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THERMODYNAMIC INTERACTION STUDIES OF GYCINE ,D(+)ALANINE AND D(+)VALINE IN AQUEOUS METFORMIN HYDROCHLORIDE AT CONSTANT TEMPERATURE

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ABSTRACT

Density and viscosity ofglycine D(+) alanine and D (+) valine in 0.05 and 0.15 in aqueousmetformin hydrochloride solutions have been determined experimentallyat 308.15 K.The results obtained from density and viscosity measurement have been used to calculate the Hydration number H_n, apparent molar volume ϕ_v, , partial molal volume ϕ^0_v , at infinite dilution, transfer volume $\Delta\phi^0_{tr}$, free energy of activation per mole of solute$\Delta \mu_2^{0\#}$and solvent $\Delta \mu_1^{0\#}$and B- coefficient. It has been observed that there exist strong solute-solvent interaction and complex formation between in these ternary systems.They have strong structure making ability

KEYWORDS: glycine D(+) alanine , D (+) valine, metformin hydrochloride Hydration number, apparent molal volume, and transfer volume and , free energy of activation per mole of solute.

1. INTRODUCTION

In continuation of our earlier work [1] on the study of interaction between amino acids and electrolytes in aqueous medium, we present in this paper, the study of interaction between glycine ,D(+) alanine and D (+) valine, in aqueous metformin hydrochloride at 308.15 K. There has been an increased interest in the physicochemical properties of amino acids in aqueous as well as aqueous electrolyte media to understand the role played by the biological molecules in living organismThe stabilization of proteins are due to several non-covalent interaction that include hydrogen bonding, electrostatic and hydrophobic interactions . When proteins are present in salt solutions some of their properties such as solubility , denaturation and dissociation into sub units and stability, show remarkable variations[2,3]. Amino acids have zwitter-ion and are the constituents of the most important class of biopolymers, i.e. Proteins. Disarrangement water and electrolyte balance in living systems cause a wide variety of health problems. In physiological media such as blood, membranes, cellulose fluids etc., the dipolar character of amino acids has an important bearing on their biological functions.Therefore, a knowledge of water-amino acid interaction the effects on several biological processes occurring in living organism.Metformin hydrochloride ($C_4H_{11}N_5HCl$) is an anti-diabetic and anti-hyperglycemic agent [4,5] that lowers both basal postprandial elevated blood glucose in patients with non-insulin dependent diabetes mellitus (Type 2- diabetes) whose hyperglycemia cannot be satisfactorily managed by diet alone. In recent years, a number of workers have utilized density and viscosity data to deduce the thermodynamic properties (relative viscosity, Jones -Dole coefficient and free energy of activation of viscous flow) for a number of mixtures solutions[6-9]. Structural interactions of non-ionic solutes with ionic ones in different solvents are important in many fields of chemistry and bio-chemistry. Very recently, we have made systematic effort to investigate the ultrasonic and volumetric properties of amino acids in concentrated electrolytic solution [10-12]. It was found that NaCl and $MgCl_2$ increase the apparent molar volume and decrease the adiabatic compressibilityof glycine. This increase could be attributed to the interactions of the ions of the NaCl and $MgCl_2$ electrolytes and zwitter-ion head group of glycine, causing the transfer of hydrated water molecule to the bulk state.

In the present paper, we report densities, ρ and *viscosities,*ηofglycine, D(+) alanine andD (+) valine,. (0.02, 0.04, 0.06, 0.08and 0.10)min aqueous Metformin hydrochloride have been determined experimentallyat 308.15

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K.From these experimental data a number of thermodynamic parameters namely,Hydration number H_n, apparent molar volume ϕ_v, partial molal volume ϕ^0_v , transfer volume $\Delta\phi^0_{tr}$ at infinite dilution ,free energy of activation per mole of solute $\Delta u_2^{0\#}$ and solvent $\Delta u_1^{0\#}$and B-coefficient respectively have been calculated. These parameters were utilized to study various interactions taking place in the solutions of glycine , D(+) alanine and D (+) valine, in aqueous metformin hydrochloride at 308.15 K.

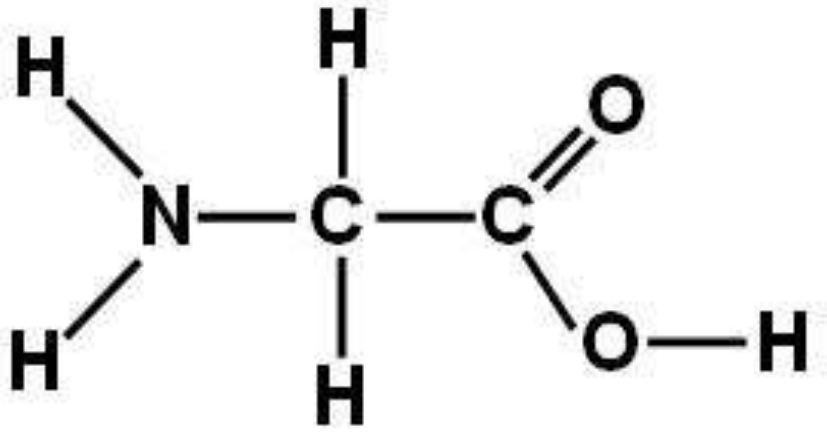
2. EXPERIMENTAL METHODS

Glycine, D(+) alanine andD (+) valine (>99% purity), were procured and S d Fine Ltd and metformin hydrochloride (99.8% purity)from Acumen Pharmaceuticals was used without any pretreatment . They were used as such without further purification, after drying over calcium chloride in desiccators for more than 48 hours. The double distilled de-ionized water were used to measured experimentally and making the solution. Aqueous solutions ofmetformin hydrochloridewere prepared and these were used as solvents to prepare glycine , D(+) alanine and D (+) valine solutions on mass basis covering the whole composition range. All the solutions were prepared by mass in dry box and were stored in special air-tight bottles and kept in dark to avoid photo chemical degradation. The weighing was done on an Afcoset ER-120A electronic balance with an accuracy ± 0.1 mg.

The densities were measured with a single capillary pycnometer (made of Borosil glass) of bulb capacity of $8x10^{-6}$ m^3. The marks of the stems were calibrated using double distilled water at 308.15 K. The pycnometer was kept for about 30 minutes in a thermostatic water bath so that the thermal fluctuation in density was minimized. The viscosities in solutions were measured using Ostwald viscometer. At least three time recorded were obtained , and the average value was used as the experimental flow time. Poiseuille's equation was employed to calculate the viscosity of the amino acid + metformin hydrochloride + water solutions.

$$\eta = \frac{\pi\rho hgr4t}{8lV} = \rho\beta t \qquad (1)$$

Hereρ is the density of the amino acids solutions, h the height of the column in the viscometer, g is the acceleration due to gravity, r is the radius of the capillary , l the length capillary and t is the time of falloff the solution of volume V. The term h, g, r, l and V are constant for a given viscometer therefore these have been replaced by single term β.The temperature of the test solutions was maintained at 308.15 K± 0.01K in an electronically controlled thermostatic water bath.



Glycine

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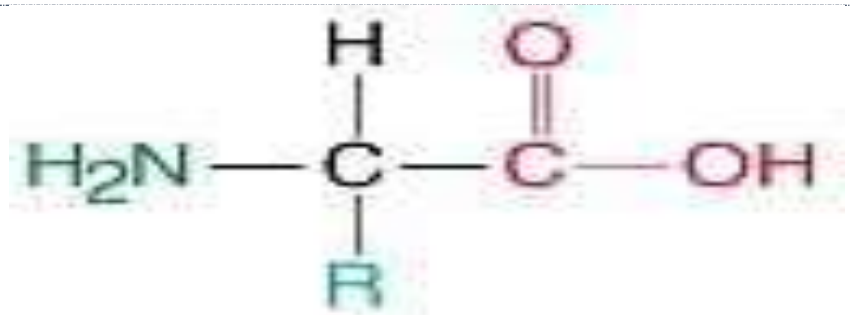

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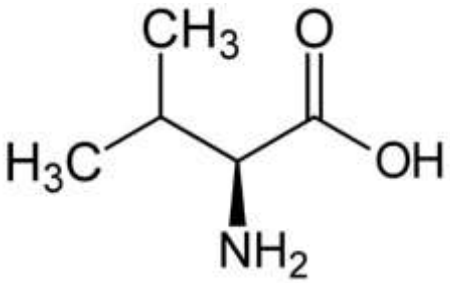
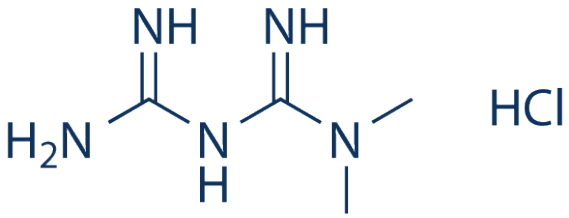


Valine



Metformin hydrochloride

3. RESULT AND DISCUSSION

The densities (ρ) and viscosities, (η)ofglycine , D(+) alanine and D (+) valine (0.02, 0.04, 0.06, 0.08 and 0.10)min aqueous metformin hydrochloridesolution have been determined experimentallyat 308.15 Kare presented in Table 1.It is observed from Table 1 that densitiesand viscositiesfor all the ternary systems increase with increase in molalities ofglycine , D(+) alanine and D (+) valine. The values of ρ andη increase with increase in concentration of amino acid in all the ternary systems under investigation, which appear to be due to hydrophobic properties of solutes i.e.H-bond forming . This may be attributed to the formation of clusters by the amino acids and strong intermolecular forces in the solute. The changes in structure of solvent or solution as a result of H- bond formation lead to decrease in intermolecular free length [13]. Solute may occupy the interstitial spaces in solvent or get solvated forming new weaker bonds. It was suggested [14-16] that what is experimentally observed for any system, reflects the compromise between the tendency for the ion and the peptide to interact with each other and inclination of the solutes to associate with the solvent.

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The apparent molal volume, ϕ_v were calculated from measured density data ofglycine , D(+) alanine and D (+) valine in aqueous metformin hydrochloridesolution have been determined experimentallyat 308.15 Kusing the following equation :

$$\phi_v = [1000 (\rho^0-\rho) / m\rho \rho^0]+M/\rho \quad (2)$$

Where M is the molecular mass of the solutes,$\rho^0$and ρ are densities of solvent and solution. The calculated values of ϕ_v of these ternary systems are given in Table 1. In these cases where molality dependence of ϕ_v , having no definite trend points, the ϕ_v values increase due to reduction in the electrostriction effect at terminals, whereas it decreases due to disruption of side group hydration by that of the charged end.

The partial molal volume at infinite dilution ϕ^o_v was calculated by taking an average data points. The linear variation is obtained by least square fitting to the following equation.

$$\Phi_v = \phi^0_v + S_v m^{½} \quad (3)$$

The intercept ϕ^0_vwhich is the partialmolal volume at infinite dilution and $S_v$is the experimental slope, which is considered to be volumetric pair wise coefficient. The derived values ϕ^0_v are summarized in Table 2.Table 2 shows that the values of $\phi^0_v$are positive of these ternary systems which indicate ion-solvent interactions are strong. The positivevalue of ϕ^0_v with metformin hydrochlorideconcentration of water molecules as a result of shielding of polar terminal groups of glycine , D(+) alanine and D (+) valinemolecules is due to increased interaction between the metformin hydrochlorideaqueous solution. These results can be explained by the co-sphere overlap model as developed by Friedman et al**.**[17] the properties of water molecules in the hydration co-spheredepends on the nature of solute species.

The types of interactions occurring between the zwitter ions of glycine , D(+) alanine and D (+) valineand metformin hydrochloridecan be classified as follows:

i. hydrophilic interaction between $-NH_3^+$ and $COO^-$of glycine , D(+) alanine and D (+) valinewith $C_4H_{11}N_5H^+$ ion.The terminal groups of zwitter ions of amino acid $-NH_3^+$ and COO^- are hydrated in electrostatic manner .
ii. The overlap of hydration co - spheres of terminal groups (NH_3^+ and COO^-) and of adjacent groups results in volume change.
iii. Hydrophilic and hydrophobic interactions between themetformin hydrochloride and non-polar group-(CH_2) ofglycine , D(+) alanine and D (+) valine.
iv. Hydrophobic and hydrophobic interactions between the non-polar group of metformin hydrochlorideand non-polar group-(CH_2) of glycine , D(+) alanine and D (+) valine.

The transfer volume $\Phi^0_{v(tr)}$of glycine , D(+) alanine and D (+) valinefrom pure water and aqueous metformin hydrochloride solution were calculated using the following equation

$$\Phi^0_{v(tr)} = \phi^0_v(\text{aq.metformin}) - \phi^0_v(\text{aq}) \quad (4)$$

These values are presented in Table 2, which shows to be positive in metformin hydrochloride systems at 308.15 K . The change in values of $\Phi^0_{v(tr)}$are interpreted on the basis of co-sphere overlap model given be Friedman and Krishnan [17]. According to this model overlap of co-spheremake the positive contribution to the transfer volume in metformin hydrochloride , since the overlap of hydration co-sphere of two terminal groups (NH_3^+ and OH^-) leads to increase the in magnitude of hydrogen bonding interaction which reflect increase in volume.Mishra et al [18] have suggested eq. (5) which shows that limiting apparent molar volume of amino acids is made up of vander volume (V_{vw}), volume associated with empty space (V_v) and volume due to shrinkage (V_s), mainly due to electrostriction of solvent by the terminal charge center of the amino acids.

$$V_\Phi = V_{vw} + V_v + V_s \quad (5)$$

These tendencies can also be explained using the co-sphere overlap model [17] . According to this model, hydrophilic-ionic group interactions contribute positively, whereasionic hydrophobic group interaction

contribute negatively values of $\Phi^0_{v(tr)}$. The values of transfer volume of glycine , D(+) alanine and D (+) valineare positive in aqueous metformin hydrochloride solution due to hydrophilic-ionic group interactions

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which leads of a decrease in the structure breaking tendency of the ion and a reduction in the electrostriction of the water caused by these ions.

The standard partial molal volumes of glycine , D(+) alanine and D (+) valine can be expressed from a simple model [19]

$$\phi^0_v = \phi^0_v(intr) + \phi^0_v(elect) \quad (6)$$

where $\phi^0_v(intr)$ is the intrinsic partial molal volume and $\phi^0_v(elect)$is the electrostriction partial molal volume due to hydration of glycine , D(+) alanine and D (+) valine. The $\phi^0_v(intr)$ is made up of two terms ., the Van der waals volume due to packing effects . The $\phi^0_v(intr)$ can be calculated by crystal molal volumes. According to the suggestion of Millero et al[20], the values of $\phi^0_v(intr)$ for glycine , D(+) alanine and D (+) valine can be estimated from crystal molal volumes .

$$\phi^0_v(intr) = (0.7/ 0.634)\ \phi^0_v(cryst) \quad (7)$$

where 0.7 is the packing density for molecules in organic crystals and 0.634 is the packing density for random packing spheres. The crystal molal volume can be calculated by the following equation:

$$\phi^0_v(cryst) = M_s /\rho(cryst) \quad (8)$$

where ρ(cryst) is the crystal density of glycine , D(+) alanine and D (+) valine [21] . The $\phi^0_v(elect)$ can be estimated by

$$\phi^0_v(elect) = \phi^0_v - \phi^0_v(intr) \quad (9)$$

The decrease in volume due to electrostriction can be related to the hydration number of water molecules (H_n) is hydrated [21].

$$H_n = \phi^0_v(elect)/ (\phi_{v\,(E)} - \phi_{v\,(B)}) \quad (10)$$

Where $\phi_{v\,(E)}$ is the molal volume of electrostricted water and $\phi_{v\,(B)}$ is the molal volume of bulk water at 308.15K are described by Millero et.al $\phi_{v\,(E)} - \phi_{v\,(B)} = -3.3\ cm^3/mol$ (11)

Therefore , as an approximation , the hydration number of water molecules can be obtained as

$$H_n = \phi^0_v(elect)/ -3.3 \quad (12)$$

The H_n value of glycine , D(+) alanine and D (+) valine in aqueous metformin hydrochloride solutions are shown in Table 3 . It can be seen that H_n of glycine , D(+) alanine and D (+) valinedecreases with increasing concentration of metformin hydrochloride . These shows that metformin hydrochloride have dehydration effect on the glycine , D(+) alanine and D (+) valine.

The viscosity data were used to calculate the relative viscosity using Jones- Dole equation[]

$$\eta_{rel} = \eta /\eta_o = [1 + Bm] \quad (13)$$

Where, η and η_o viscosities of the solutions and solvent respectively. B, is the Jones- Dole coefficient [22] , an empirical constant, and is measure of ion-solvent interaction. Its values depend on the size and shape of the solute particles. They were obtained by a least square treatment as the intercepts and slopes of the linear plots of $\eta /\eta_o - 1/m^{1/2}$ versus $m^{1/2}$ and their values are given in Table 2.

For a dilute solution of unsolvated spherical colloidal suspension, has derived by Einstein relation

$$\eta_{rel} = 1 + 2.5\varphi \quad (14)$$

Where φ is the volume fraction of the solute . If this equation is valid or amino acids, Eq. (3) becomes

$$\eta_{rel} = 1 + 0.0025\ V_hC \quad (15)$$

Where V_h is the hydrodynamic volume. For a dilute solution, the following relation holds

$$B = 0.0025\ V_h \quad (16)$$

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Hakin et.al(23) may be assumed that the partial molar volume at infinite dilution of the unsolvated solute particle in a continuum solvent. The more B values in the mixed solvent might mean a more hydrodynamic volume in the mixed solvent. The viscosity B-coefficient is valuable to provide information concerning of solvation of solute and their effects on the structure of the solvent. The B- coefficient values are positive and large which indicates the solute-solvent interaction are strong are shown in Table 2.The B- coefficient increase when water is replaced by metformin hydrochloride are acting as a water structure maker through the H-bonding

According to the transition state theory of the relative viscosities of glycine , D(+) alanine and D (+) valine solutions proposed by Feakins et al [24],the B- coefficient given as

$$B = (\bar{V}_1^0 - \bar{V}_2^0) / 1000 + \bar{V}_1^0[(\Delta \mu_2^{0\#} - \Delta \mu_1^{0\#}) / RT] \quad (17)$$

Where $\bar{V}_1^0$and $\bar{V}_2^0$ are the partial molar volumes of the solvent and solute at infinite dilution , respectively ,$\Delta \mu_1^{0\#}$ the free energy of activation per mole of the solvent and $\Delta \mu_2^{0\#}$ is the free energy of activation per mole of the solute . The $\Delta \mu_1^{0\#}$ and $\Delta \mu_2^{0\#}$ were calculated from the equation

$$\Delta \mu_1^{0\#} = RT \ln (\eta^0 \bar{V}_1^0 / hN_A) \quad (18)$$

and

$$\Delta \mu_2^{0\#} = \Delta \mu_1^{0\#} + RT/\bar{V}_1^0 [1000 B - (\bar{V}_1^0 - \bar{V}_2^0)] \quad (19)$$

Where R, h and N are the gas constant, Planck's constant and Avogadroa's constant respectively and T is the absolute temperature. The values of $\Delta \mu_1^{0\#}$ and $\Delta \mu_2^{0\#}$ for different compositions of glycine , D(+) alanine and D (+) valine in aqueous metformin hydrochloride are given in Table 3. Table 3 shows that $\Delta \mu_2^{0\#}$ are larger than $\Delta \mu_1^{0\#}$ suggesting that the formation of transition state is accompanied by the breaking and distortion of the intermolecular bonds. Moreover, the greater values of $\Delta \mu_2^{0\#}$ than $\Delta \mu_1^{0\#}$ suggest that the metforminhydrochloride under study , behave as structure makers/promoters in different concentration ranges of glycine , D(+) alanine and D (+) valine. Greater values of $\Delta \mu_1^{0\#}$ have also been reported in mixtures of Ni, Cu, Co and Zn chlorides in aqueous in aqueous glycine [24].

A comparison of $\Delta \mu_1^{0\#}$ and $\Delta \mu_2^{0\#}$ values of three solutes result the structure making ability of D (+) valineis greater than glycine , D(+) alanine which may be due to stronger solute-solvents interaction in D (+) valine. Therefore the hydration of D (+) valinewill is much more than that of glycine , D(+) alanine. The greater values of $\Delta \mu_2^{0\#}$at concentration forglycine , D(+) alanine and D (+) valine in aqueous metformin hydrochloride which indicates the maximum structure making ability. Increase in concentrations of metformin hydrochloride from *0.1& 0.15 M* probably causes disruption of the intermolecular bonds of the solvent, thereby decreasing the values of $\Delta \mu_2^{0\#}$.

It can be concluded that the existence of molecular interaction is in the order:

valine>alanine>glycine

Thus the trends and magnitude of the various parameters obtained from viscosity measurement reported in this paper. The studies suggest that ion- solvent interactions are stronger and ion-ion interaction are weak .The extent of interactions and structure making ability is greater in case of D (+) valine. The dB/dT is a better criterion for determining the structure making/ breaking nature of any electrolyte rather than simply the B-coefficient.

4. CONCLUSION

The volume data have been used to study of solute-solvent interaction in these ternary systems. The polar terminal groups of glycine , D(+) alanine and D (+) valinemolecules is due to increased interaction between these metformin hydrochloride aqueous solution It can be concluded that the existence of molecular interaction is in the ordervaline>alanine>glycine.This suggests glycine , D(+) alanine and D (+) valinein aqueous metformin hydrochloride solution is strong structure maker . The positive value of $\Delta\Phi^0_{tr}$ of glycine , D(+)

alanine and D (+) valinefrom water to aqueous metformin hydrochloride solutions show that the strong interactions involving the charged Centre of peptide as well as ions are dominating.The extent of interactions and structure making ability is greater in case of D (+) valine than glycine , D(+) alanine

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Table -1

Densities (ρ) and Viscosities (η) of glycine D(+) alanine,D (+) in aqueous metformin hydrochloridesolutions at 308.15 K

m (mol.Kg^{-1})	ρ× 10 $^{-3}$(Kg m^{-3})	η (mPa.s)	ϕ_v × 10 $^{-3}$(m^3.mol^{-1})
		Glycine+aqueous metformin hydrochloride	
		(0.1) m	
0.00	0.9990	0.7390	-
0.02	0.9995	0.7421	45.05
0.04	1.0002	0.7443	46.07
0.06	1.0006	0.7471	46.69
0.08	1.0011	0.7492	47.48
0.10	1.0016	0.7522	47.95
		Glycine +aqueous metformin hydrochloride	
		(0.15) m	
0.00	1.0004	0.7500	-
0.02	1.0011	0.7532	45.04
0.04	1.0015	0.7553	46.02
0.06	1.0019	0.7581	48.33
0.08	1.0024	0.7610	48.72
0.10	1.0032	0.7632	46.92
		D(+) alanine +aqueous metformin hydrochloride	
		(0.1) m	
0.00	0.9990	0.7390	-
0.02	0.9997	0.7425	59.07
0.04	1.0001	0.7489	61.54
0.06	1.0005	0.7511	64.02
0.08	1.0010	0.7532	65.22
0.10	1.0014	0.7592	65.97

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D(+) alanine +aqueous metformin hydrochloride

(0.15) m

0.00	1.0004	0.7500	-
0.02	1.0011	0.7544	59.03
0.04	1.0015	0.7592	61.52
0.06	1.0018	0.7631	63.97
0.08	1.0022	0.7662	65.21
0.10	1.0027	0.7712	66.93

Valine +aqueous metformin hydrochloride

(0.1) m

0.00	0.9990	0.7390	-
0.02	0.9994	0.7452	92.16
0.04	1.0001	0.7523	92.19
0.06	1.0004	0.7571	93.76
0.08	1.0007	0.7654	94.56
0.10	1.0012	0.7712	96.03

Valine +aqueous metformin hydrochloride

(0.15) m

0.00	1.0004	0.7500	-
0.02	1.0009	0.7570	96.92
0.04	1.0013	0.7633	94.38
0.06	1.0018	0.7772	95.17
0.08	1.0020	0.7781	94.32
0.10	1.0024	0.7782	95.76

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Table -2

Limiting apparent molal volume (Φ^0_v),Transfer volume ($\Delta\Phi^0_{tr}$), and viscosity B-coefficient of glycine D(+) alanine,D (+) in aqueous metformin hydrochloridesolutions at 308.15 K

m	Φ^0_v 10^{-3}	$\Delta\Phi^0_{tr}$	B
mol.Kg-1	(m^3mol^{-1})	(m^3mol^{-1})	($dm^3.mol^{-1}$)
glycine + aqueous metformin hydrochloride			
0.10	46.45	2.48	0.162
0.15	46.81	2.89	0.167
D (+) Alanine+ aqueous metforminhydrochloride			
0.10	63.19	2.79	0.264
0.15	*64.34*	*3.94*	*0.271*
D (+) Valine+ aqueous metformin hydrochloride			
0.10	93.74	3.44	0.497
0.15	94.70	4.42	0.507

Table -3

Hydration number (H_n),free energy of activation of solvent($\Delta\ \mu_1^{0\#}$) , and free energy of activation of solute ($\Delta\ \mu_2^{0\#}$)of glycine D(+) alanine,D (+) in aqueous metformin hydrochloridesolutions at 308.15 K

m	H_n	$\Delta\ \mu_1^{0\#}$KJ. mol^{-1}	$\Delta\mu_2^{0\#}$KJ.mol^{-1}
glycine + aqueous metformin			
0.10	2.02	26.72	53.24
0.15	1.92	26.77	53.83
Alanine+ aqueous metformin			
0.10	2.76	26.72	69.78
0.15	2.48	26.77	70.76
Valine+ aqueous metformin			
0.10	4.35	26.72	98.92
0.15	4.11	26.77	100.06