energy, reference RMSD of the molecule have been calculated by using molecular modelling performances. The thermodynamical parameters such as internal pressure and free volume show as strong interactions taking place in the non-aqueous solutions. The molecular docking output shows that the lowest binding energy for the peptide-drug-amide molecule is -8.16 kcal/mol and maximum docked inhibitors interacted with the ligand within the 4HOE binding site. Hence, the ternary solutions have pharmacological properties. The molecular docking analysis supports the experimental findings and calculations of peptide - amide - drug interactions of ultrasonic methods.

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CONFLICTS OF INTEREST: Nil

REFERENCES:

1. Umadevi M and Kesavasamy R: Molecular interaction studies on ester with cyclohexane in alcohol at 303, 308

and 313K. Int Journal of Chemical, Environmental and Pharmaceutical Research 2012; 3(1): 72-82.
2. Thennarasu J and Meenakshi G: Evaluation of ultrasonic velocity and percentage deviation of ternary mixtures at different temperatures. Rasayan Journal of Chemistry 2011; 4(4): 904-09.
3. Thirumaran S, Jayakumar JE and Dhanasundaram BH: Molecular interaction studies on N-Alkanols with DMA in Toluene at 303K. Journal of Chemistry 2010; 7(2): 465-72.
4. Vigneswari M, Saravanakumar SS, Sureshbabu VN and Sankarrajan S: Molecular interactions in solutions of poly vinyl alcohol; an ultrasonic study. International Journal of Advanced Chemistry 2016; 4(2): 15-21.
5. Halder S, Agarwal PS, Thakur S and Paliwal LJ: Ultrasonic study of molecular interaction in ternary liquid mixtures at 293K. IOSR J of App Chem 2014; 7(9): 36-40.
6. Chen XG, Pusheng L, Holtz JSW, Zhenhuan C, Vasil P, Asher SA and Kelly LA: Resonance Raman examination of the electronic excited states of Glycylglycine and other Dipeptides: observation of a Carboxylate amide charge transfer transition. Journal of American Chemical Society 1996; 118: 9705-15.
7. Prajapat G, Gupta R and Bhojak N: Thermal, spectroscopic and antimicrobial properties of novel Nickel (II) complexes with sulfanilamide and sulfamerazine drugs. Chemical Science International Journal 2018; 24(2): 1-13.
8. Mohammed MH, Mahdi MF, Naser NH and Ali SM: Design, synthesis and pharmacological evaluation of sulfanilamide-ciprofloxacin conjugates utilizing hybridization approach as new antibacterial agents. Journal of Natural Sciences research 2015; 5(4).
9. Saleem H, Maryam A, Bokhari SA, Ashiq A, Rauf SA, Khalid RR, Qureshi FA and Siddiqi AR: Design, synthesis, characterization and computational docking studies of novel sulfonamide derivatives. EXCLI Journal 2018; 17.
10. Prajapat, Gupta R and Bhojak N: Thermal, spectroscopic and antimicrobial properties of novel Nickel (II) complexes with sulfanilamide and sulfamerazine drugs. Chemical Science International Journal 2018; 24(2): 1-13.
11. Tack A, Nikolic V, Nikolic L and Slavic I: Antimicrobial sulfonamide drugs. Advanced Technologies 2017; 6(1): 58-71.
12. Muthuselvi C, Selvi I and Pandiarajan S: Growth, characterization and antibacterial study of sulfanilamide doped by 4-aminobenzoic acid. Journal of Chemical and Pharmaceutical Research 2017; 9(5): 122-30.

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