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AN INVESTIGATION OF ACOUSTIC, THERMODYNAMIC AND ANTIMICROBIAL ACTIVITY STUDY OF A DRUG IN A PEPTIDE

R. Padmavathy [*1], K. Dhanalakshmi [2], E. Jasmine Vasantha Rani [3] and N. Radha [4]

PG and Research Department of Physics, Seethalakshmi Ramaswami College, Tiruchirappalli - 620002, Tamil Nadu, India.

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Amino acids, Acoustical parameters, Antimicrobial study

Correspondence to Author:
R. Padmavathy

PG and Research
Department of Physics, Seethalakshmi Ramaswami College, Tiruchirappalli - 620002, Tamil Nadu, India.

E-mail: shreepadmaram@gmail.com

ABSTRACT: Peptides are recognized for being highly selective efficacious and at the same time, relatively safe and well tolerated. Consequently, there is an increased interest in peptides, in pharmaceutical research and development (R&D) and approximately 140 peptide therapeutics are currently being evaluated in clinical trials. Ultrasonic is an area of intense scientific and technological research, science and technology of ultrasonics are widely used in the recent years for industrial and medical applications. Ultrasonic investigation in non- aqueous solutions of electrolytes and non- electrolytes with amino acids provide useful information in understanding the behaviour of liquid/solution system. In the present work, the ultrasonic velocity of a peptide with drug in formamide have been measured at various concentrations and at different temperatures. Using the measured value the thermodynamic parameters and acoustical parameters are calculated. These parameters have been utilized to study the interionic and intermolecular interactions existing in the system. The results arrived from ultrasonic methods have been corroborated with antimicrobial study.

INTRODUCTION: Proteins and peptides are ubiquitous in every cell and thus are vital for various biological functions. In recent years, there is an extensive growth in the development of various biological and large molecules like proteins and peptides [1]. More than seven thousand naturally occurring peptides have been identified and these often have crucial roles in human physiology, including actions as hormones, neurotransmitters, growth factors, ion channel ligands or anti-infective [2 - 5].

During the past decade, peptides have gained a wide range of applications in medicine, biotechnology and therapeutic peptide research is also currently experiencing a renaissance for commercial reasons [6]. One of these systems is peptide-mediated drug delivery which has been extensively applied to a wide range of cargo molecules [7].

Cell penetrating peptides (CPPs) provides a promising solution to the problems commonly related to the drug delivery of conventional cancer chemotherapeutics as well as oligonucleotide based treatments. Many sulpha drugs like sulphadiazine, sulphamethoxazole, sulphamerazine possess SO_2NH moiety as an important toxophoric function [8]. Sulfadiazine is a useful antibacterial drug with a typical sulphonamide structure.

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It has been shown selectively by Yoshida Sarcoma [9] and new antitumor agents can be designed by combining sulfadiazine and antitumor agents in one compound [10].

Formamide is a constituent of cryo protectant verification mixtures used for cryopreservation of tissues and organs. Formamide is also used as an RNA stabilizer in gel electrophoresis, it is used for stabilizing (single) strands of denatured DNA. Formamide is used to prepare primary amines directly from ketones. The ultrasonic measurements are useful in determining number of acoustical parameters. Using the measured values of ultrasonic velocity, viscosity and density, the acoustical parameters such as adiabatic compressibility, specific acoustic impedance and intermolecular free length have been calculated. Solution of Glycyl-L- glycine + Sulfadiazine in polar solvent is taken for present investigation. This study has been carried out at different temperatures concentrations. The antimicrobial activity have been analysed the solution Glycyl-L- glycine + Sulfadiazine + formamide.

Experimental details:

Ultrasonic velocity measurement: Ultrasonic velocity was measured using digital ultrasonic interferometer of fixed frequency 2 MHz with on accuracy of ± 0.2 m/s (Model F-81 Mittal enterprises, New Delhi). The measuring cell of the interferometer is a specially designed double walled vessel with provision for maintaining temperature constant. A digital constant temperature bath operating in the temperature range 0 °C to 90 °C with an accuracy of ± 0.1 °C has been used to circulate water through the outer jacket of double walled measuring cell containing the experimental liquid/solution.

Density measurement: The density of the peptide + drug solutions are measured using DMA 4100 Anton Paar digital densitometer with an accuracy of ± 0.0001 gm/cc.

Viscosity measurement: The viscosities of the solutions are measured using a specially designed Cannon Fenske viscometer (±0.1 % error) with the experimental solution is 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 viscosity was determined using the relation, $\eta_1 = [\rho_1 t_1/\rho_2 t_2] \times \eta_2$

MATERIALS AND METHODS: Glycyl-L-glycine and Sulfadiazine of molecular weight 132.12 and 278.42 g/mole respectively were purchased from Sisco research laboratories Pvt. Ltd. and Moly chemicals Mumbai respectively. Formamide has been used as a solvent for preparing sulfadiazine (0.01 m) fixed molality and Glycyl – L – glycine solutions of various concentrations.

Computation: Using the measured data, the following transport and acoustical parameters have been computed using the standard relations,

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

Free volume $(V_f) = [Meff\ u/K\eta]^{3/2}\,(m^3)\,(10^{-9})$

Adiabatic compressibility $(\beta) = (1/u^2\,\rho)\,(10^{-12}\ Pa^{-1})$

Specific acoustic impedance $(Z) = \rho u (kg.m^{-2}.S^{-1})\,(10^3)$

Intermolecular free length $(L_f) UL_f\,(\rho)^{1/2} = K\,(10^{-11})$ m

Where π_i is the internal pressure, 'K' is a constant equal to 4.28×10^9, 'η' viscosity of the solution in pascal, 'u' ultrasonic velocity of the solution in m/sec, M_{eff} is given by $M_{eff} = (M_1X_1+M_2X_2+M_3X_3)$, and 'ρ' the density of solution at the given temperature.

RESULTS AND DISCUSSION:

Internal pressure and free volume: The internal pressure is a single factor which is measured quantitatively. Internal pressure and free volume are easily measurable and fundamentally responsible for various interactions occurring in the solution. In the present investigation the internal pressure increases with increasing concentration initially and then decreases at 0.015 molality, at all the temperatures. This decrease may be due to weak solute-solvent interaction that exist between the Glycyl-L-glycine and Sulphadiazine solutes in the non-aqueous solvent. Hence the cohesion between the solutes and the solvent is reduced whereas the interaction between the Glycyl-L-Glycine + Sulphadiazine + formamide *i.e.* solute-solute interaction is dominating the solute-solvent interaction.

Thus the solute-solute interaction favour's the dipole-dipole, dipole-induced dipole and electrostrictive forces hence the structure breaking nature of the peptide and drug is identified (Glycyl-L-glycine + Sulphadiazine+ formamide).

Free volume is one of the significant factors in explaining the variations in the physico-chemical properties of liquids, solutions and liquid mixtures. The free space and its dependent properties have close connection with molecular structure.

Adiabatic compressibility: The adiabatic compressibility is found to decrease with increase in concentration and temperatures. The calculated values of (β_{ad}) have been presented in **Table 1**. It is clear that the compressibility of a solvent is higher than that of a solution and it decreases with increase in concentrations. The primary effect of dissolving a peptide along with a drug is to lower the compressibility of the solvent. 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 the number of incompressible molecule and (ii) structural change 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.

Specific acoustic impedance: For a given concentration the values of acoustic impedance (z) increases with increase in concentration in the system, Glycyl-L-glycine + Sulphadiazine + formamide. The increase in 'z' with the increase in concentrations of solutes can be explained in terms of inter and intra molecular interactions between the molecules of the solution. This linear increase in 'Z' may be due to the presence of solute-solvent interaction occurring in the peptide-drug solution.

Intermolecular Free Length: The decrease in intermolecular free length with increase of concentration in solution indicates that there are significant interactions between the peptide and drug solution, *i.e.* the distance covered by the two neighboring molecules is reduced. Thus the intermolecular distance decreases with concentration. The decrease in free length may due to the gain of dipolar association and making up of hydrogen bonds in the molecules of the solutions.

TABLE 1: INTERNAL PRESSURE, FREE VOLUME, ADIABATIC COMPRESSIBILITY, SPECIFIC ACOUSTIC IMPEDANCE AND INTERMOLECULAR FREE LENGTH OF NON- AQUEOUS TERNARY SOLUTIONS AT DIFFERENT TEMPERATURES

Molality (m)	Internal Pressure (10^9Pa)	Free Volume (m^3) (10^{-9})	Adiabatic Compressibility (10^{-11})(m^2/N)	Specific acoustic impedance ($kg.m^2.S^{-1}$) (10^{-3})	Intermolecular free length (10^{-11})m
298.15K					
0.001	1.444	25.6	34.246	0.1821	1.159
0.005	1.459	24.8	34.203	0.1823	1.158
0.1	1.469	24.3	34.168	0.1824	1.157
0.25	1.414	27.2	34.103	0.1826	1.156
0.05	1.469	24.2	33.985	0.183	1.154
308.15K					
0.001	1.340	34.9	35.365	0.1786	1.198
0.005	1.353	33.9	35.312	0.1788	1.197
0.1	1.382	31.8	35.281	0.1789	1.197
0.25	1.312	37.2	35.22	0.1791	1.196
0.05	1.389	31.1	35.12	0.1794	1.194
318.15K					
0.001	1.226	49.4	36.592	0.1749	1.237
0.005	1.239	47.9	36.536	0.1751	1.236
0.1	1.252	46.3	36.499	0.1752	1.235
0.25	1.192	53.7	36.401	0.1755	1.234
0.05	1.261	45.2	36.326	0.1757	1.233

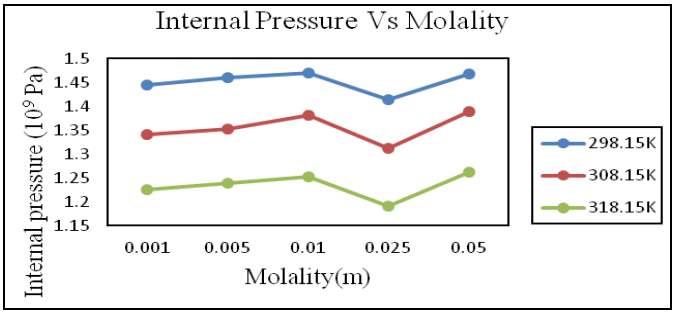


FIG. 1: INTERNAL PRESSURE VS MOLALITY

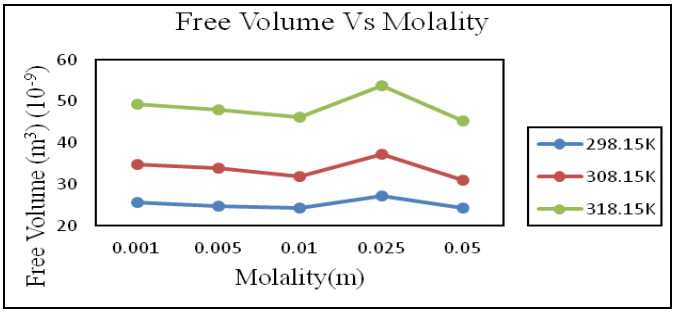


FIG. 2: FREE VOLUME VS MOLALITY

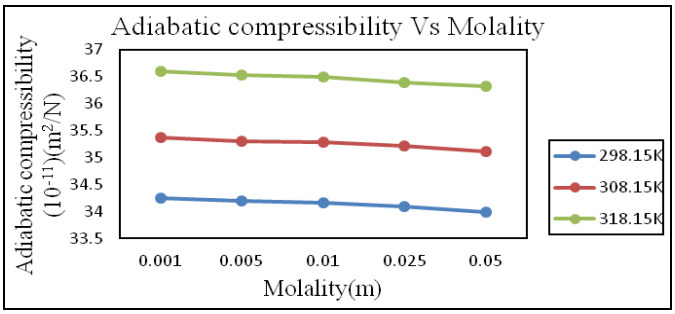


FIG. 3: ADIABATIC COMPRESSIBILITY VS MOLALITY

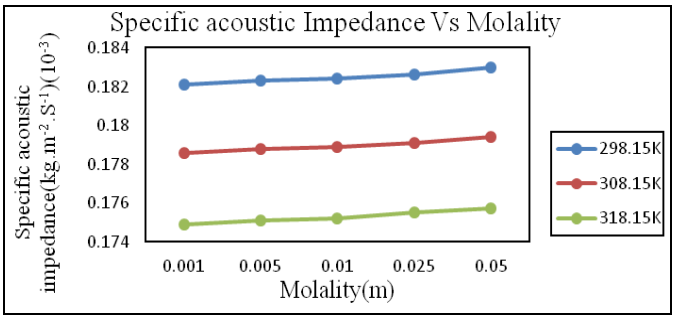


FIG. 4: SPECIFIC ACOUSTIC IMPEDANCE VS MOLALITY

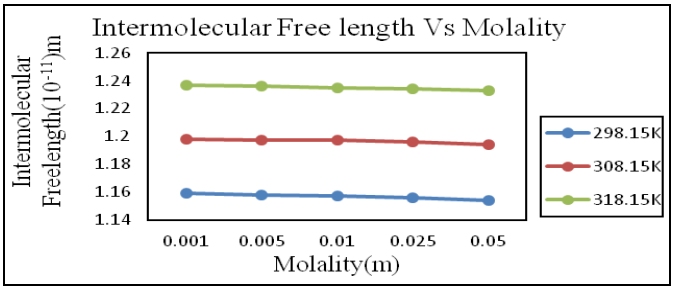


FIG. 5: INTERMOLECULAR FREE LENGTH VS MOLALITY

Salient features of samples:

Microbial and Micro fungal activity: Antibacterial and antifungal activities of plant part extracts against four pathogenic bacteria (two Gram-positive and negative) and three pathogenic fungi were investigated by the agar disk diffusion method.

Selection of microorganisms: Totally 3 human pathogenic microorganisms namely two bacterial strains such as, *Escherichia coli* and *Streptococcus pyogenes* and one fungal strains such as, *Aspergillus niger* were selected for the present investigation. The human pathogenic bacteria and fungi were originally obtained from Microbial Germ Plasm Culture Collection Unit (MGPCCU), Sri Gowri Biotech Research Academy, Thanjavur and used for the present investigation.

Preparation of microbial inoculums: The young microbial inoculums was prepared and used during the research period. The nutrient broth (NB) and potato dextrose broth (PDB) were prepared and poured into several tubes. Then these tubes were sterilized. The pure microbial cultures inoculated in the tubes by using inoculation loops. After that these tubes were incubated at 37 °C for 24-28 hrs and 28 °C for 48-72 hours for bacteria and fungi respectively. After incubation the cultures were used for the experiments.

Media preparation:

Composition of nutrient agar medium;

Peptone	-	5gms
Beef extract	-	3gms
NaCl	-	5 gms
Agar	-	18gms
Distilled water	-	1000ml
pH	-	6.8

Preparation of nutrient agar medium: The ingredients were weighed and taken in a conical flask contains 1000 ml distilled water. Then pH of the medium was adjusted to 6.8 using a pH meter by the addition of either acid (or) alkali. The flask was sterilized in an autoclave at 121^{o}C for 15 lbs pressure for 15 minutes and allowed to cool.

Composition of potato dextrose agar medium:

Potato (Peeled)	-	200gms
Dextrose	-	20gms
Agar	-	15gms

Distilled water	-	1000ml
pH	-	5.6

Preparation of potato dextrose agar medium: The potato tubers were peeled and weighed for about 200 g. The tubers were chopped into small pieces with the help of sterile knife. The chopped potatoes were transferred into a conical flask containing about 100 ml of distilled water. The content was boiled for 20 mins. The supernatant were decanted and filtered by muslin cloth and the filtrate was collected. Dextrose agar were transferred into the extract and shacked to dissolve the ingredients. The medium was made up to 1litre by addition of distilled water. The pH of the medium was adjusted to 5.6 by using 1N hydrochloric acid or sodium hydroxide drop wise. Finally, the medium was poured into two conical flasks, cotton plugged and sterilized in pressure cooker for 20 minutes.

Screening of antibacterial activity: The antibacterial activities of the (GG+ SDZ) were tested against the selected bacterial strains. The petriplates were washed and placed in an autoclave for sterilization. After sterilization, nutrient agar medium was poured into each sterile petriplate and allowed to solidify in a laminar air flow chamber. After solidification, using a sterile cotton swab, fresh bacterial culture with known population count was spread over the plate by spread plate technique. One well of 5 mm size made into the agar plates with the help of sterile corkborer, the wells were loaded with 200 µl of extracts (GG+SDZ). All the plates were incubated at 37 ^{o}C for 24 hours. After incubation, the plates were observed for formation of clear inhibition zone around the well indicated the presence of antibacterial activity. The zone of inhibition was calculated by measuring the diameters of the inhibition zone around the well.

Screening of antifungal activity: In the freshly prepared and sterilized potato dextrose agar medium, a pinch amount of streptomycin was added to prevent bacterial contamination and mixed well. The medium was poured into each petriplate and allowed to solidify. The test fungal cultures were evenly spread over the appropriate media by using sterile cotton swab. Then wells 5mm was made in the medium by using sterile cork borer, 200 µl of the GG+SDZ extracts were transferred into separate wells. Then these plates were incubated at 28 °C for 48-72 hours. After incubation period the results were observed and measured the diameter of inhibitor zone around the each well.

Antibiotic sensitivity test on microbes (Positive control): The antibiotic sensitivity test was analysed using standard antibiotics (Streptomycin) (10 µg/µl) for bacteria and Fluconazole (10 µg/µl) for fungi. The sterilized nutrient agar and PDA medium were poured into each sterile petriplates and allowed to solidify. By using a sterile cotton swab, a fresh bacterial and fungal culture was spread over the plates by following spread plate technique. Then the selected standard antibiotics were placed on the bacterial and fungal culture plates. Then, the plates were incubated for 24 hours at 37 ^{o}C for bacteria and 28 ^{o}C for 48 – 72 hours for fungi. After the incubation period, the results were observed and the diameter of the inhibition zone was measured around the wells.

Antimicrobial effects of solvents (Negative control): The antimicrobial activity of FMA were tested against the selected bacterial and fungal strains. The sterilized nutrient agar medium and PDA medium was poured into each sterile petriplates and allowed to solidify. By using a sterile cotton swab, a fresh bacterial and fungal culture was spread over the plates by following spread plate technique. 200 µl of FMA were loaded in to separated wells. The plates were incubated for 24 hours at 37 ^{o}C for bacteria and 28 ^{o}C for 48 - 72 hours for fungi. After the incubation period, the results were observed and the diameter of the inhibition zone was measured around the wells.

RESULTS AND DISCUSSIONS:

TABLE 2: ANTIBACTERIAL ACTIVITY OF GG+ SDZ

S.no	Name of bacterial pathogen	Zone of inhibition (mm)		
		GG+SDZ	FMA	Streptomycin
1.	*Escherichia coli*	10	-	15
2.	*Streptococcus pyogenes*	9	-	14

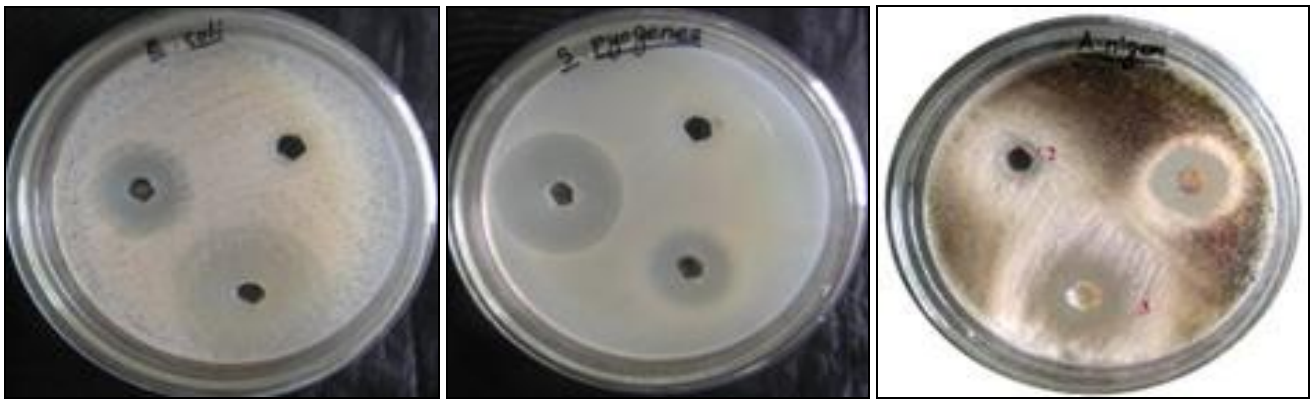
TABLE 3: ANTIFUNGAL ACTIVITY

S.no	Name of fungal pathogen	Zone of inhibition (mm)		
		GG+SDZ	FMA	Fluconazole
1.	*Aspergillus niger*	13	-	9

The antimicrobial activity of the Glycyl-L-glycine +Sulfadiazine was assayed in agar well diffusion test against human pathogenic gram positive bacteria such as streptococcus pyogenes and gram negative bacteria such as *Escherichia coli* and human pathogenic fungi such as *Aspergillus niger* was studied.

Glycyl-L-glycine +Sulfadiazine showed maximum zone of inhibition against *Escherichia coli* (10 mm) and minimum zone of inhibition against streptococcus pyogenes (9 mm). The formamide showed absence of zone of inhibition against streptococcus pyogenes and *Escherichia coli.* The streptomycin antibiotic showed the maximum zone of inhibition against *Escherichia coli* (15 mm) and minimum zone of inhibition against streptococcus pyogenes was observed as (14 mm standard).

The antifungal activity of Glycyl-L-glycine + sulfadiazine showed maximum zone of inhibition against *Aspergillus niger* (13 mm), the formamide showed absence of zone of against Aspergillus niger and the Flucozole (standard) antibiotic showed zone of inhibition against *Aspergillus niger* (9 mm). An attempt is made to corroborate the behavior of the samples with antifungal and antimicrobial activity. The biological applications of the samples are revealed in this study [11] as the solution of Glycyl-L-glycine + formamide + sulfadiazine may act as a disinfectant.



1. Glycyl-L-glycine+ Sulfadiazine
2. FMA
3. Streptomycin

FIG. 6: ANTIFUNGAL AND ANTIMICROBIAL ACTIVITY OF GLYCYL-L-GLYCINE+ SULFADIAZINE+FMA

CONCLUSION: The peptide and protein based pharmaceuticals thus are rapidly becoming very important class of therapeutic agents. The peptides and proteins based drugs effectively treats various diseases and life-threatening conditions. The improvement in therapies and cure is definite by increasing the alteration in the properties of peptides and proteins. Sulfonamides also known as sulfa drugs, represent a kind of typical antibiotics and have been widely used in human and vertinary medicine to treat and prevent infections of bacterial diseases. Sulfadiazine is a rapidly absorbed and readily excreted sulfonamide antibacterial agent used commonly in triple sulfa preparations. Sulphadiazine is a sulfonamide antibiotic. It is rapidly absorbed from the Gastrointestinal tract and widely distributed in the body. Glycyl-L-glycine has also been reported to be helpful in solubilizing recombinant proteins in *Escherichia coli.*

An understanding of the structure of formamide is of some importance because formamide is the simplest amide containing an N-C-O configuration. 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. The N-C bond distance in formamide is also of some interest because of its partial double bond character. This behavior of the solutes in the solvent reveals ion-dipolar group interaction between the ions of the sulfadiazine and non-polar parts of peptides results in the reduction of internal pressure. The above results are confirmed by the analysis of acoustical parameters and antimicrobial activity.

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CONFLICTS OF INTEREST: Nil

REFERENCES:

1. Anubha Mahajan, Ashwani Singh Rawat, Nidhi Bhatt and Meenakshi, K., Chauhan. Structural modification of protein and peptides, Pharmaceutical Research. 2014; 48(3).
2. Padhi, A., *et al.,* Antimicrobial peptides and proteins in mycobacterial therapy: current status and future prospects. Tuberculosis .2014; 94,363-373.
3. Buchwald, H., *et al.,* Effects on GLP-1, PYY, and leptin by direct stimulation of terminal ileum and cecum in humans: implications for ileal transposition. Surg.obes. Relat. Dis.2014.
4. Giordano, C., *et al.,* Neuroactive peptides as putative mediators of antiepileptic ketogenic diets. Front. Neurol. 2014; 5, 63-68.
5. Robinson, S.D., *et al.,* Diversity of conotoxin gene superfamilies in the venomous snail, Conus victoriae plos 2014.
6. Kaspar, A.A., and Reichert, J.M. Future directions for peptide therapeutics development. Drug Discovery. 2014; 807-817.
7. Nasrolahi Shirazi, A., Tiwari, RK., Oh D., Sullivan, B., Mccaffrey, K., Mandal, D., *et al*. Surface decorated gold nanoparticles by linear and cyclic peptides as molecular transporters. Mol Pharm.2013; 10(8): 3137-3151.
8. Mukta Jain., Sampat Nehra., Nematicidal. Fungicidal and Bactericidal Activities of Manganese (II) complexes with Heterocyclic Sulphonamide Imine, Metal Based Drugs. 2002; 9: 1-2.
9. Zhaohua Huang., Zhaoliang Lin., and Junlian Huang. A novel kind of antitumor drugs using sulphonamide as parent compound; Eur.J.Med.Chem.2001; 36: 863-872.
10. Zhaohua Huang., Fenjin Yang., Zhaoliang Lin., *et al.*, 2-[N1-2 Pyrimidyl- amino benzene sulfonamido] Ethyl 4-Bis (2- chlorethyl) Aminophenyl Butrate: A Potent Antitumor Agent, Bioorganic and Medicinal Chemistry Letters. 2001; 11, 1099-1103.
11. Abdalla, M., KHEDR Fawaz, A. SAAD Synthesis, structural characterization, and antimicrobial efficiency of Sulphadiazine azo- azomethine dyes and their bi- homo nuclear uranyl complexes for chemotherapeutic use. 2015; 39: 267-280.

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