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INTERACTIONS OF L-THREONINE WITH AN AQUEOUS ANTI-DIABETIC DRUG SOLUTION AT VARIOUS TEMPERATURES: A VOLUMETRIC APPROACH

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ABSTRACT
Densities ρ, have been measured for L-Threonine in aqueous Metformin Hydrochloride solutions of higher concentrations (0.10, 0.15 and 0.20M) mol kg^{-1} at T = 298.15 – 318.15K. Apparent molal volumes V_φ have been determined from the experimental densities. Several thermodynamical parameters such as standard partial molal volume $V\varphi^0$, transfer volume $\Delta_{tr}V\varphi^0$, hydration number n_H, the second derivative of infinite dilution of partial molal volume with respect to temperature, viz., $\partial^2V\varphi^0 / \partial T^2$ have been calculated using the density data. Furthermore apparent molal expansibility E_0, thermal expansion coefficient, α_2 and the pair and triplet interaction coefficients V_{AD} and V_{ADD} have been calculated and reported. These results interpreted through a co-sphere overlap model indicate the presence of strong solute-co-solute interactions in the reported systems.

KEYWORDS: Apparent molal volume, standard partial molal volume, transfer volume, hydration number, L-Threonine, Metformin hydrochloride.

1. INTRODUCTION
Most biochemical processes are temperature dependant and occur in aqueous media. The studies on the physicochemical properties of biomolecules like amino acids, peptides, sugars provide useful information about the complex mechanism of molecular interactions. In our bodies, drug interactions with proteins play a very vital role in the metabolic pathways or the biological processes occurring inside it. When certain molecules are added to protein solutions they either stabilize or destabilize the proteins.[1,2] Sometimes protein conformation is affected by co-solutes due to the solvent effects or their direct binding. The physicochemical behaviour of protein are strongly influenced by its interaction with the surrounding solvent molecules.[3,4]

Because of the complex structure of proteins, direct studies of solute–solvent effects on these biological macromolecules are quite difficult. Therefore, to study the interactions of these macromolecules, some simple model compounds such as amino acids which are the basic structural units of proteins are generally taken. There is a difference in the side chains of these model compounds due to the size, shape, charge, hydrogen-bonding capacity, hydrophobicity and chemical reactivity, due to which these side chains contribute to the structure and function of proteins, individually and collectively. Thermodynamic properties of amino acids in aqueous drug solutions can provide valuable information regarding the stability of proteins in these solutions, their solubility, separation and purification, solute– solvent and solute–co-solute interactions. Therefore, it may be interesting to investigate variations of these thermodynamic properties of amino acid with temperature which will help us to understand the phenomena like drug action, drug absorption, and drug transport. Hence, several authors have used amino acids instead of proteins to study the molecular interactions with aqueous drugs solutions.[5-10]

For example, Rajagopal and Jayabalakrishnan (2010) have reported the effect of temperature on volumetric and viscometric properties of homologous amino acids in aqueous Metformin Hydrochloride solutions[11] and concluded that second derivative of V_φ^0 with respect to temperature shows the structure making property of the studied amino acids in aqueous Metformin Hydrochloride solutions. Rajagopal and Edwin Gladson (2010) also studied and reported Partial Molar volume and partial molar compressibility of four homologous α – amino aicds in aqueous sodium fluoride solutions at different temperatures.[12] Amalendu Pal and Nalin

Chauhan (2012) investigated the interactions of amino acids and peptides with the drug pentoxifylline at various temperatures and have confirmed the strong solute-cosolute interactions.[13] Densities and speeds of sound of L‑Serine with aqueous solutions of antibacterial drugs at different temperatures have been reported (2012) by H Kumar and Kirtanjot kaur.[14] Volumetric and viscometric properties of glycine and methionine (amino acids) in a 0.2 vol. % amikacin sulphate (antibiotic drug) aqueous solution with the molality range of 0.025 mol kg^{-1}–0.25 mol kg^{-1} have been reported by S Chauhan (2013) over the temperature range of 20°C–40°C.[15] Partial molar volume and partial molar compressibility of homologous amino acids in aqueous xylose solutions at different temperatures have been reported[16] by Rajagopal and Johnson(2015). Chunli Liu and Li Zhou (2015) have investigated transfer properties of Glycylglycine from water to aqueous $NaNO_3$, $NaClO_4$, and Na_2SO_4 Solutions.

Furthermore, Rajagopal and Roy Richi (2017) have investigated and reported[17] the state of solute-cosolute interaction of L-phenylalanine in aqueous Metformin Hydrochloride solutions at temperature range of 298.15 – 318.15K. Xu wang[18] reported the interactions of amino acids with aqueous hydroxypropyl-β-cyclodextrin solutions at different temperatures through volumetric and viscometric studies. The solute – solvent interactions in the aqueous ternary systems containing some amino acids (Glycine or L-serine) and ionic liquid (1-butyl-3-methylimidazolium tetra fluoroborate[19] have been studied by Hamid Reza Rafiee and Farshid Frouzesh (2017). H Kumar et al[20] reported the interactions of L-serine and L-threonine with the drug Metformin hydrochloride at low concentrations (0.03, 0.06 and 0.09 $mol.kg^{-1}$) as a function of temperature (305.15, 310.15 and 315.15K) through the volumetric and acoustic studies. The positive values of both partial molar volume and transfer volume have been related to the interactions are due to the ion+hydrophilic and hydrophilic+hydrophilic in the (L-Threonine+Metformin Hcl + water) mixtures. However, H Kumar et al have not reported the viscometric studies of the above ternary (L-Threonine+Metformin Hcl + water) system.

In continuation of our earlier work with amino acids such as glycine, L-leucine, L-alanine, L-valine, L-Phenylalanine and L-Proline in aqueous Metformin hydrochloride[21,25], recently, we have reported[26] the viscometric studies of L-threonine in aqueous Metformin hydrochloride (0.05, 0.10, 0.15 and 0.20 $mol.kg^{-1}$) solutions at the temperature range 298.15 – 318.15K and observed strong interactions between solute and co-solute molecules. As we feel that depending on the intensity of type 2 diabetes, higher dosage of Metformin hydrochloride has to be used by the diabetic patients, it is reasonable to study and report the volumetric parameters at slightly higher concentrations of Metformin hydrochloride. However, to the best of our knowledge, no one has reported the volumetric studies of L-threonine in aqueous Metformin hydrochloride solutions of higher concentrations (0.10, 0.15 and 0.20 $mol.kg^{-1}$) at the temperature range 298.15 – 318.15K. In order to further substantiate our findings with viscometric studies for the ternary (L-Threonine+Metformin Hcl + water) system, in this paper, we report the volumetric studies of L-Threonine in aqueous Metformin Hydrochloride solutions by experimentally measuring and reporting the density of L-Threonine in aqueous Metformin Hydrochloride solutions of higher concentrations (0.10 to 0.20) mol^{-1} kg^{-1} and at various temperatures, T = (298.15, 303.15, 308. 15, 313.15 and 318.15) K. It is emphasised that our work on L-Threonine in aqueous solutions of Metformin hydrochloride is totally different from H Kumar et al's work[20] in terms of concentration range of Metformin hydrochloride & temperature. However our volumetric finding closely matches with the results of the above author.

Using the measured values of density various thermodynamical parameters such as partial molal volume $V\varphi$, standard partial molal volume $V\varphi^0$, transfer volume $\Delta V\varphi^0$, hydration number n_H, the second derivative of infinite dilution of partial molal volume with respect to temperature, viz., $\partial^2 V\varphi^0 / \partial T^2$ have been estimated. Furthermore Partial molal expansivity E_2^0, Isobaric Thermal Expansion Coefficient α_2 and Pair & triplet coefficients V_{AD}, V_{ADD} have also been estimated. All these parameters are used to discuss the solute-co-solute and solute-solvent interactions occurring in the ternary (amino acids + Metformin Hydrochloride + water) system and the structure making/breaking tendency of the solutes in the given solution.

2. EXPERIMENTAL

2.1 Materials

L-threonine is a polar amino acid and one of the two proteinogenic amino acids that contain alcohol groups. L-threonine is an essential amino acid which is biologically active in humans, however, cannot be synthesized by the human body. It is primarily found in animal protein such as beef, poultry and fish. Dairy products also contain significant levels of L-threonine, especially cottage cheese. Vegetable sources of L-threonine include black beans, lentils and sesame seeds. L-threonine is often used to support the production of connective tissue. Additional benefits of L-threonine supplements include the support of bone and liver health as well as the immune system. Chemical structure of L-Threonine is shown in figure 1.

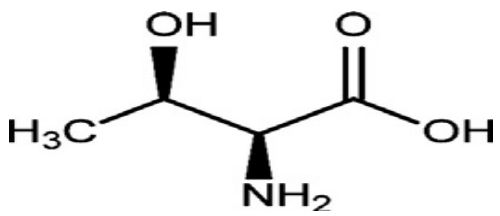

Figure 1: Chemical structure of L-Threonine.

Metformin hydrochloride, a white crystalline powder, has a molecular formula of ($C_4H_{11}N_5HCl$) belongs to a group of medicines called biguanide anti−hyperglycaemic agents.[27,28] It works by lowering human blood−sugar level, i.e. lowering both basal and postprandial plasma glucose. Its pharmacologic mechanisms of action are different from other classes of oral anti hyperglycemic agents. Metformin hydrochloride decreases both the hepatic glucose production and intestinal absorption of glucose and improves insulin sensitivity by increasing peripheral glucose uptake and utilization. Metformin hydrochloride is also used for the treatment of polycystic ovary syndrome and is only anti-diabetic drug that has been conclusively shown to prevent the cardiovascular complications of diabetes.[29] Metformin Hydrochloride has a structure shown in figure 2.

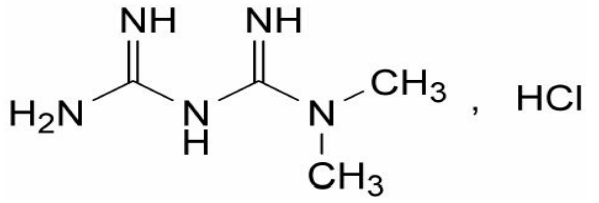

Figure 2: Chemical structure of Metformin Hydrochloride.

2.2 Equipment and Procedures

L-Threonine (CAS no. 80-68-2) with mass fraction of purity > 0.99 purchased from Avra Synthesis Pvt. Ltd., Hyderabad, India have been used as such. Metformin hydrochloride with mass fraction purity > 0.985 purchased from Accumen Pharmaceuticals Pvt. Ltd., India has been used without further purification. Doubly distilled deionized water with a conductivity of 1 $\mu\Omega^{-1}\cdot cm^{-1}$ has been used in our experiments and degassed prior to preparation of solutions. The densities of the solutions have been measured using a single stem pycnometer (Borosil glass) of bulb capacity of ~ 10mL. The capillary, with graduated marks, had a uniform bore and could be closed by a well-fitting airtight glass cap. A standard microscope is used to calibrate the volume in the pycnometer. Once the pycnometer is calibrated, then the calibrated standard values may be used to calculate the volume of the solution[30] and its density in turn.

The solutions of Metformin hydrochloride (0.10, 0.15 and 0.20) $mol\cdot kg^{-1}$ have been prepared in double distilled water and used as solvents to prepare the L-Threonine solutions of five different molal concentrations (ranging from 0.05 to 0.15 $mol\cdot kg^{-1}$). The solutions so prepared have been gently stirred on a magnetic stirrer before being subjected to measurements. The weighing has been done on a high precision SHIMADZU electronic balance (model TXC623L, Philiphines) with a precision of ±0.1 mg.

The pycnometer filled with solutions without bubbles has been allowed to stand for about 30 minutes in a thermostatic water bath so as to minimize thermal fluctuations. The density is extremely sensitive to temperature and hence has been controlled to $\pm 1\times10^{-2}$ K by an electronically controlled thermostatic water bath (Eurotherm, INSCIN, Chennai). The apparatus has also been tested with the density of a known molality of aqueous NaCl. The standard partial molal volumes (V_φ^0) (see table 2) calculated using the experimentally measured density values (see table 1) of L-Threonine in water at the studied temperatures agrees very well with the literature values[31-34] thus validating our experimental procedures. The reproducibility of density measurements has been $\pm$ 2.5 $\times10^{-4}$ $kg\cdot m^{-3}$.

3. RESULTS AND DISCUSSIONS

3.1 Apparent Molal Volume

The values of the density, ρ, for L-Threonine in aqueous solutions of Metformin Hydrochloride at T = 298.15-318.15 K has been reported in Table 1. The apparent molar volumes V_φ of L - Threonine in aqueous Metformin Hydrochloride solutions, (with m_H = 0.10, 0.15, and 0.20 mol kg^{-1}, where m_H is the molality of drug) have been calculated from the experimentally measured densities using the following equation,

$V_\varphi = (M/\rho)\text{-}1000\ (\rho - \rho_0)\ /m\ \rho\ \rho_0$ (1)

Where *M*, *m*, ρ and ρ_0 are the molar mass of solute (amino acid), the molality of solute, densities of solution and solvent (aqueous metformin hydrochloride solutions) respectively.

The calculated apparent molal volumes, V_ϕ as a function of molalities of L- Threonine in different concentrations of aqueous solutions of metformin hydrcochloride at various temperatures are also listed in Table 1.

The V_ϕ value is sensitive to measure solute–solvent interaction occurring in the solution. The variation of V_ϕ is found to be linear dependent on the molality (m_A) of amino acid, in the different concentrations of water + metformin hydrochloride solutions studied. A sample plot of V_ϕ for L - Threonine in different concentrations of aqueous Metformin Hydrochloride solutions at temperature 313.15 K is shown in Figure 3.

It is observed from Table. 1 that apparent molal volume V_φ values are positive and increase with increase in concentration of Metformin hydrochloride as well as temperature for L-threonine in the studied system, thereby showing the presence of strong solute -solvent interactions.[35] This further suggests that the strength of solute–co-solute interactions is increasing with increase in the concentration of aqueous Metformin Hydrochloride solutions.

Table 1: Densities, ρ and Apparent molal volumes, V_ϕ, of L-Threonine in aqueous Metformin Hydrochloride solutions as a function of concentration of L-Threonine at different temperatures.

m_A	$\rho \times 10^{-3}$ (kg m^{-3})					$V_\phi \times 10^6$ (m^3 mol^{-1})				
mol·kg^{-1}	298.15 K	303.15 K	308.15 K	313.15 K	318.15 K	298.15 K	303.15 K	308.15K	313.15 K	318.15 K
	L-Threonine+0.10 mol·kg^{-1} (m_H)									
0	1.00077	0.99932	0.99767	0.99582	0.99383					
0.05	1.00281	1.00135	0.99969	0.99783	0.99583	78.17	78.49	78.67	78.98	79.30
0.075	1.00380	1.00235	1.00069	0.99882	0.99681	78.37	78.56	78.75	79.06	79.37
0.10	1.00480	1.00334	1.00168	0.99980	0.99779	78.49	78.67	78.83	79.18	79.45
0.125	1.00578	1.00432	1.00266	1.00077	0.99876	78.59	78.78	78.91	79.28	79.53
0.15	1.00675	1.00529	1.00362	1.00174	0.99972	78.71	78.89	79.05	79.34	79.66
	L-Threonine+0.15 mol·kg^{-1} (m_H)									
0	1.00266	1.00121	0.99956	0.99769	0.99569					
0.05	1.00465	1.00319	1.00153	0.99965	0.99764	79.14	79.33	79.60	79.86	80.18
0.075	1.00563	1.00416	1.00250	1.00062	0.99860	79.23	79.49	79.70	79.98	80.26
0.10	1.00659	1.00512	1.00346	1.00157	0.99955	79.42	79.63	79.83	80.10	80.39
0.125	1.00755	1.00608	1.00441	1.00252	1.00049	79.54	79.71	79.92	80.19	80.49
0.15	1.00850	1.00703	1.00536	1.00346	1.00143	79.64	79.80	80.04	80.27	80.57
	L-Threonine+0.20 mol·kg^{-1} (m_H)									
0	1.00460	1.00312	1.00146	0.99959	0.99760					
0.05	1.00654	1.00505	1.00338	1.00150	0.99950	80.03	80.33	80.42	80.76	81.01
0.075	1.00749	1.00600	1.00433	1.00244	1.00044	80.16	80.37	80.56	80.88	81.13
0.10	1.00843	1.00694	1.00527	1.00338	1.00137	80.32	80.51	80.65	80.93	81.22
0.125	1.00936	1.00786	1.00620	1.00429	1.00229	80.46	80.68	80.75	81.16	81.29
0.15	1.01028	1.00878	1.00711	1.00521	1.00320	80.58	80.77	80.91	81.20	81.44

m_A – molality of amino acid L-Threonine m_H – molality of Metformin Hydrochloride.

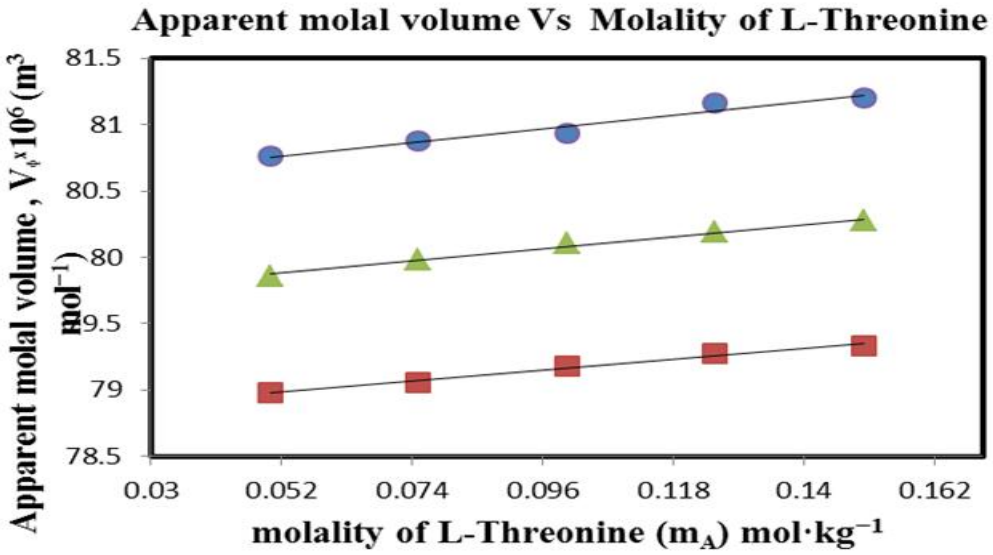

Figure 3: Apparent molar volumes, Vφ as a function of molality, m_A of L - Threonine with different concentrations of (water + Metformin Hydrochloride) solutions (a) 0.10 mol.kg^{-1}(■), (b) 0.15 mol.kg^{-1} (▲), (c) 0.20 mol.kg^{-1} (●) at temperature 313.15K.

3.2 Partial molal volume and transfer volume of L-Threonine in aqueous Metformin Hydrochloride solutions

The values of partial molal volume for the amino acids are generally represented[36] by a linear equation,

$$V_\varphi^0 = V_\varphi + S_v\, m \qquad (2)$$

Where V_φ^0 is the infinite dilution value that is equal to the partial molal property at infinite dilution. It gives information about solute – solvent interactions[37] and Sv is the experimental slope that gives information about the solute-solute interactions. The V_φ^0 values of L-Threonine in pure water at all the studied temperatures are found to agree fairly well with the reported literature values (see table 2) and thus validating our experimental procedure.

The evaluated values of V_φ^0 using equation (2) are listed in Table 2. A perusal of Table 2 reveals that the V_φ^0 values of L- Threonine increase (See Figure.4) with increase in Metformin Hydrochloride concentration.

Furthermore, V_φ^0 values are positive and having higher magnitudes indicating the presence of strong solute–solvent interactions.

The partial molal volume (V_φ^0) values increase with increase in concentration of solute may be related to the reduction in the electrostriction at terminals. The increase in V_φ^0 values (Table 2.) with increase in temperature may be attributed to the solvation effect of L-Threonine zwitterions in the solvent.

Table 2: Standard Partial Molal Volumes (V_φ^0) of L-Threonine in aqueous Metformin Hydrochloride solutions at different temperatures.

Temperature	Standard Partial Molal Volume V_φ^0 ×10^6/m^3·mol^{-1}				
T/K	m_H = 0 (water)		m_H = 0.10	m_H = 0.15	m_H = 0.20
Temperature	Present work	Literature [31,32,33,34]	mol·kg^{-1}	mol·kg^{-1}	mol·kg^{-1}
298.15	75.668	75.03[a]	77.949	78.870	79.752
303.15	76.066	76.30[c]	78.267	79.132	80.061
308.15	76.401	75.67[a], 77.33[d]	78.469	79.382	80.192
313.15	76.768	77.31[c]	78.797	79.665	80.524
318.15	77.145	77.67[b]	79.111	79.976	80.808

a – Ref 31, b – Ref 32 c – Ref 33 d – Ref 34.

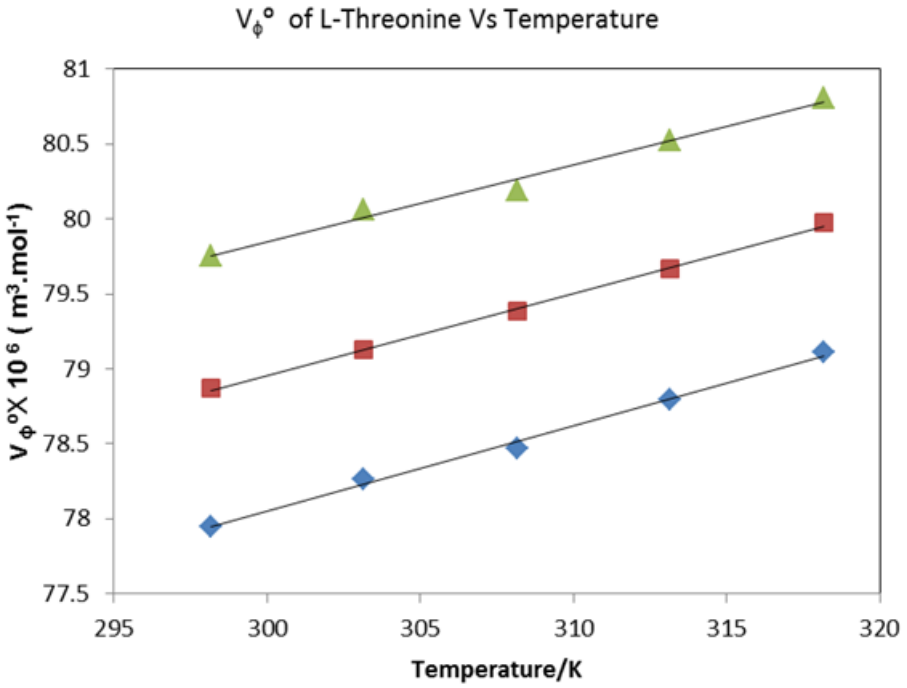

Figure 4: Plot of Partial Molal Volume of L-Threonine (V_φ^0) against different temperatures at various molal Concentrations (m_H) of aqueous Metformin Hydrochloride solutions (a) 0.10 mol.kg^{-1}(♦), (b) 0.15 mol.kg^{-1} (■), (c) 0.20 mol.kg^{-1} (▲)

Partial molal properties of transfer, $\Delta_{tr}V\varphi^0$ at infinite dilution of L-Threonine from water to aqueous solutions of Metformin Hydrochloride have been determined by the equation.[11]

$\Delta_{tr}V_\varphi^0 = V_\varphi^0$ (amino acids in aqueous drug solution) - V_φ^0 (amino acid in water) (3)

The calculated values of partial molal transfer volume, $\Delta_{tr}V_\varphi^0$, have been given in Table 3. and illustrated in figure 5. Generally values of $\Delta_{tr}V_\varphi^0$ can be justified by the Co-sphere model suggested by Friedman and Krishnan.[12,38] Depending on the nature of the intermolecular interactions the properties of water molecules in the hydration co-spheres around amino acids and metformin hydrochloride molecules will differ. The interactions between the L-Threonine and metformin hydrochloride may be classified into four types as discussed below.[39,40]:

(i) (ion + hydrophobic) interactions (between zwitterionic centres of amino acids and nonpolar groups of Metformin-Hcl) and
(ii) (hydrophobic + hydrophobic) interactions (between non-polar groups of amino acids and non-polar groups of Metformin-Hcl).
(iii) (ion+ hydrophilic) interactions (between zwitterionic centres of amino acids and polar groups of Metformin-Hcl)

(iv) (hydrophilic + hydrophilic) interactions (between polar groups of amino acids and polar groups of Metformin-Hcl)

According to co-sphere overlap model, (ion + hydrophilic) and (hydrophilic + hydrophilic) interactions contribute to positive $\Delta_{tr}V_{\varphi}^{0}$ values whereas (ion + hydrophobic) interactions and (hydrophobic + hydrophobic) interactions contribute negatively to transfer volumes. In the present study of L-Threonine in aqueous Metformin Hydrochloride solutions, the $\Delta_{tr}V_{\varphi}^{0}$ values are positive thereby indicating the predominance of the type (iii & iv) interactions over type (i) & (ii) interactions[12,22] and complementing further the same results obtained through our earlier viscometric studies.[26]

Table 3: Partial Molal Volumes of transfer ($\Delta_{tr}V_{\varphi}^{0}$) and Hydration Number ($n_H$) of L-Threonine in aqueous Metformin Hydrochloride solutions at different temperatures.

Temperature	Partial Molal Volume Transfer $\Delta_{tr}V\varphi^{0}10^{6}/m^{3}\cdot mol^{-1}$			Hydration Number n_H		
T/K	m_H = 0.10	m_H = 0.15	m_H = 0.20	m_H = 0.10	m_H = 0.15	m_H = 0.20
	$mol\cdot kg^{-1}$	$mol\cdot kg^{-1}$	$mol\cdot kg^{-1}$	$mol\cdot kg^{-1}$	$mol\cdot kg^{-1}$	$mol\cdot kg^{-1}$
298.15	2.281	3.202	4.084	2.45	2.22	2.00
303.15	2.201	3.066	3.995	2.37	2.15	1.92
308.15	2.068	2.981	3.791	2.32	2.09	1.89
313.15	2.029	2.897	3.756	2.24	2.02	1.80
318.15	1.966	2.831	3.663	2.16	1.94	1.73

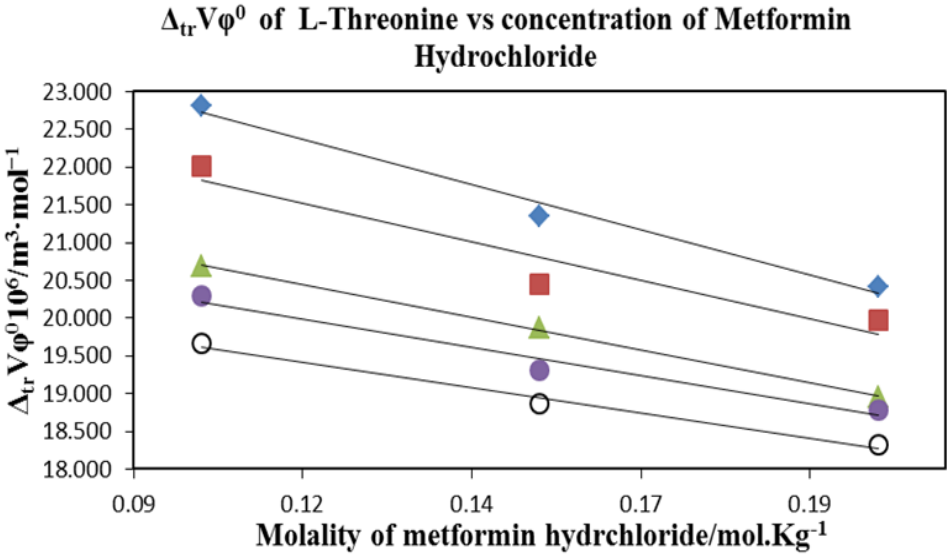

Figure 5: Representation plots of $\Delta_{tr}V_{\varphi}^{0}$ of L- Threonine versus concentration of Metformin Hydrochloride at different temperatures 298.15K(◆), 303.15K(■), 308.15K(▲), 313.15K(●) 318.15K (o)

3.3 Hydration number n_H

The information on hydration effect may be obtained by evaluating the electrostriction partial molal volume $V_{\varphi}^{0}{}_{(elec)}$ from the experimentally measured V_{φ}^{0} values as

$V_{\varphi}^{0}{}_{(elec)} = V_{\varphi}^{0} - V_{\varphi}^{0}{}_{(int)}$ (4)

Where $V_{\varphi}^{0}{}_{(int)}$, the intrinsic partial molal volume has been calculated by[11,41,42] using the following expressions,

$V_{\varphi}^{0}{}_{(int)} = (0.7/0.634)^{x} V_{\varphi}^{0}{}_{(cryst)}$ (5)

Where $V_{\varphi}^{0}{}_{(cryst)} = (M/\rho_{(cryst)})$ (6)

M and ρ being molecular weight and density values of amino acids respectively. In eqn (5) 0.7 is the packing density for molecules in the organic crystal and 0.634 is the packing density for random packing spheres. The decrease in volume due to electrostriction can be related to the number of water molecules n_H hydrated to the amino acids and are estimated (Millero et al 1974, Zhao et al 2005)[11,12,42-45] using the relation given by,

$n_H = V_{\varphi}^{0}{}_{(elec)} / (V_E^{\circ} - V_B^{\circ})$ (7)

Where V_E° is the molal volume of the electrostricted water and V_B^{0} is the molal volume of bulk water at T = 308.15 K.[46]

$(V_E^{\circ} - V_B^{\circ}) = -4 cm^2.\ mol$ (8)

This value of $(V_E^{\circ} - V_B^{\circ})$ has been retained at the other studied temperatures following the work of Lark et al (2006)[11,46] and the evaluated values of n_H are included in Table 3 and illustrated in figure 6.

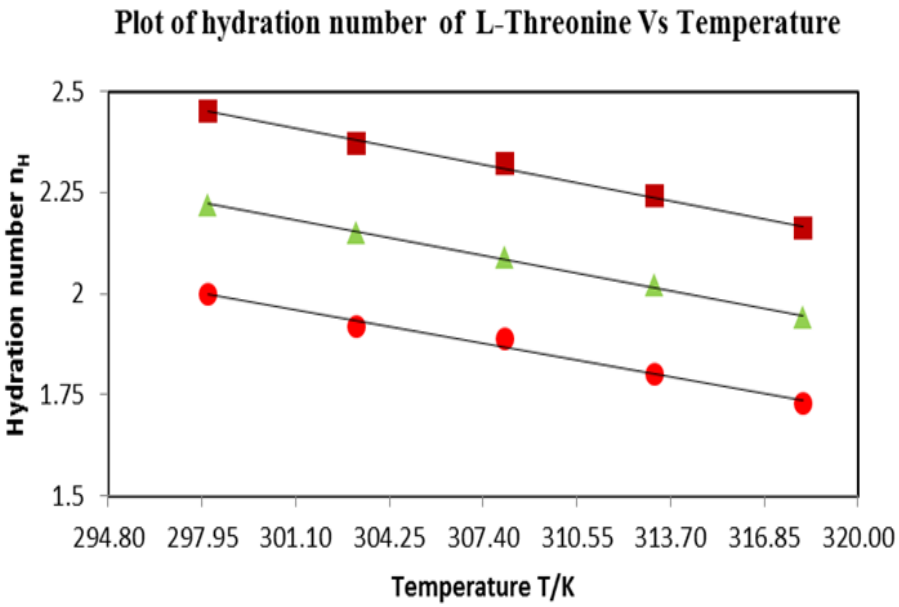

Figure 6: Hydration number, n_H vs. Temperature, T/K, for L- Threonine in Metformin hydrochloride + water solutions at concentrations concentration (a) 0.10 mol.kg^{-1}(■), (b) concentration 0.15 mol.kg^{-1} (▲), (c) concentration 0.20 mol.kg^{-1} (●)

It is seen from Figure 6. that the hydration number n_H of L-Threonine in aqueous Metformin hydrochloride decreases with increasing concentration of Metformin hydrochloride and temperature, which again substantiate the predominance of solute-co-solute interactions. Further this establishes the fact that Metformin hydrochloride has a dehydration effect on L-Threonine.

3.4 Hepler's constants

The structure making/breaking property of the solute (L-Threonine) in aqueous Metformin hydrochloride may be determined from the temperature dependence of the standard partial molal volume at infinite dilution. The temperature dependence of V_φ^0[47] for L-Threonine in aqueous Metformin Hydrochloride solution can be expressed by the equation (9).

$V_\varphi^0 = a + bT + cT^2$ (9)

Where a, b and c may be estimated by the least squares fitting of partial molal volume in the above equation. The value $\partial^2 V_\varphi^0/\partial T^2$, called Hepler's constant[47,48] gives the information about the structure making / breaking properties of solute in aqueous Metformin hydrochloride solution. On the basis of these criteria, a structure making solute will exhibit positive ($\partial^2 V_\varphi^0/\partial T^2$) values and structure breaking solute will show opposite trend. In our present study the following equations (10-13) at four concentrations of metformin hydrochloride are obtained.

V_φ^o = 51.166 + 0.0907 T - 0.0000286 T^2 (for m_H = 0.0 mol.kg^{-1}) (10)

V_φ^o = 92.926 – 0.1507 T + 0.00337 T^2 (for m_H = 0.10 mol.kg^{-1}) (11)

V_φ^o = 98.009 – 0.1757 T + 0.000374T^2 (for m_H = 0.15 mol.kg^{-1}) (12)

V_φ^o = 105.343 – 0.214 T + 0.000431 T^2 (for m_H = 0.20 mol.kg^{-1}) (13)

Table 4: Partial molal expansivity E_2^0, Hepler's constants ($\partial^2 V_\varphi^0/\partial T^2$) of L- Threonine in aqueous Metformin Hydrochloride solutions for a range of temperatures from 298.15 to 318.15K.

m_H/ (mol·kg^{-1})	$\partial^2 V_\varphi^0/\partial T^2$ / (m^6. mol^{-2} · k^{-2})	10^6 E_2^0/ (m^3 ·mol^{-1}·K^{-1})
0.0	-0.000029	0.07380
0.1	0.000337	0.05810
0.15	0.000374	0.05530
0.2	0.000431	0.05280

The values of Hepler's constant are given in Table 4. It is clear that the values of $\partial^2 V_\varphi/\partial T^2$ are positive for all concentrations of Metformin Hydrochloride (see Table 4), indicating the structure-making ability of L-Threonine in aqueous Metformin Hydrochloride solutions[43,49] complementing our earlier viscometric studies on these systems.[26]

3.5 Partial molal expansivity and Isobaric Thermal Expansion Coefficient

The values of partial molal expansivity[43] have been calculated from the partial molal volume using the relation (14) and are included in Table 4.

$E_2^0 = (\partial V_\varphi^0/\partial T)_P$ (14)

The values of partial molar expansivity E_2^0 are considered to be an important and sensitive indicator of solute-solvent interactions and the structure making or breaking properties of solute.[50] Positive values of E_2^0 (see Table 4) indicate that the studied amino acid (L-Threonine) is a structure maker in aqueous Metformin hydrochloride solvent.[43]

The results of the partial molar volume V_φ^0 have been used for the calculation of the isobaric thermal expansion coefficient α_2 using the following equation (15) and are shown in table 5.

$\alpha_2 = E_2^0 / V_\varphi^0$ (15)

Table 5: Isobaric Thermal Expansion Coefficient α_2 of L- Threonine in aqueous Metformin Hydrochloride solutions at different temperatures.

	Isobaric Thermal Expansion Coefficient $\alpha_2 = (E_2^0 / V_\varphi^o) / K^{-1}$			
T/K	m_H = 0.0 mol•kg−1	m_H = 0.10 mol•kg−1	m_H = 0.15 mol•kg−1	m_H = 0.20 mol•kg−1
298.15	0.00097597	0.00074536	0.00070115	0.00066205
303.15	0.00097087	0.00074233	0.00069883	0.00065950
308.15	0.00096661	0.00074042	0.00069663	0.00065842
313.15	0.00096199	0.00073734	0.00069416	0.00065571
318.15	0.00095729	0.00073441	0.00069146	0.00065340

The values of isobaric thermal expansion coefficient (α_2) (see table 5) are found to decrease with increase in temperature thereby showing the predominance of hydroxyl group interactions in the reported systems.

3.6 Pair and Triplet interaction coefficients, V_{AD} and V_{ADD} of L-Threonine in aqueous of Metformin Hydrochloride

To calculate the interaction coefficients Friedman and Krishnan[38] proposed a formalism based on the Mcmillan and Mayer theory[51] which permits the separation effects due to interaction between the pairs of solute molecules and those due to interactions between three or more solute molecules expressed by the following equation.

ΔV_φ^0 (water to aqueous Metformin Hydrochloride solution) = $2\, V_{AD}\, m_H + 3\, V_{ADD}\, m_H^2$ + ---- (16)

Where A stands for L-Threonine and B stands for metformin hydrochloride and m_H is the molality of metformin hydrochloride in water (cosolute). The constants V_{AD}, V_{ADD} are pair and triplet volumetric interaction parameters obtained by fitting data to the above equation[16], the volumetric interaction parameters V_{AD}, V_{ADD} have been summarized in Table 6.

Table 6: Values of pair (V_{AD}, V_{ADD}) of L- Threonine in aqueous Metformin Hydrochloride solutions at different temperatures.

T/K	$V_{AD}\, x10^6$ / $m^3 . mol^{-2} . kg$	$V_{ADD}\, x10^6$ / $m^3 . mol^{-3} . kg^2$
298.15	12.556	-7.967
303.15	11.931	-6.783
308.15	11.212	-5.750
313.15	10.863	-5.033
318.15	10.484	-4.483

It is clear from Table 6 that V_{AD} is positive while V_{ADD} is negative. The higher positive V_{AD} in comparison to V_{ADD} shows that the interactions between amino acids and Metformin Hydrochloride are mainly pair interactions due to the overlap of hydration spheres of solute-co-solute molecules, which again support the conclusion drawn from the co-sphere overlap model & through viscometric studies.[26]

4. CONCLUSION

In this paper, we have reported the density of L-Threonine in aqueous Metformin Hydrochloride solutions of higher concentrations (0.10, 0.15 and 0.20M) mol kg^{-1} at T = 298.15 – 318.15K. The partial molal volume of L – Threonine values are positive in aqueous drug solution, predicting the presence of strong solute – co-solute interactions which are increasing with the higher concentrations of Metformin Hydrochloride. The second derivative of V_φ^0 with respect to temperature shows structure making property of L- Threonine in aqueous Metformin Hydrochloride solutions. The increasing positive values of $\Delta_{tr}V\varphi^0$ indicate predominance of hydrophilic – hydrophilic, ion-hydrophilic interactions between the amino acid and drug molecules reported. The volumetric study of L-Threonine in aqueous Metformin Hydrochloride complements fairly well with our earlier viscometric studies on the same systems.

Declarations: None.

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