

CHAPTER VIII

GOUT PAIN CAUSED BY URIC ACID MOLECULE AND ITS SOLUTION CAUSED BY CITRIC ACID MOLECULE: A PHYSICOCHEMICAL INVESTIGATION

8.1. Introduction

Gout is a very painful experience in our modern era. The underlying cause of gout is due to the crystallization of uric acid, often related to relatively high levels in the blood of human body. This can occur for number of reasons, including diet, genetic predisposition, or under excretion of urate, the salts of uric acid. The remedial methods are both lifestyle changes and medications can decrease uric acid levels. Doctor's are generally advice to reduce intake of food such as meat and seafood, limiting alcohol and consuming citrus fruits [1]. Citric acid is one of the major ingredients of citrus fruits. The chemistry of solutions deals with solutes and solvents and how solutes interact with solvents as they move about in solutions. So that we choose this biologically active compound, citric acid as a solute and aqueous uric acid as a solvent to examine the interaction between these two. Studies on the apparent molar volumes and viscosity *B*-coefficients at infinite dilution provide valuable information regarding solute-solute, solute-solvent and solvent-solvent interactions [2]. The addition of solute could break or make the structure of a liquid [3-5] as viscosity being a property of the solution depending upon the intermolecular forces, the structural aspects of the liquid can be inferred from the viscosity of solutions at different concentrations and temperatures.

Citric acid, $C_6H_8O_7$ (CA), i.e. 2-hydroxypropane-1, 2, 3-tricarboxylic acid, is a tribasic, environmentally suitable and versatile chemicals. As it occurs in metabolism of almost all living beings, its interactions in an aqueous solution is of great value to the biological scientists. In the Pharmaceutical industry, citric acid is used as stabilizer in various formulations, as a drug component and as anticoagulant in blood for transfusions and also used as an acidifier in many pharmaceuticals. Citric acid can be used as flavouring and preservative in food and beverages, especially soft drinks [6].

Citric acid exists in greater than trace amounts in variety of vegetables, most notably citrus fruits.

Uric acid is a heterocyclic compound with the molecular formula $C_5H_4N_4O_3$ (UA), i.e. 7,9-Dihydro-1H-purine-2,6,8 (3H)-trione. It is a diprotic acid. It was first isolated from kidney stones in 1776 by Scheele [7]. Uric acid is deprotonated at a nitrogen atom and uses a tautomeric keto/hydroxyl group as an electron-withdrawing group to increase the pK_a value while most organic acids are deprotonated by the ionization of a polar hydrogen-to-oxygen bond. In general, the water solubility of uric acid is low. This low solubility is significant for the etiology of gout. Uric acid is a strong reducing agent and potent antioxidant. In humans, over half the anti oxidant capacity of blood plasma comes from uric acid [8].

To the best of our knowledge, the studies in the present ternary solution systems have not been reported earlier. Therefore, in present study we have endeavoured to make certain nature of interaction of solute itself (citric acid) and with co-solute (uric acid) in $w_1=0.00001$, 0.00002 and 0.00003 mass fraction of aqueous uric acid mixture at different temperatures (298.15-313.15)K with 5 interval to explain various non covalent interactions prevailing in the ternary systems under investigation.

8.2. Experimental Section

8.2.1 Source and purity of materials

Citric acid monohydrate was purchased from HiMedia. Uric acid (UA) was purchased from S D Fine-Chem. Ltd. The mass fractions purity of both was ≥ 0.99 . The reagents were always placed in the desiccators over P_2O_5 to keep them in dry atmosphere. These chemicals were used as received without further purification. The provenance and purity of the chemical used has been depicted in table 1.

8.2.2 Apparatus and procedure

Solubility of the uric acid in water (deionised, doubly distilled water with a specific conductance of $1 \cdot 10^{-6} S \cdot cm^{-1}$) and the citric acid in aqueous uric acid had been checked precisely, prior to start of the experimental work and seen that citric acid soluble in all proportion of aqueous uric acid solution. The mother solutions of citric acid were prepared by mass (Mettler Toledo AG-285 with uncertainty 0.0003g) and

then the working solutions (six sets) were prepared by mass dilution. The conversions of molarity into molality [9] had been done using experimental density values of respective solutions and adequate precautions were taken to reduce evaporation losses during mixing and throughout the experiment.

The densities (ρ) of the solutions were measured by means of vibrating u-tube Anton Paar digital density meter (DMA 4500M) with a precision of $\pm 0.00005 \text{ g.cm}^{-3}$ maintained at $\pm 0.01 \text{ K}$ of the desired temperature. It was calibrated by passing deionised, triply distilled water and dry air [10].

The viscosities (η) were measured using a Brookfield DV-III Ultra Programmable Rheometer with fitted spindle size-42. The detail description has already been described earlier [11].

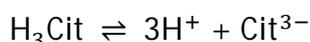
Refractive index (n_D) was measured with the help of a Digital Refractometer Mettler Toledo. The light source was LED, $\lambda=589.3\text{nm}$. The refractometer was calibrated twice using distilled water and calibration was checked after every few measurements [12a]. The uncertainty of refractive index measurement was ± 0.0002 units.

The pH values of the experimental solutions were measured by a Mettler Toledo Seven Multi pH meter [12b]. The measurements were made in a thermostated water bath maintaining the temperature at 298.15K, 303.15K, 308.15K and 313.15K with the uncertainty 0.01 K.

8.3. Result and Discussion:

The physical parameters of binary mixtures in different mass fractions ($w_1=0.00001, 0.00002, 0.00003$) of aqueous uric acid (UA) solutions at four different temperatures (298.15K, 303.15K, 308.15K and 313.15K) and at 1.013 bar have been reported in table 2. The experimental measured values of density, viscosity of citric acid (CA) as a function of concentration (molality), in different mass fractions of aqueous uric acid mixture at four above mentioned temperatures have been listed in table 3. According to the pH data of ternary solution citric acid in different mass fraction of aqueous uric acid is found within the range of 5.90-6.63 (table 3); which is equivalent or higher than the value of pK_{a3} value of citric acid ($pK_{a1}=3.13$ [13a]; $pK_{a2}=4.76$ [13a]; $pK_{a3}=6.39$ [13b], 6.40 [13c]). On the other hand, the estimated pK_a

value of the solution is greater than pK_{a3} of citric acid (table 3), and it predicting that the citric acid is dissociated at and above the pH. Thus it clearly indicated that the citric acid is completely dissociated and exists as $3H^+ + Cit^{3-}$ form (scheme 1) in ternary solutions *i.e.*,



8.3.1 Apparent molar volume:

Volumetric properties, like, apparent molar volume (φ_V) and limiting apparent molar volume (φ_V^ρ) consider important tools for understanding of interactions taking place in solution systems. The apparent molar volume can be regarded to be the sum of the geometric volume of the central solute molecule and changes in the solvent volume due to its interaction with the solute around the peripheral or co-sphere. Therefore, the apparent molar volumes (φ_V) have been determined from the solutions densities using the suitable equation [14] and the values are given in table 4.

$$\varphi_V = M/\rho - 1000(\rho - \rho_0)/m\rho\rho_0 \quad (1)$$

where M is the molar mass of the solute, m is the molality of the solution, ρ and ρ_0 are the density of the solution and aqueous uric acid mixture respectively.

The values of (φ_V) are positive and large for all the systems, signifying strong solute-solute interactions. The apparent molar volumes (φ_V) are found to decrease with increasing concentration (molality, m) of citric acid in same mass fraction of aqueous uric acid at same temperature. It is also found that apparent molar volumes (φ_V) increase with both increasing temperature as well as mass fraction of aqueous uric acid solution and varied with \sqrt{m} and could be least-squares fitted to the extended Masson equation [15] from where limiting molar volume, φ_V^ρ (infinite dilution partial molar volume) have been estimated and the values have been represented in table 5.

$$\varphi_V = \varphi_V^\rho + S_V^* \sqrt{m} + S_{V^\#} \cdot m \quad (2)$$

Here φ_V^ρ is the apparent molar volume at infinite dilution, S_V^* and $S_{V^\#}$ is the experimental slope. At infinite dilution each solute molecule is surrounded only by the solvent molecules and remains infinite distant from each other. As a consequence, that φ_V^ρ is unaffected by itself interaction of solute molecules (either

uric acid itself or citric acid) and it is a measure only of the solute-cosolute (uric acid -citric acid) interaction.

An inspection of table 5 and fig 1 shows that φ_V^0 are large and positive for all citric acid at all the studied temperatures, suggesting the presence of strong solute-cosolute interaction (scheme 2). Comparing φ_V^0 with S_V^* and S_{VV}^\neq values show that the magnitude of φ_V^0 is greater than S_V^* and S_{VV}^\neq , suggesting that solute-cosolute interactions dominates over itself interaction of solute molecules in all solutions at all studied temperatures. Moreover, S_V^* values are negative at all temperatures indicates force of itself interaction of solute molecules is very poor. Positive and significant magnitude of S_{VV}^\neq is indicating the ternary interaction of solute-cosolute-solute, cosolute-solute-cosolute (scheme 3).

The variation of φ_V^0 with temperature are fitted to a polynomial of the following

$$\varphi_V^0 = a_0 + a_1 T + a_2 T^2 \quad (3)$$

Where T is the temperature in K and a_0 , a_1 and a_2 are the empirical coefficients depending on the solute, mass fraction of cosolute uric acid. Values of coefficients of the above equation for the citric acid in aqueous uric acid mixtures are reported in table 6.

The limiting apparent molar expansibilities, φ_E^0 , can be evaluated by the following equation,

$$\varphi_E^0 = (\delta\varphi_V^0/\delta T)_P = a_1 + 2a_2 T \quad (4)$$

The limiting apparent molar expansibilities, φ_E^0 , change in magnitude with the change of temperature. The values of φ_E^0 for different solutions of studied citric acid at ($T=298.15, 303.15, 308.15$ and 313.15) K are reported in table 7.

All the values of φ_E^0 shown in the table 7 are positive for citric acid in aqueous uric acid and studied temperature. This fact helps to explain the absence of caging or packing effect for the citric acid in solution [16].

The long-range structure-making and breaking capacity of the solute in mixed system can be determined by examining the sign of $(\delta\varphi_E^0/\delta T)_P$ developed by Hepler [17].

$$(\delta\varphi_E^0/\delta T)_P = (\delta^2\varphi_V^0/\delta T^2)_P = 2a_2 \quad (5)$$

The positive sign or small negative of $(\delta\varphi_{E^0}/\delta T)_P$ signifies the molecule is a structure-maker; otherwise, it is a structure-breaker [18]. The perusal of table 6 shows that, $(\delta\varphi_{E^0}/\delta T)_P$ values of citric acid are all positive under investigation. It shows the more symmetric rearrangement of the interacting molecules (citric acid and uric acid) with the formation of H-bonding, van der waal forces, dipole-dipole interactions etc. The plausible sites of different interactions playing in the ternary solution are shown in scheme 2. This symmetric arrangement is signifies the molecules of citric acid and uric acid is definitely interacting with structure-making tendency in all of the studied solution systems. The table 6 also showing the positively magnitude of $(\delta\varphi_{E^0}/\delta T)_P$ values in of citric acid is depicting this structure-making tendency.

8.3.2 Viscosity:

The experimental viscosity data for studied systems are listed in table 3. The relative viscosity (η_r) has been calculated using extended Jones-Dole equation [19] for non electrolytes.

$$(\eta/\eta_0 - 1)/\sqrt{m} = (\eta_r - 1)/\sqrt{m} = A + B \cdot \sqrt{m} + D \cdot m \quad (6)$$

Where $\eta_r = \eta/\eta_0$ is the relative viscosity, η and η_0 are the viscosities of ternary solutions (citric acid + aqueous uric acid) and solvent (aqueous mixture of uric acid) respectively and m is the molality of citric acid in ternary solutions. Where A is known as Falkenhagen coefficient [20] as it is determined by the ionic attraction theory of Falkenhagen-Vernon and B is empirical constants known as viscosity B -coefficients, which are specifying to the interaction of solute itself and/or with cosolute molecules respectively. The values of A -, B - and D -coefficients are estimated by least-square polynomial method by plotting $(\eta_r - 1)/\sqrt{m}$ against \sqrt{m} with second order and reported in table 4. It is observed from table 4 the values of the A -coefficient are found to decrease with increase in temperature. This fact indicates the presence of very weak solute-solute interaction and also in excellent agreement with those obtained from S_V^* values.

The valuable information about the solvation of the solvated solutes and their effects on the structure of the cosolute uric acid in the local vicinity of the solute (citric acid) molecules in solutions has been obtained from viscosity B -coefficient [21]. It is found

from table 4 and fig 2; the values of B -coefficient are positive and much higher than A -coefficient which signifies solute-cosolute interaction is dominant over solute-solute and cosolute-cosolute interaction. It is also observed that the positive magnitude of viscosity B -coefficient increases with increasing temperature and also increases with an increase in mass fraction of aqueous uric acid mixture which suggests that solute-cosolute interaction is strengthened with rise in temperature as well as mass fraction of aqueous uric acid mixture. These results are in good agreement with those obtained from limiting apparent molar volume φ_{V^0} values.

It is observed from table 4 that the values of the B -coefficient of citric acid increases with temperature, i.e., the dB/dT values are positive. From table 8, the small positive dB/dT values for the citric acid behaves almost as structure-maker.

The free energy of activation of viscous flow per mole of solvent, $\Delta\mu_1^{0\ddagger}$ as proposed by Eyring and co-workers [22] could be calculated from the following equation:

$$\eta_0 = (hN_A/V_1^0) \exp(\Delta\mu_1^{0\ddagger}/RT) \quad (7)$$

Where h , N_A and V_1^0 are the Planck's constant, Avogadro's number and partial molar volume of the solvent respectively. The equation (7) can be rearranged as follows we get

$$\Delta\mu_1^{0\ddagger} = RT \ln (\eta_0 V_1^0 / hN_A) \quad (8)$$

Feakins et al.[23-25] suggested that if equations (6) and (8) are obeyed, then

$$B = (V_1^0 - V_2^0) + V_1^0 [(\Delta\mu_1^{0\ddagger} - \Delta\mu_2^{0\ddagger})/RT] \quad (9)$$

where V_2^0 is the limiting partial molar volume (φ_{V^0}) of the solute and $\Delta\mu_2^{0\ddagger}$ is the ionic activation energy per mole of solute at infinite dilution . Rearranging the equation (9) we get

$$\Delta\mu_2^{0\ddagger} = \Delta\mu_1^{0\ddagger} + (RT/V_1^0)[B - (V_1^0 - V_2^0)] \quad (10)$$

From table 8, it is evident that $\Delta\mu_2^{0\ddagger}$ values are all positive and much larger than $\Delta\mu_1^{0\ddagger}$, suggesting that interaction between solute (citric acid) and solvent (aqueous uric acid mixture) molecules in the ground state is stronger than in the transition state. According to free energy terms the salvation of solute in the transition state is unfavourable.

The entropy of activation ($\Delta S_2^{0\ddagger}$) [24] for the solution has been calculated using relation:

$$\Delta S_2^{0\ddagger} = -d(\Delta\mu_2^{0\ddagger})/dT \quad (11)$$

Here $\Delta S_2^{0\ddagger}$ has been obtained from the negative slope of the plots of $\Delta\mu_2^{0\ddagger}$ against T by using a least-squares treatment.

The enthalpy of activation ($\Delta H_2^{0\ddagger}$) [24] has been obtained from the relation:

$$\Delta H_2^{0\ddagger} = \Delta\mu_2^{0\ddagger} + T\Delta S_2^{0\ddagger} \quad (12)$$

The values of $\Delta S_2^{0\ddagger}$ and $\Delta H_2^{0\ddagger}$ are also reported in table 8.

It is evident from table 9, that $\Delta\mu_1^{0\ddagger}$ is practically constant at all the mass fraction of the aqueous uric acid mixture, suggesting that $\Delta\mu_2^{0\ddagger}$ is mainly dependent on the viscosity coefficients and $(V_1^0 - V_2^0)$ terms. Positive $\Delta\mu_2^{0\ddagger}$ values at all studied temperature and solvent composition suggests that the process of viscous flow becomes difficult as the temperature and mass fraction of aqueous uric acid mixture increases. Therefore, the formation of transition state becomes less favourable. Feakins et al [24] proposed that, $\Delta\mu_2^{0\ddagger} > \Delta\mu_1^{0\ddagger}$ for solutes having positive B -coefficients and indicates a stronger solute –solvent interactions, thereby suggesting that the formation of transition state is accompanied by the rupture and distortion of the intermolecular forces in the solvent structure [24, 26]. The negative values of both $\Delta S_2^{0\ddagger}$ and $\Delta H_2^{0\ddagger}$ suggest that the formation of transition state is associated with bond-making and an increase in order. Although a detailed mechanism for this is not easily advanced, it may be suggested that the slip-plane is in the disordered state [24, 27]. According to Feakins et al. model, as $\Delta\mu_2^{0\ddagger} > \Delta\mu_1^{0\ddagger}$, the solute (citric acid) behaves as structure makers. This again supports the behaviour of dB/dT for the solute in aqueous uric acid mixture.

Furthermore, it is attractive to observe that there is linear correlation between viscosity B -coefficients of the studied citric acid with the limiting apparent molar volumes (φV^0) in different mass fraction of aqueous uric acid solutions (fig 3). From the above fact it means

$$B = A_1 + A_2 \varphi V^0 \quad (13)$$

The coefficients A_1 and A_2 are listed in table 8. As both viscosities B -coefficient and limiting apparent molar volumes define the solute-solvent interaction in solution. The linear variation of viscosity B -coefficient and limiting apparent molar volume (φ_V^0) reflects the positive slope (or A_2).

It is evident from this study, that there is a strong interaction between citric acid and uric acid and it becomes stronger with rise in temperature. As molecules of uric acid are engaged with the citric acid's molecules, the accumulation among the uric acid molecules becomes less effective. Therefore, the process of crystallization and deposition of uric acid gets hampered in presence of citric acid (Scheme 3). The above fact suggests that the relief of painful effect of gout can be achieved by consumption of more citrus fruits and by making warmth the affected area.

As we know that the gout is the disease occurred due to the crystallization of uric acid in the joint of human body. Therefore the interaction of citric acid with uric acid in aqueous solution at human body temperature (37°C or 310.15K) is important. We have obtained the derived parameters like, limiting apparent molar volume (φ_V^0), viscosity B -coefficient by interpolation and presented in table 5. The positive and significant magnitude of φ_V^0 and B -coefficient from table 5 clearly indicates that the limiting apparent molar volume (φ_V^0), viscosity B -coefficient is increases with increasing mass fraction of citric acid, which indicates the positive effect of hampering in crystallization and deposition of uric acid in joint of the human body, as a result presence of citric acid relief the painful effect of gout. The effect also evidence from the values of free energy of activation ($\Delta\mu_1^{0\#}$ and $\Delta\mu_2^{0\#}$), entropy ($\Delta S_2^{0\#}$) and enthalpy ($\Delta H_2^{0\#}$) (table 9). The positive values and increasing order of free energy of activation and negative magnitude and decreasing degree of entropy ($\Delta S_2^{0\#}$) and enthalpy ($\Delta H_2^{0\#}$) also suggesting the positive effect for pain relief of gout in presence of citric acid.

8.3.3 Refractive Index:

The measurement of refractive index is also a suitable method for investigating the molecular interaction existing in solution. The molar refraction (R_M) (fig 4) can be evaluated from the Lorentz-Lorenz relation [28]. The refractive index of a substance is defined as the ratio c_0/c , where c and c_0 is the velocity of light in the medium and in vacuum respectively. Stated more simply that the refractive index of a compound

describes its ability to refract light as it passes from one medium to another and thus, the higher the refractive index of a compound, the more the light is refracted [29]. As stated by Deetlefs et al. [30] the refractive index of a substance is higher when its molecules are more tightly packed or in general when the compound is denser. Hence, a perusal of table 10 we found that the refractive index and the molar refraction are higher for the studied citric acid in all the mass fraction of aqueous uric acid, indicating to the fact that the molecules are more tightly packed in the solution.

The Limiting molar refraction (R_M^0) estimated from the following equation (14) and presented in table 10.

$$R_M = R_M^0 + R_S \sqrt{m} \quad (14)$$

Accordingly, we found that the higher values of refractive index and R_M^0 which representing the fact that the molecules of citric acid are more tightly packed and greater solute-solvent interaction with uric acid molecules than solute solvent interaction. This is also in good agreement with the results obtained from apparent molar volume and viscosity B -coefficients discussed above.

All the above derived parameters suggest that there is strong interaction between citric acid (solute) and uric acid (solvent) and these increases with rise in temperature. The solute-solvent interaction is much greater than the solute-solute and solvent-solvent interactions.

8.4. Conclusion:

It is evident from this study, that there is a strong interaction between citric acid and uric acid and it becomes stronger with rise in temperature. As molecules of uric acid are engaged with the citric acid molecules, the accumulation among the uric acid molecules becomes less effective. The table S1 was given a strong evidence of enhances solubility of uric acid in the presence of citric acid. Therefore, the process of crystallization and deposition of uric acid gets hampered in presence of citric acid (Scheme 3). The above fact suggests that the relief of painful effect of gout can be achieved by consumption of more citrus fruits and by making warmth the affected area.

Tables

Table 1: Source and purity of the chemicals

Chemical name	Source	mass fraction purity	Purification Method
Citric acid monohydrate	HiMedia	≥0.99	Used as procured
Uric acid	SD Fine-Chem Ltd.	≥0.99	Used as procured

Table 2: Experimental values of density (ρ), viscosity (η) and pH at different temperatures, refractive index (n_D) at 298.15 K and at pressure 1.013 bar of different mass fraction (w_1) of aq. uric acid mixtures*

Aq. Uric acid Mixture (w_1)	Temperature (K)	$\rho \times 10^{-3}$ /kg·m ⁻³	η /mP·s	n_D	pH
0.00001	298.15	0.99698	0.90	1.3316	6.58
	303.15	0.99558	0.82		6.50
	308.15	0.99401	0.73		6.41
	313.15	0.99209	0.63		6.33
0.00002	298.15	0.99704	0.90	1.3321	6.60
	303.15	0.99566	0.83		6.52
	308.15	0.99407	0.75		6.43
	313.15	0.99239	0.65		6.35
0.00003	298.15	0.99712	0.91	1.3327	6.63
	303.15	0.99573	0.84		6.53
	308.15	0.99414	0.76		6.44
	313.15	0.99244	0.66		6.36

*Standard uncertainties u are: $u(\rho) = 0.00002 \text{ kg}\cdot\text{m}^{-3}$, $u(\eta) = 0.02 \text{ mP}\cdot\text{s}$, $u(n_D) = 0.0002$, $u(\text{pH}) = 0.01$ and $u(T) = 0.01\text{K}$, (0.68 level of confidence)

Table 3: Experimental values of density (ρ) and viscosity (η), pH and pKa of citric acid in different mass fractions of aqueous uric acid mixture (w_1) at three different temperatures at pressure 1.013 bar*

am /mol·kg ⁻¹	$\rho \times 10^{-3}$ /kg·m ⁻³	η /mP·s	pH	pKa	am /mol·kg ⁻¹	$\rho \times 10^{-3}$ /kg·m ⁻³	η /mP·s	pH	pKa	am /mol·kg ⁻¹	$\rho \times 10^{-3}$ /kg·m ⁻³	η /mP·s	pH	pKa
$w_1=0.00001$					$w_1=0.00002$					$w_1=0.00003$				
T = 298.15 K					T = 298.15 K					T = 298.15 K				
0.0100	0.99721	0.91	6.51	6.87	0.0100	0.99723	0.92	6.53	6.92	0.0100	0.99727	0.92	6.56	6.99
0.0252	0.99789	0.92	6.42	7.17	0.0252	0.99791	0.93	6.44	7.21	0.0252	0.99794	0.93	6.47	7.27
0.0404	0.99896	0.93	6.32	7.20	0.0404	0.99894	0.94	6.34	7.24	0.0404	0.99894	0.95	6.37	7.30
0.0556	1.00021	0.93	6.23	7.16	0.0556	1.00021	0.95	6.25	7.20	0.0556	1.00014	0.96	6.28	7.26
0.0709	1.00149	0.94	6.14	7.09	0.0709	1.00155	0.96	6.16	7.13	0.0709	1.00152	0.97	6.19	7.19
0.0863	1.00303	0.95	6.06	7.01	0.0863	1.00307	0.97	6.08	7.05	0.0863	1.00312	0.98	6.10	7.10
T = 303.15 K					T = 303.15 K					T = 303.15 K				
0.0101	0.99578	0.83	6.43	6.68	0.0101	0.99582	0.84	6.45	6.73	0.0101	0.99584	0.85	6.48	6.80
0.0252	0.99647	0.84	6.34	6.99	0.0252	0.99649	0.85	6.36	7.04	0.0252	0.99645	0.86	6.39	7.10
0.0404	0.99751	0.85	6.25	7.03	0.0404	0.99748	0.86	6.27	7.08	0.0404	0.99745	0.87	6.30	7.14
0.0557	0.99874	0.86	6.16	7.00	0.0557	0.99873	0.87	6.18	7.04	0.0557	0.99862	0.88	6.20	7.10
0.0710	1.00004	0.86	6.07	6.93	0.0710	1.00010	0.88	6.09	6.97	0.0710	1.00004	0.89	6.12	7.03
0.0864	1.00155	0.87	5.98	6.85	0.0864	1.00161	0.88	6.00	6.89	0.0864	1.00164	0.90	6.03	6.95
T = 308.15 K					T = 308.15 K					T = 308.15 K				
0.0101	0.99416	0.74	6.35	6.44	0.0101	0.99419	0.76	6.37	6.49	0.0101	0.99421	0.77	6.39	6.55
0.0253	0.99481	0.75	6.25	6.80	0.0253	0.99482	0.77	6.27	6.84	0.0253	0.99477	0.78	6.29	6.88
0.0405	0.99577	0.75	6.16	6.84	0.0405	0.99580	0.77	6.18	6.89	0.0405	0.99573	0.79	6.20	6.93
0.0558	0.99706	0.76	6.07	6.82	0.0558	0.99703	0.78	6.09	6.86	0.0558	0.99693	0.80	6.11	6.90
0.0712	0.99839	0.77	5.98	6.75	0.0712	0.99842	0.79	6.00	6.79	0.0712	0.99836	0.80	6.02	6.83
0.0866	0.99990	0.77	5.90	6.67	0.0866	0.99995	0.80	5.92	6.71	0.0866	0.99991	0.81	5.94	6.75
T = 313.15 K					T = 308.15 K					T = 308.15 K				
0.0101	0.99219	0.64	6.29	6.26	0.0101	0.99246	0.66	6.31	6.32	0.0101	0.99247	0.67	6.33	6.38
0.0253	0.99277	0.65	6.19	6.66	0.0253	0.99302	0.66	6.21	6.71	0.0253	0.99304	0.67	6.23	6.75
0.0406	0.99374	0.65	6.10	6.72	0.0406	0.99398	0.67	6.12	6.76	0.0406	0.99393	0.68	6.14	6.80
0.0559	0.99501	0.66	6.01	6.69	0.0559	0.99527	0.68	6.03	6.73	0.0559	0.99511	0.69	6.05	6.77
0.0713	0.99639	0.67	5.93	6.63	0.0713	0.99655	0.68	5.95	6.67	0.0713	0.99649	0.70	5.96	6.71
0.0867	0.99794	0.67	5.84	6.54	0.0867	0.99817	0.69	5.86	6.59	0.0867	0.99809	0.71	5.88	6.63

* Standard uncertainties u are: $u(\rho) = 0.00002 \text{ kg}\cdot\text{m}^{-3}$, $u(\eta) = 0.02 \text{ mP}\cdot\text{s}$ and $u(T) = 0.01 \text{ K}$ (0.68 level of confidence)

am molality has been expressed per kilogram of (uric acid + water) solvent mixture

Table 4: Apparent molar volume (φ_V) and $(\eta_r-1)/\sqrt{m}$ of citric acid in different mass fraction (w_1) of aqueous uric acid mixtures at three different temperatures*

^a molality /mol·kg ⁻¹	$\varphi_V \times 10^6$ /m ³ mol ⁻¹	$(\eta_r-1)/\sqrt{m}$ /kg ^{1/2} mol ^{-1/2}	^a molality /mol·kg ⁻¹	$\varphi_V \times 10^6$ /m ³ mol ⁻¹	$(\eta_r-1)/\sqrt{m}$ /kg ^{1/2} mol ^{-1/2}	^a molality /mol·kg ⁻¹	$\varphi_V \times 10^6$ /m ³ mol ⁻¹	$(\eta_r-1)/\sqrt{m}$ /kg ^{1/2} mol ^{-1/2}
w ₁ =0.00001			w ₁ =0.00002			w ₁ =0.00003		
T = 298.15 K			T = 298.15 K			T = 298.15 K		
0.0100	188.21	0.12	0.0100	191.71	0.13	0.0100	195.70	0.13
0.0252	174.27	0.14	0.0252	175.86	0.16	0.0252	177.85	0.16
0.0404	161.13	0.17	0.0404	163.12	0.18	0.0404	165.12	0.19
0.0556	151.87	0.18	0.0556	152.96	0.20	0.0556	155.68	0.21
0.0709	146.15	0.20	0.0709	146.14	0.23	0.0709	147.71	0.24
0.0863	139.38	0.22	0.0863	139.61	0.24	0.0863	139.95	0.26
T = 303.15 K			T = 303.15 K			T = 303.15 K		
0.0101	190.98	0.08	0.0101	194.99	0.08	0.0101	199.99	0.08
0.0252	175.40	0.11	0.0252	177.71	0.11	0.0252	182.12	0.12
0.0404	162.61	0.14	0.0404	165.36	0.14	0.0404	167.86	0.16
0.0557	153.36	0.15	0.0557	154.99	0.17	0.0557	158.27	0.18
0.0710	147.08	0.17	0.0710	147.35	0.19	0.0710	149.21	0.20
0.0864	140.53	0.19	0.0864	140.75	0.20	0.0864	141.21	0.23
T = 308.15 K			T = 308.15 K			T = 308.15 K		
0.0101	196.32	0.07	0.0101	199.32	0.06	0.0101	204.34	0.08
0.0253	179.13	0.10	0.0253	181.21	0.10	0.0253	186.03	0.12
0.0405	167.14	0.13	0.0405	167.89	0.13	0.0405	171.39	0.16
0.0558	155.62	0.15	0.0558	157.25	0.16	0.0558	160.35	0.19
0.0712	148.46	0.17	0.0712	148.88	0.18	0.0712	150.74	0.21
0.0866	141.69	0.18	0.0866	141.80	0.20	0.0866	143.10	0.23
T = 313.15 K			T = 313.15 K			T = 313.15 K		
0.0101	201.74	0.07	0.0101	204.70	0.04	0.0101	208.72	0.02
0.0253	184.40	0.11	0.0253	186.36	0.09	0.0253	187.56	0.08
0.0406	170.24	0.14	0.0406	171.70	0.12	0.0406	174.21	0.12
0.0559	158.30	0.17	0.0559	158.99	0.14	0.0559	162.83	0.15
0.0713	149.90	0.19	0.0713	151.87	0.17	0.0713	153.44	0.18
0.0867	142.44	0.21	0.0867	143.23	0.19	0.0867	144.76	0.22

* Standard uncertainties u are: $u(T) = 0.01\text{K}$, the accuracy of φ_V is $1.75 \times 10^{-6} \text{ m}^3 \text{ mol}^{-1}$ and $(\eta_r-1)/\sqrt{m}$ is $0.005 \text{ kg}^{1/2} \text{ mol}^{-1/2}$ (0.68 level of confidence)

^amolality has been expressed per kilogram of (uric acid + water) solvent mixture

Table 5: Limiting apparent molar volume (φ_V^0), experimental slope (S_V^*), viscosity A - and B -coefficient of Citric acid in different mass fraction (w_1) of aqueous uric acid mixtures at five different temperatures*

Mass fraction (w_1)	T /K	$\varphi_V^0 \times 10^6$ /m ³ mol ⁻¹	$S_V^* \times 10^6$ /m ³ mol ^{-3/2} kg ^{1/2}	$S_V^\#$ /m ³ mol ⁻² kg	B /kg mol ⁻¹	A /kg ^{1/2} mol ^{-1/2}	D /kg ^{3/2} mol ^{-3/2}
0.00001	298.15	218.20	-307.10	129.83	0.30	0.08	0.56
	303.15	223.44	-339.72	194.74	0.48	0.03	0.14
	308.15	229.27	-339.97	139.42	0.59	0.01	0.03
	310.15	232.33	-339.53	100.13	0.70	0.00	-0.06
	313.15	235.52	-339.19	72.33	0.80	-0.01	-0.15
0.00002	298.15	223.89	-332.69	151.99	0.43	0.07	0.40
	303.15	228.36	-347.48	165.08	0.59	0.02	0.15
	308.15	234.17	-360.97	156.76	0.71	-0.01	0.08
	310.15	237.36	-366.01	141.98	0.78	-0.02	-0.05
	313.15	241.18	-372.21	132.41	0.84	-0.04	-0.17
0.00003	298.15	228.79	-344.90	147.05	0.51	0.07	0.39
	303.15	233.57	-343.32	99.36	0.73	0.01	0.05
	308.15	239.03	-350.76	79.37	0.82	-0.01	-0.01
	310.15	242.11	-371.00	60.01	0.93	-0.05	-0.10
	313.15	246.19	-390.71	59.69	1.08	-0.09	-0.19

* Standard uncertainties values of u are: $u(T) = 0.01K$

Table 6: Values of various coefficients and standard deviation of equation-3 for citric acid in different aqueous uric acid solutions*

Aq. Uric acid Mixture (w_1)	$a_0 \times 10^6$ /m ³ mol ⁻¹	$a_1 \times 10^6$ /m ³ mol ⁻¹ K ⁻¹	$a_2 \times 10^6$ /m ³ mol ⁻¹ K ⁻²	$(\delta\varphi_E^0/\delta T)_P \times 10^6$ /m ³ mol ⁻¹ K ⁻²	σ
0.00001	814.79	-5.01	0.01	0.02	0.0002
0.00002	2249.69	-14.36	0.03	0.06	0.0003
0.00003	2102.48	-13.37	0.02	0.04	0.0001
Average standard deviation	4.2	0.013	0.0003	0.0001	

Table 7: Limiting apparent molar expansibilities (φ_E^0) for citric acid in different mass fraction of aqueous uric acid (w_1) at different temperature

Aq. Uric acid Mixture (w_1)	$\varphi_E^0 \times 10^6$ /m ³ mol ⁻¹ K ⁻¹					σ
T/ K	298.15	303.15	308.15	310.15	313.15	0.01
0.00001	0.953	1.053	1.153	1.201	1.253	0.0001
0.00002	3.529	3.829	4.129	4.277	4.429	0.0003
0.00003	-1.444	-1.244	-1.044	-0.941	-0.844	0.0002
Average standard deviation	0.003	0.003	0.002	0.002	0.001	

Table 8: Values of dB/dT , A_1 , A_2 coefficients and standard deviation (σ) for the citric acid in different mass fraction of aqueous uric acid (w_1) at studied temperatures*

Aq. Uric acid Mixture (w_1)	dB/dT	A_1	A_2	σ
0.00001	0.031	-5.674	0.027	0.0003
0.00002	0.026	-4.676	0.022	0.0002
0.00003	0.035	-6.529	0.030	0.0002
Average standard deviation	0.002	0.015	0.003	

* Standard uncertainties values of u are: $u(T) = 0.01K$ Table 9: Values of V_1^0 , $(V_1^0 - V_2^0)$, $\Delta\mu_1^{0\#}$, $\Delta\mu_2^{0\#}$, $T\Delta S_2^{0\#}$ and $\Delta H_2^{0\#}$ and standard deviation (σ) for citric acid in different mass fraction (w_1) of aqueous uric acid mixture at different temperatures*

Mass fraction	T/K	$V_1^0 \cdot 10^6$ /m ³ ·mol ⁻¹	$(V_1^0 - V_2^0) \cdot 10^6$ /m ³ ·mol ⁻¹	$\Delta\mu_1^{0\#}$ /KJ.mol ⁻¹	$\Delta\mu_2^{0\#}$ /KJ.mol ⁻¹	$T\Delta S_2^{0\#}$ /KJ.mol ⁻¹	$\Delta H_2^{0\#}$ /KJ.mol ⁻¹
$w_1=0.00001$	298.15	18.05	-200.15	9.19	78.41	-1468.09	-1389.68
	303.15	18.08	-205.36	9.11	104.93	-1492.71	-1387.78
	308.15	18.11	-211.17	8.97	121.61	-1517.33	-1395.72
	310.15	18.13	-214.27	8.85	138.02	-1527.18	-1389.16
	313.15	18.14	-217.38	8.73	154.44	-1541.95	-1387.51
$w_1=0.00002$	298.15	18.05	-205.84	9.20	96.77	-416.00	-319.23
	303.15	18.08	-210.28	9.14	120.99	-520.14	-399.15
	308.15	18.11	-216.06	9.04	139.50	-599.70	-460.20
	310.15	18.12	-219.55	8.93	150.52	-647.09	-496.57
	313.15	18.14	-223.04	8.82	161.55	-694.49	-532.94
$w_1=0.00003$	298.15	18.05	-210.74	9.22	108.18	-603.12	-494.94
	303.15	18.08	-215.49	9.17	141.55	-789.16	-647.61
	308.15	18.11	-220.92	9.07	155.65	-867.75	-712.10
	310.15	18.12	-224.49	8.96	175.92	-980.76	-804.84
	313.15	18.14	-228.05	8.85	196.19	-1093.76	-897.57
σ	0.01	0.03	7.22	0.08	10.85	10.57	0.65

Table 10: Refractive index (n_D), molar refraction (R_M) and limiting molar refraction (R_M^ρ) citric acid in different mass fraction of aqueous uric acid solutions at 298.15 K and at pressure 1.013 bar *

a molality /mol·kg ⁻¹	n_D	$R_M \times 10^6$ / m ³ mol ⁻¹	$R_M^\rho \times 10^6$ / m ³ mol ⁻¹
$w_1=0.00001$			
0.0100	1.3319	43.22	
0.0252	1.3322	43.22	
0.0404	1.3327	43.23	43.19±0.03
0.0556	1.3332	43.24	
0.0709	1.3337	43.24	
0.0863	1.3343	43.25	
$w_1=0.00002$			
0.0100	1.3323	43.26	
0.0252	1.3326	43.27	
0.0404	1.3331	43.29	43.23±0.03
0.0556	1.3337	43.29	
0.0709	1.3342	43.30	
0.0863	1.3348	43.30	
$w_1=0.00003M$			
0.0100	1.3331	43.35	
0.0252	1.3335	43.37	
0.0404	1.3342	43.41	43.28±0.02
0.0556	1.3349	43.43	
0.0709	1.3355	43.45	
0.0863	1.3362	43.47	

*Standard uncertainties u are: $u(n_D) = 0.0002$ and $u(T) = 0.01K$ (0.68 level of confidence)

^amolality has been expressed per kilogram of (uric acid + water) solvent mixture

Figures:

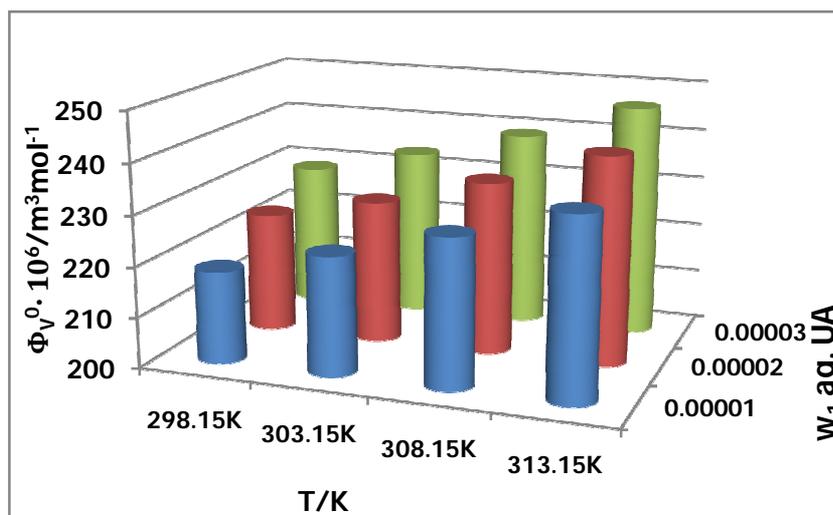


Fig 1: Plot of ϕ_V^ρ as a function of different mass fraction (w_1) of aqueous uric acid solutions at different temperature

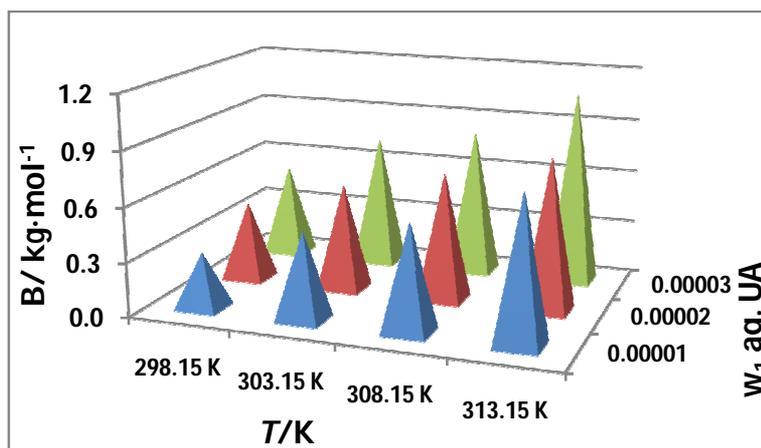


Fig 2: Plot of viscosity B -coefficient as a function of different mass fraction (w_1) of aqueous uric acid solutions at different temperature

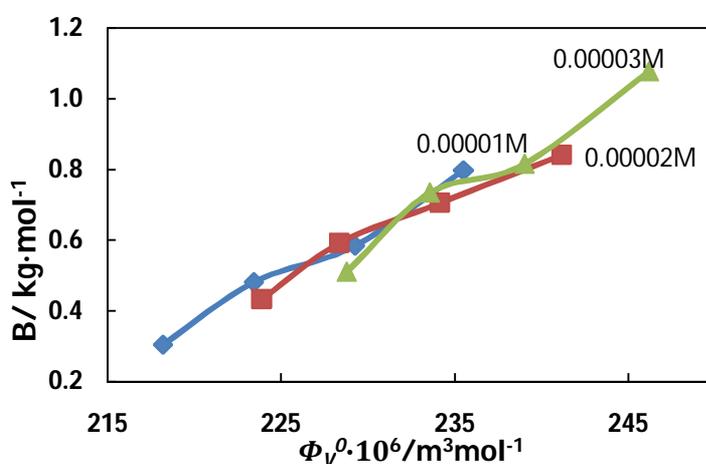


Fig 3: Plot of B against ϕ_v^0 of different mass fraction (w_1) of aqueous uric acid solutions at different temperature

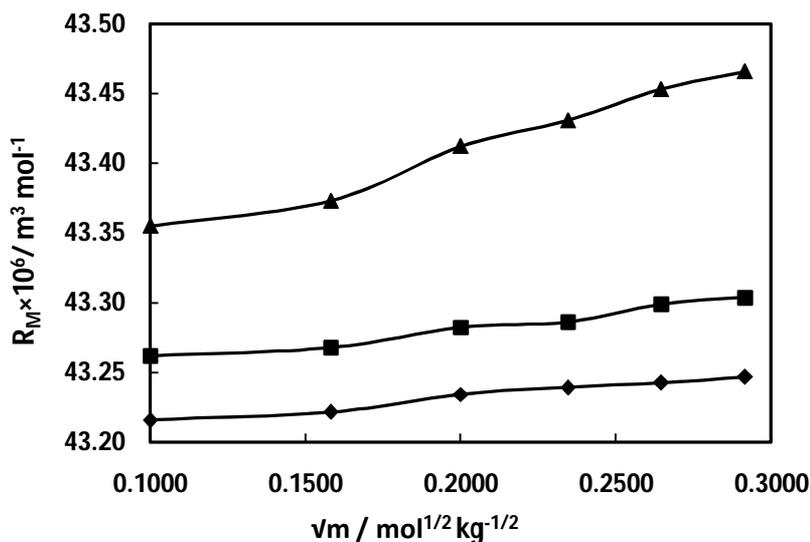
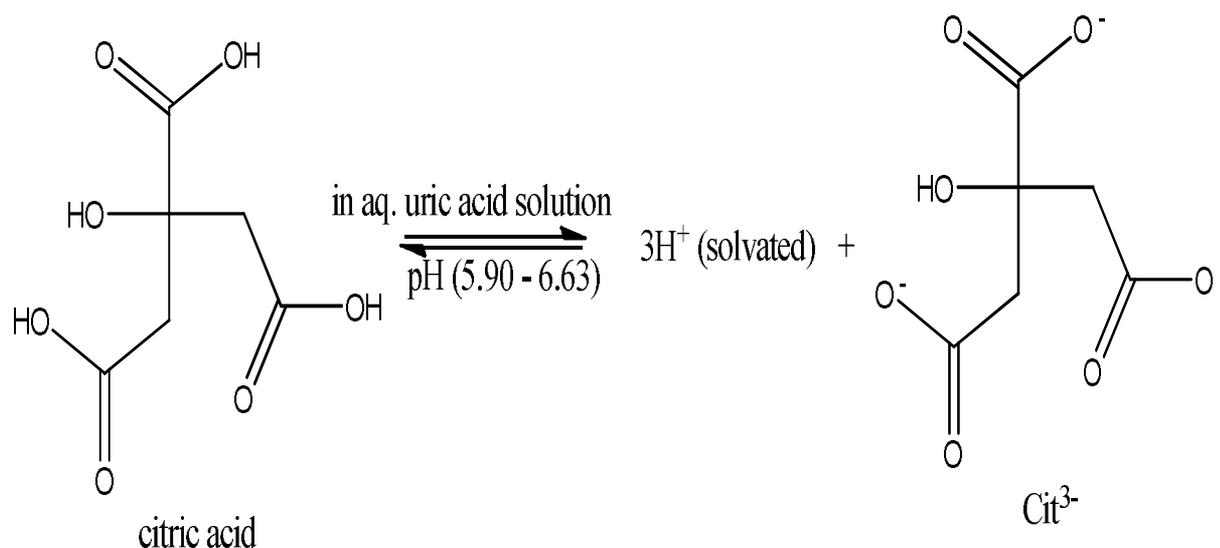
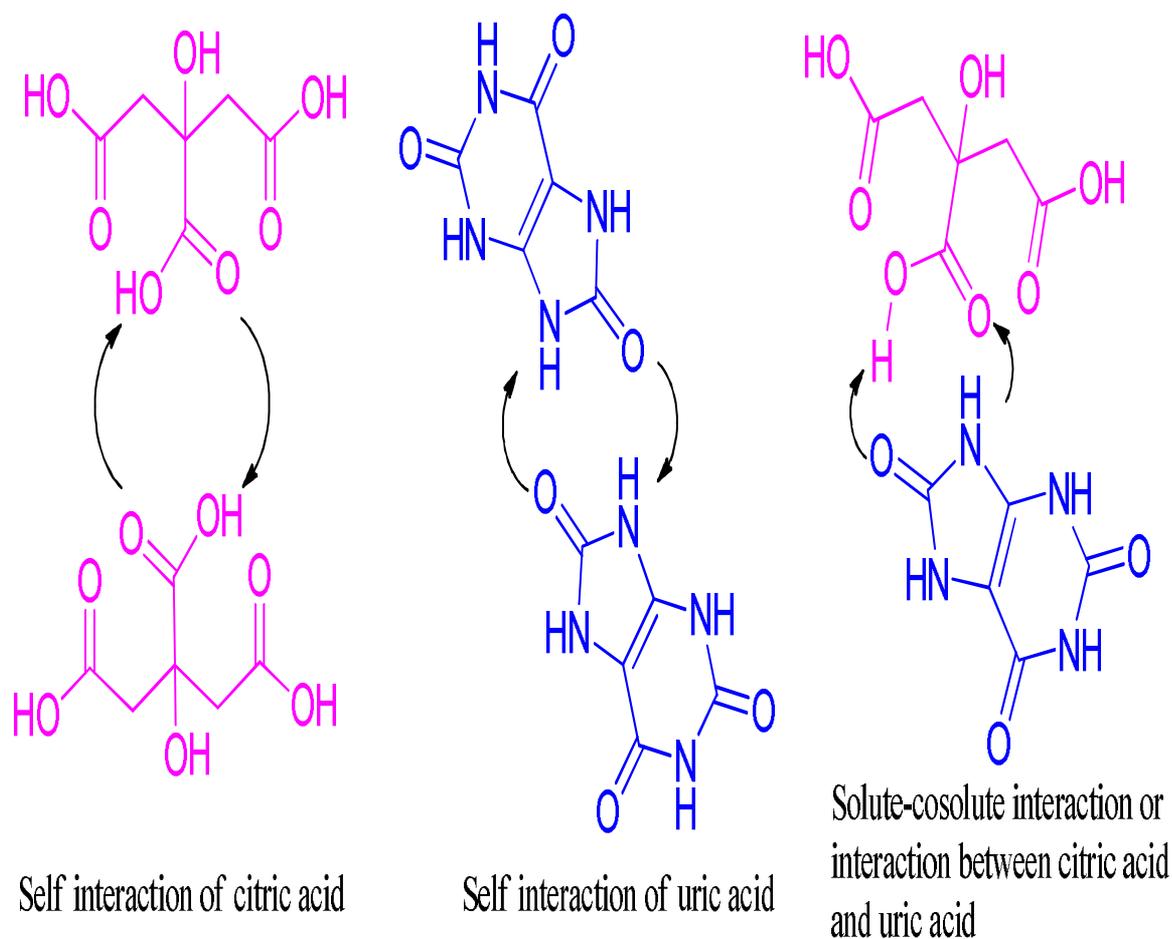


Fig 4: Plot of molar refractive index (R_M) against square root of concentration (\sqrt{m}) for citric acid in different mass fraction (w_1) at different temperature

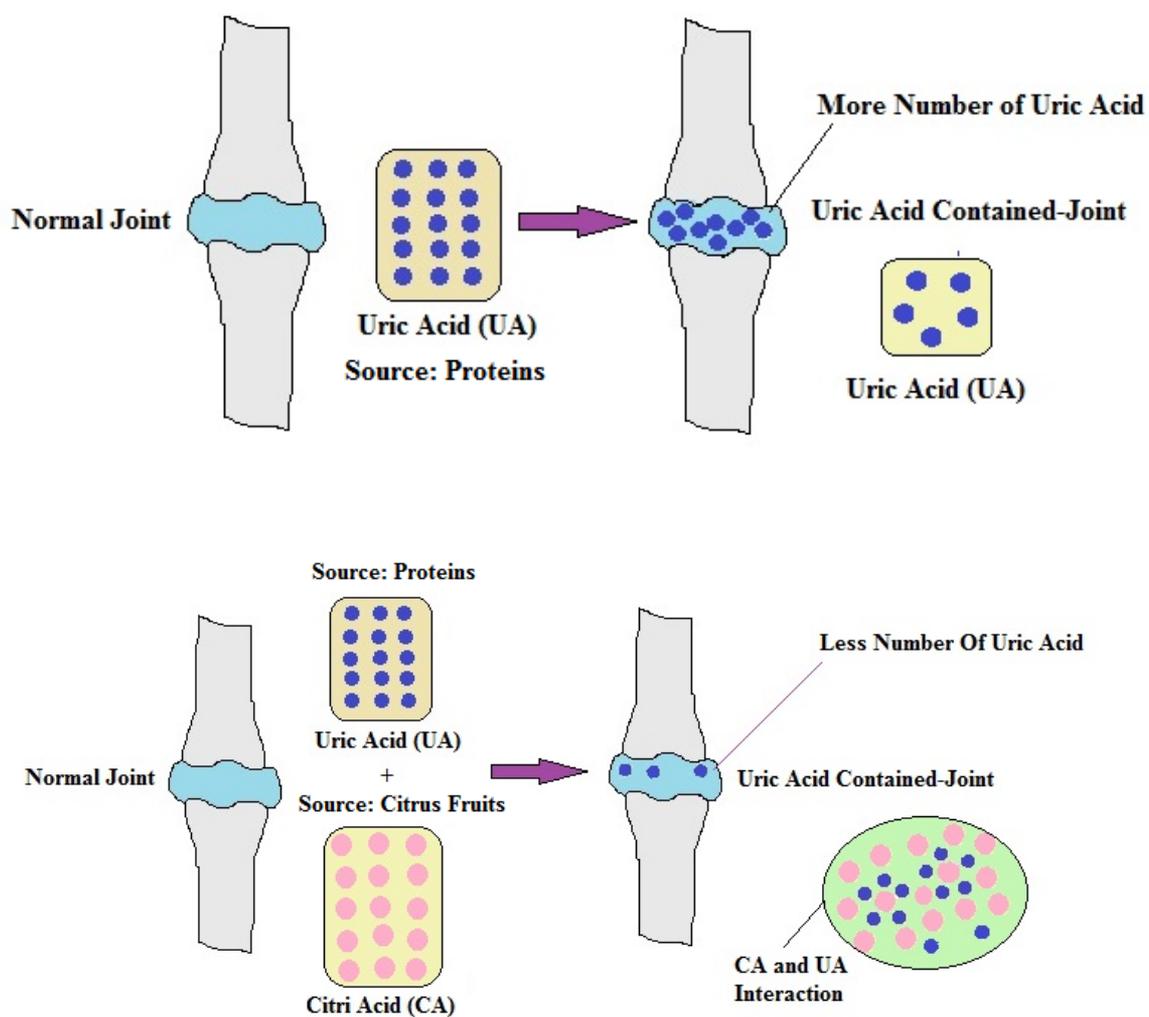
Schemes



Scheme 1. 3H⁺+ Cit³⁻ form of the citric acid in aqueous uric acid solutions.



Scheme 2. Plausible sites of interactions between solute-solute (citric acid-citric acid), cosolute-cosolute (uric acid-uric acid) and solute-cosolute molecules (citric acid-uric acid)



Scheme 3. Deposition of uric acid in joints in absence of citric acid (top) and in presence of citric acid (bottom)