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Thermoacoustic, Electrochemical, Solvation and Antimicrobial Analysis of Ternary Potassium Salts Solutions at 308.15K

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ABSTRACT

Introduction: Amino acids join with each other by peptide bonds and build polymers referred to peptides and proteins. Peptides are recognized for being very therapeutic, selective and comparably safe. As a result, peptides are receiving more attention in the pharmaceutical research and development. The scientific progress of ultrasonics has been employed extensively in past decades for both industrial and medical applications.

Aim/Objectives: Ultrasonic investigation in non- aqueous solutions of electrolytes with peptides provide information that is helpful in understanding the behaviour of liquids/solutions system. In the present work, the ultrasonic velocity of a peptide with electrolyte in formamide has been measured at various concentrations at 308.15K.

Methods: Utilizing the measured data the thermoacoustical, electrochemical and solvation analysis are carried out. The interionic and intermolecular interactions existing in the present system are studied in detail.

Conclusion: The results arrived from ultrasonic methods have been corroborated with antimicrobial activity of the samples.

Key Words: Ultrasonic velocity, Thermodynamic& acoustic parameters, Solvation, Equivalent conductance, Zone of inhibition.

INTRODUCTION

The solvation or mixing effects of solutes in different solvents are widely used in many fields and hence the understanding of thermoacoustic, electrochemical properties and solvation analysis are of considerable importance in solutions/liquids. The behaviour of liquid systems/solutions, intermolecular and intermolecular association, complex formation and related structural changes can be understood using information from the interrogations of ultrasonic velocity of aqueous and non-aqueous solutions of amino acids with electrolytes and non-electrolytes.[1] The measurement of ultrasonic velocity is to comprehend the qualitative data fetched on the strength and physical characteristics of molecular interactions.[2] As amino acids and peptides are the building blocks of proteins, their study provides important information which can be related to the behaviour of larger biomolecules such as proteins. The effect of electrolytes on structure and function of proteins and nucleic acids has been widely studied in terms of their structure-making and structure breaking properties.[3]

Formamide is a polar protic solvent and has a quite high dipole moment and dielectric constant. In addition to the size and shape differences, there may also be hydrogen bonding interactions between dissimilar molecules that cause the thermodynamic parameters of binary and ternary mixtures/solutions containing polar and related components to deviate significantly from ideality.[4]

The simplest of all the dipeptides, glycyl-glycine, is used as a theoretical base for the creation of more complex peptides. Potassium acetate is classified into "Synthetic flavouring substances and adjuvants" that may be safely used in food.[5] Potassium ascorbate is a non-citrus, and one form of vitamin (c).[6] Several E. coli strains are now characterized as food-borne pathogens[7], hence biological study of microbes play a vital role.

In the present investigation, the basic parameters such as density (ρ), ultrasonic velocity (u) and viscosity (η) of ternary systems of glycyl – glycine added with potassium salts in non – aqueous formamide medium were measured at 308.15

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K. From these experimental data, internal pressure (π_i), free volume (V_f), adiabatic compressibility (β), intermolecular free length (L_f), specific acoustic impedance (Z), solvation number and equivalent conductance (Λ_c) have been calculated. These parameters were analysed to identify various interactions taking place in the ternary solutions of electrolytes (potassium acetate and potassium ascorbate) and the peptide (glycyl–glycine) in non-aqueous medium (formamide). This study has been carried out for different concentrations at 308.15K. The antimicrobial activity hasalso been analysed for the ternary solutions.

MATERIALS AND METHODS

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 non-aqueous peptide added with electrolyte solutions were measured using Anton Paar DMA 4100 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 which 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.

Solute–I (Glycyl – glycine), co-solutes potassium acetate and potassium ascorbate of molecular weight 132.12, 98.15 and 150.22 g/mole respectively were purchased from Siscom research laboratories Pvt. Ltd. Formamide has been used as a solvent, potassium acetate & potassium ascorbate (0.01 m) offixed molality and Glycyl – glycine solutions of various concentrations.

Computation

Using the measured basic data, the following thermoacoustic and solvation parameters have been calculated using the standard formulae,

Viscosity $\eta_2 = [\rho_1 t_1 / \rho_2 t_2]\ \eta_1$ Pa.S

Ultrasonic velocity $u = f\lambda$ m/sec

Internal pressure $\pi_i = bRT \left(\frac{K\eta}{u}\right)^{2/3} \left(\frac{\rho^{2/3}}{M_{eff}^{7/6}}\right)$ (10^9Pa)

Free volume $V_f = \left(\frac{M_{eff} u}{K\eta}\right)^{3/2}$ (m^3) (10^{-9})

Adiabatic compressibility $\beta = \frac{1}{\rho u^2}\left(10^{-12}\right) Pa^{-1}$

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

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

Solvation number $n_s = \frac{n_i}{n_f}\left(1 - \frac{\beta_{so\ln}}{\beta_{solv}}\right)$

Equivalent conductance $\Lambda_c = kc\ [\frac{1000}{N}]\ (10^{-7})$ siemens.m^2

where 'K' is a constant equal to 4.28×10^9, M_{eff} is given by $M_{eff} = (M_1X_1 + M_2X_2 + M_3X_3)$.

RESULTS

The internal pressure is the resultant of all the internal interactions of the system. With increasing concentration, the non-aqueous solutions of the peptide with electrolytes have increasing values for density (ρ), viscosity (η), and ultrasonic velocity (u) which are presented in Table 1 and graphically depicted in figures 1, 2 and 3. Variations in ultrasonic velocity provide information about the structural changes of the samples. The observed increase in ultrasonic velocity is due to molecular interactions of different molecules present in the solutions, which may be closely linked to the cohesion established in the samples utilized for the investigation.[8] The density of non-aqueous solutions of the samples progressively rises as concentration increases which may be caused by the molecules of disc size having closer packing in the solutions. As the concentration increases the viscosity increases may be due to the increase in movement of molecules and ions present in the solutions for both the systems.

Internal Pressure and Free volume

Cohesive energy is the measure of the internal pressure of the system.[9] In both the non-aqueous solutions of potassium acetate and potassium ascorbate with Glycyl-glycine, the internal pressure increases with increasing concentration as shown in figure 4. The increase in cohesive energy indicates that there is a significant interaction between the solvent, solute and the co-solutes. Furthermore, this increase may be attributed to the structure making nature of the solute and co-solutes added to the solvent when solution is formed.[10]Interionic and intermolecular interactions of potas-

sium ascorbate solutions is greater than potassium acetate solutions. The free volume is the actual volume in which a particular molecule can move around without restrictions. The free volume decreases with increasing concentration outlined in figure 5. The free volume decreases with rise in concentrations also confirm the ion-solvent interactions occurring in both the systems. The variation in the free volume with respect to concentrations of the ternary solutions act in a way that is in a direction opposite to internal pressure. The free volume is often ascribed to the chain ends because of their higher mobility. Therefore, shorter chains have a higher fraction of free volume compared to longer chains. A decrease in fractional free volume accompanying the increase in chain length is commonly referred to be the reason behind the molecular weight dependence[11] of the samples. It is to be noted that molecular weight of potassium ascorbate is higher than potassium acetate.

The decrease in free volume and the increase in internal pressure indicates that the cohesive forces are dominating in the solutions. The Na^+, K^+ and Cl^- ions furnished by electrolytes interact electrostatically with NH_4 and COO^- group of amino acids and dipeptide zwitterions.[12]

Adiabatic compressibility

The adiabatic compressibility is found to decrease with concentration increase and the calculated values of β have been presented in Table 2 and represented in figure 6. It is clear that the compressibility of a solvent is higher and it decreases with increase in concentrations. The primary effect of dissolving a peptide along with an electrolyte (both potassium acetate and potassium ascorbate) 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 amount of molecules that are incompressible and (ii) structural transformation taking place in the solution. This may be due to the association taking place between the disparate molecules. When the concentration increases, the associated groups of molecules breakdown increasingly and the forces of attraction between the molecules decrease. This leads to a decrease in the adiabatic compressibility of the system.[13]

Intermolecular Free Length:

The decreasing intermolecular free length with increasing concentration in solutions were shown in table 2 and in figure 7. It indicates that there are significant interactions between the peptide and electrolytes solution, i.e. the distance covered by the solutes and solvent neighbouring molecules is reduced,[14] suggesting a structure promoting behaviour on the addition of solute. As a result, the intermolecular distance decreases with increasing concentration.

Specific Acoustic Impedance:

Acoustic Impedance in solutions can be used to gauge the potency of intermolecular interactions. The variations of specific acoustic impedance with respect to the molality are given in table 2 and illustrated in figure 8. Specific acoustic impedance may exhibit linear behaviour[15] with concentration as a result of solute-solvent interactions.

Solvation Number analysis:

The solvation number is calculated from compressibility measurements. The solvation approach is used to interpret ion-solvent interactions. Only at 0.001 molality for potassium acetate exhibits negative solvation number and for the remaining concentrations for both electrolytes they exhibit positive solvation number as depicted in figure 9. The positive value of solvation number suggests that the compressibility of the solution at all the molalities will be less than that of the solvent. The negative value of solvation number emphasizes that the solutions are more compressible than solvent. At higher concentration, the solvation number attains the saturation. The ion solvent interaction may be equal to intermolecular interaction energy which results in zero values of solvation number.[16] The decrease in solvation number with increasing molality may be due to either not enough solvent molecules available for all the ions or preferentially ion-pairing occurred.[17]

Electrochemical Analysis:

The increase or decrease in conductivity depends on the solvent added and the extent of the resulting dissociation of the solution into the ions. The computed values of equivalent conductance of the ternary solutions are given in table (2). The variations of Λc with concentration of the solution are as shown in the figure 10. In general, in all the conducting solutions, the equivalent conductance is found to be high, at lower concentrations. By increasing the concentration, the equivalent conductance decreases.[18] The variation of equivalent conductance is found to be very small, at higher concentrations.

Salient features of the samples

Antimicrobial resistance has become a global problem and the need for a solution minimizing the number of resistant bacteria is crucial.[19] An antimicrobial is a compound that either destroys or inhibits the growth of microorganisms. Antimicrobial drugs can be categorised based on the microorganisms that they are most effective against. Disinfectants, which eliminate a variety of bacteria on inanimate surfaces to stop the spread of disease, antiseptics, and antibiotics are the three primary groups of antimicrobial agents.

Test organisms

The National Chemical Laboratory (NCL) in Pune is where the test microorganisms for E. coli and the fungus Asper-

gillus niger are obtained. By employing the disc diffusion method, the ligand and its complexes antibacterial and antifungal activities were tested in vitro against the bacterial species Escherichia coli and the fungal species *Aspergillus niger*. The effect produced by the samples were 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 in table 3.

The addition of potassium acetate into the non-aqueous peptide solution is having enhanced zone of inhibition (32 mm) than the electrolyte itself (30 mm) in the case of antibacterial study indicated in table 3. The antifungal study emphases that the peptide solution in the presence of potassium acetate is to promote its antifungal activity i.e. zone of inhibition of Glycyl-glycine is 16 mm whereas, zone of inhibition of Glycyl -glycine in formamide with potassium acetate is 26 mm. The solutions of Gycyl-glycine and Potassium ascorbate in Formamide showed zone of inhibition against Escherichia coli as 26mm which is greater than the individual responses. The antifungal activity of Gycyl-glycine with Potassium ascorbate in formamide showed maximum zone of inhibition against *Aspergillus niger* 22mm against individual response. With respect to zone of inhibition, in *Aspergillus niger* study potassium ascorbate values are nearer to standards whereas in E. coli study potassium ascorbate seems to have more inhibition than potassium acetate. In the present investigation, it is identified that the non-aqueous solutions of Glycyl-glycine with Potassium ascorbate acts as an intermediate with respect to E. coli whereas this combination is sensitive in the case of *Aspergillus niger*, i.e., this combination may act as a disinfectant. (Figure 11,12)

CONCLUSION

Formamide is the simplest amide with an N-C-O configuration, understanding its structure is quite crucial. The (N-C-O) peptide linkage, which is crucial for protein synthesis, depends on the flexibility of the molecule in consideration. The N-C bond distance in formamide is also of some interest due to its partial double bond character. The results obtained from the present study reveals that the presence of molecular interaction in liquid mixtures/solutions of peptide with electrolytes in non-aqueous medium. The solute-co solute interactions and solute-solvent interactions are present in both the systems from the cohesive energy enhancement. Due to increase in density and decrease in intermolecular free length with increase in concentration indicating the loosening of intermolecular forces in non-aqueous potassium salts. Hence it is evident that ultrasonic velocity measurement in the given medium serves as the powerful probe in characterising the physicochemical properties of the given medium. The behaviour of the solute and co-solutes present in the solvent reveals that the molecular association is dominating in potassium ascorbate than potassium acetate samples.

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Conflict of Interest: None

Authors' Contribution: R. Geetha - Execution, data analysis and article writing; Dr.R.Padmavathy - Article review and overall supervision

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Table 1: Values of density, viscosity, ultrasonic velocity, internal pressure and free volume of non- aqueous potassium acetate and potassium ascorbate ternary solutions at 308.15K

Potassium acetate					
Molality (m)	ρ kgm^{-3}	η Pa.S	u m/sec	π_i 10^9Pa	V_f $10^{-9}m^3$
0.001	1.1214	0.1509	1580	1.3152	36.5922
0.005	1.1229	0.1543	1581	1.3302	35.4410
0.01	1.1240	0.1571	1582	1.3417	34.5692
0.025	1.1245	0.1623	1583	1.3618	33.0103
0.05	1.1254	0.1643	1584	1.3670	32.5444
Potassium ascorbate					
0.001	1.1230	0.1579	1581	1.3440	34.3080
0.005	1.1234	0.1608	1583	1.3557	33.4283
0.01	1.1240	0.1664	1584	1.3781	31.8291
0.025	1.1257	0.1708	1584	1.3956	30.6602
0.05	1.1273	0.1788	1585	1.4252	28.7497

Table 2: Values of Adiabatic compressibility, Intermolecular free length, Specific acoustic impedance, Solvation number and Equivalent conductance of non- aqueous potassium acetate and potassium ascorbate ternary solutions at 308.15K

Potassium acetate					
Molality (m)	β $10^{-12}Pa^{-1}$	L_f 10^{-11}m	Z	n_s $10^3Kg.m^{-2}.s^{-1}$	Λ_c 10^{-7} Siemens.m^2
0.001	35.7331	1.2045	1772	-5.27	8.2613
0.005	35.6334	1.2028	1775	0.09	6.2454
0.01	35.5675	1.2017	1778	2.02	5.5779
0.025	35.5091	1.2007	1780	2.15	4.3572
0.05	35.4134	1.1991	1783	2.2	3.7051
Potassium ascorbate					
0.001	35.6091	1.2024	1776	1.44	5.6500
0.005	35.5350	1.2012	1778	4.01	4.6800
0.01	35.4611	1.1999	1780	5.21	4.2300
0.025	35.4061	1.199	1783	3.92	3.5800
0.05	35.3066	1.1973	1787	3.28	2.7800

Table 3: Report on antimicrobial activity of the samples

S. No.	Name of microorganism	Zone of inhibition in mm						
		FMA	GG	Pot. Ace	Pot. Asc	GG+ Pot. Ace	GG+ Pot. Asc	Standard
1	E.coli (NCIM 2065)	14	17	30	20	32	26	38
2	Aspergillus niger (NCIM 105)	16	16	20	16	26	22	30

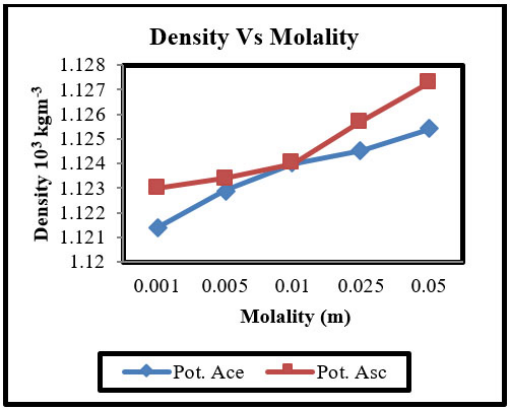


Figure 1: Density Vs Molality.

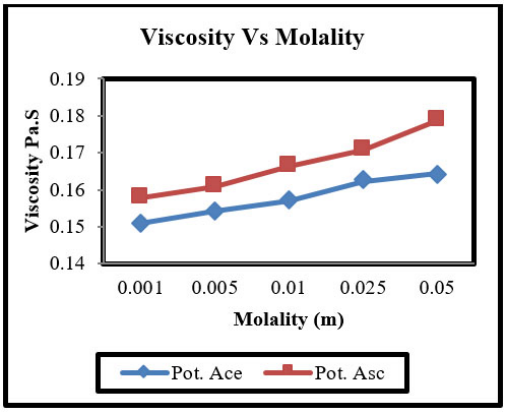


Figure 2: Viscosity Vs Molality.

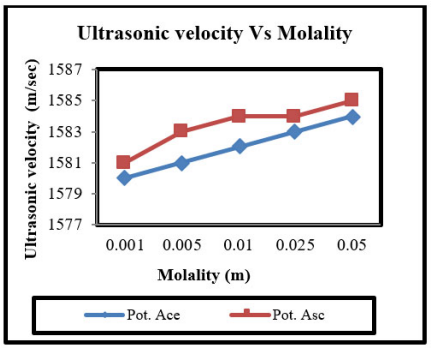


Figure 3: Ultrasonic velocity Vs Molality.

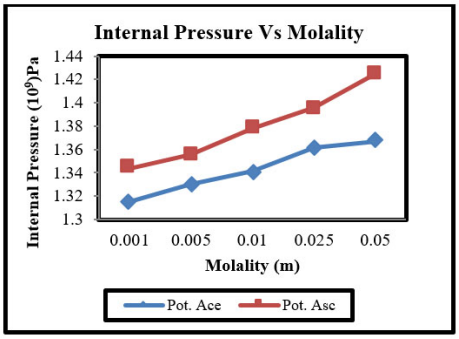


Figure 4: Internal Pressure Vs Molality.

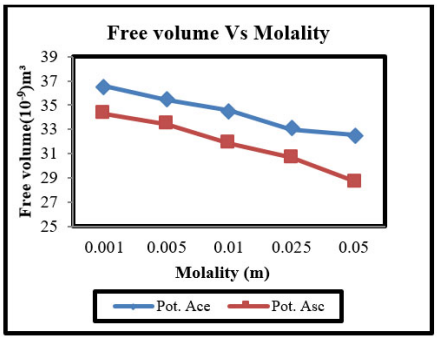


Figure 5: Free volume Vs Molality

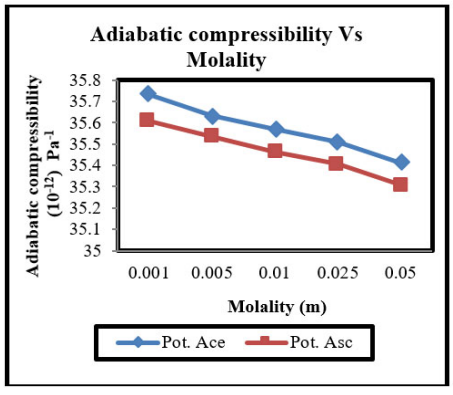


Figure 6: Adiabatic compressibility Vs Molality.

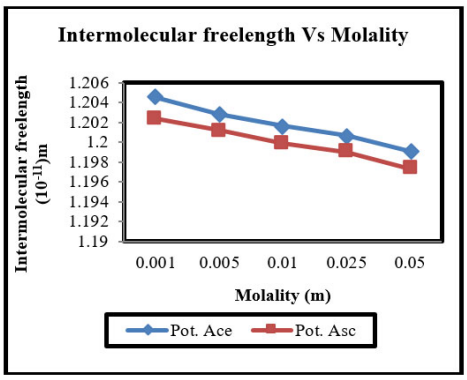


Figure 7: Intermolecular free length Vs Molality.

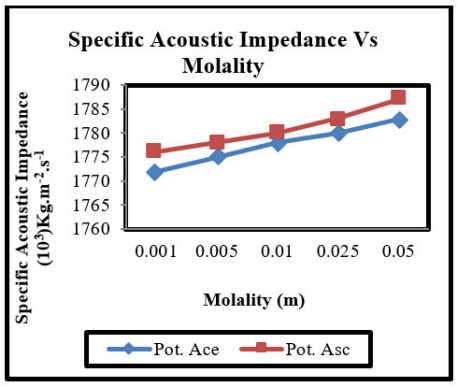


Figure 8: Specific Acoustic Impedance Vs Molality.

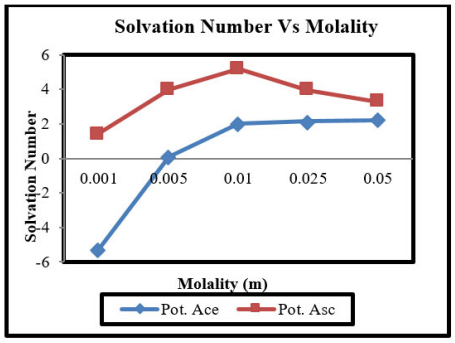


Figure 9: Solvation Number Vs Molality.

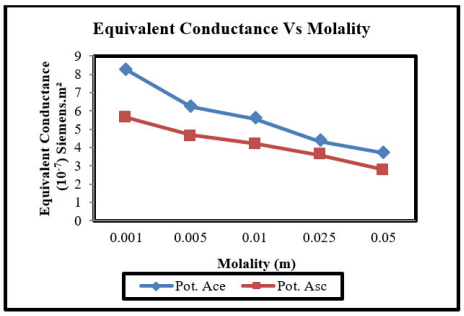
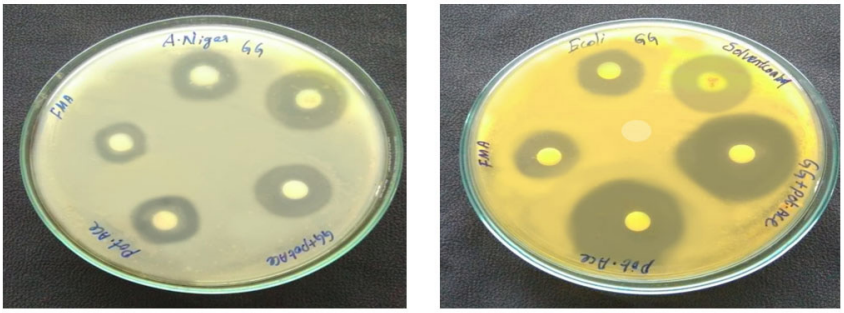
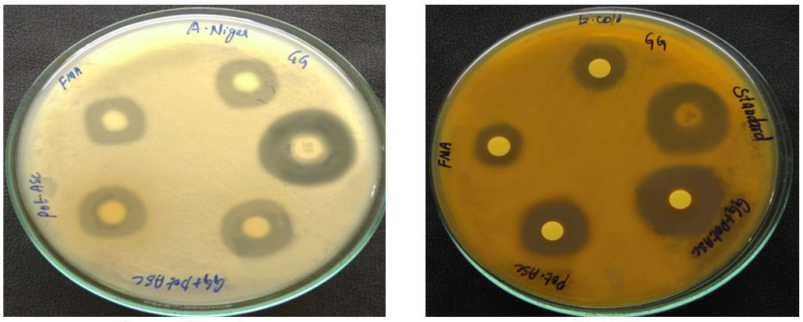


Figure 10: Equivalent Conductance Vs Molality.



Aspergillus niger E. coli

Figure 11: Non- aqueous potassium acetate ternary solutions.



Aspergillus niger E. Coli

Figure 12: Non- aqueous potassium ascorbate ternary solutions.