CHAPTER IV

EXPLORATION OF INTERACTIONS BETWEEN BIOACTIVE SOLUTES AND VITAMIN B9 IN AQUEOUS MEDIUM BY PHYSICOCHEMICAL CONTRIVANCES

4.1 Introduction:

Folic acid (also known as folate, vitamin M, vitamin B9, vitamin B_c (or folacin), pteroyl-L-glutamic acid, pteroyl-L-glutamate, and pteroylmonoglutamic acid) are the water-soluble vitamin B9. Folic acid (FA) is composed of three components: an aromatic pteridine ring system (Pteridine), a *p*-amino benzoic acid (PABA) portion and the amino acid glutamic acid (Glu). The molecular structure of FA is shown in Scheme 1. It is an essential vitamin that is yellow-orange in color, is reported to be present in photosensitive organs, various mammalian metabolic pathways, and possibly involved in photosynthesis [1]. The electrochemical behavior of folic acid has been well studied [2]. Folic acid is itself not biologically active, but its biological importance is due to tetrahydrofolate and other derivatives after its conversion to dihydrofolic acid in the liver [3].

Vitamin B9 (folic acid and folate) is essential for numerous bodily functions. Humans cannot synthesize folate de novo; therefore, folate has to be supplied through the diet to meet their daily requirements. The human body needs folate to synthesize DNA, repair DNA, and methylate DNA as well as to act as a cofactor in certain biological reactions [4]. It is especially important in aiding rapid cell division and growth, such as in infancy and pregnancy, and reproduction of cells, particularly red blood cells. Children and adults both require folic acid to produce healthy red blood cells and prevent anemia [5].

The best natural sources of folic acid are: Leafy vegetables such as spinach, asparagus, turnip greens, lettuce, peas, whole grains, nuts; Legumes such as dried or fresh beans, peas and lentils egg yolk; liver, kidneys, yeast, sunflower seeds, certain fruits (orange juice, canned pineapple juice, cantaloupe, honeydew melon, grapefruit juice, banana, raspberry, grapefruit and strawberry). Folate is also necessary for the

production and maintenance of new cells, for DNA synthesis and RNA synthesis, and for preventing changes to DNA, and, thus, for preventing cancer [6].

The stabilization of native conformations of biological macromolecules is commonly related to several non-covalent interactions including hydrogen bonding, electrostatic and hydrophobic interactions [7]. These interactions are affected by the surrounding solutes and solvent molecules; for this reason, the physico-chemical behaviors of proteins are strongly influenced by the presence of solutes. However, due to the complex conformational and configurational three-dimensional structures of proteins, direct investigations of the solute-solvent effect on these biological macromolecules are very challenging. Amino acids are basic component of proteins and are considered to be one of the important model compounds of protein molecules, which participate in all the physiological processes of living cells are quite helpful in understanding the water–protein-folic acid interactions in solutions. Especially viscometric and volumetric properties (such as viscosity B-coefficients and standard partial molar volumes) and salts solutions can provide valuable clues for comprehending the protein unfolding [8,9] and the hydrophobic interactions of non-polar side chains [10]. In the present study, we have attempted to ascertain the nature of solute–solvent/cosolute interactions of α-amino acids (glycine, L-alanine, and L-valine) in w_1= 0.0001, 0.0003, 0.0005 mass fraction of aqueous folic acid (FA) binary mixtures at 298.15K, as literature survey reveals that very rare work has been carried out in the studied ternary systems.

4.2 Experimental Section:

4.2.1 Source and purity of samples

The studied salts (glycine, L-alanine, L-valine) and cosolute folic acid (FA), puriss grade was procured from Sigma-Aldrich, Germany and was used as purchased. The mass fraction purity of salts were ≥ 0.99. The salts were dried from moisture at 353K for 24 h, and then they were cooled and store in a desiccator prior to use.

4.2.2 Apparatus and Procedure

Aqueous binary solution of folic acid (FA) was prepared by mass (Mettler Toledo AG-285 with uncertainty ±0.0003g), which are used as solvent. Stock

solutions of the salts (amino acids) were also prepared by mass and the working solutions were obtained by mass dilution. The conversion of molality into molarity was accomplished using experimental density values. All solutions were prepared afresh before use. The uncertainty in molarity of the solutions is evaluated to ±0.0001 mol kg^{-3}.

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

The viscosities were measured using a Brookfield DV-III Ultra Programmable Rheometer with fitted spindle size-42. The viscosities were obtained using the following equation

$$\eta = (100 / \text{RPM}) \times \text{TK} \times \text{torque} \times \text{SMC} \quad (1)$$

where RPM, TK (0.09373) and SMC (0.327) are the speed, viscometer torque constant and spindle multiplier constant, respectively. The instrument was calibrated against the standard viscosity samples supplied with the instrument, water and aqueous $CaCl_2$ solutions [11]. Temperature of the solution was maintained to within ± 0.01°C using Brookfield Digital TC-500 temperature thermostat bath. The viscosities were measured with an accuracy of ± 0.1 %. Each measurement reported herein is an average of triplicate reading with a precision of 0.3 %.

Refractive index was measured with the help of a Digital Refractometer Mettler Toledo. The light source was LED, λ=589.3nm. The refractometer was calibrated twice using distilled water and calibration was checked after every few measurements. The uncertainty of refractive index measurement was ±0.0002 units.

The ultrasonic speed (u) was measured by multi frequency ultrasonic interferometer (Model M-81) from Mitral Enterprises, India. The interferometer working at 5 MHz is based on the same principle as was used by Freyer et al. [12] and Kiyoharo et al. [13]. The obtained speeds were corrected for diffraction errors as given by Subrahmayan et al. [14]. The uncertainty in the speed is ±0.2 m s^{-1}. The temperature was controlled within ±0.01 K using a Lauda thermostat during the measurement.

4.3 Result and Discussion:

4.3.1 Apparent molar volume

The salts are freely soluble in all proportions of the solvent mixtures. The physical properties of binary mixtures in different mass fractions (w_1=0.0001, 0.0003, 0.0005) of aqueous FA solutions at 298.15K are reported in Table 1. The measured experimental values of densities, viscosities, refractive indices, ultrasonic speeds of simple three amino acids in different mass fractions of aqueous FA mixture at 298.15K as a function of concentration (molarity) are listed in Table 2. Volumetric properties, such as,ϕ_V,ϕ_V^0, are regarded as sensitive tools for the understanding of interactions in solutions. The apparent molar volume can be considered to be the sum of the geometric volume of the solute molecule and changes in the solvent volume due to its interaction with the solute. For this purpose, the apparent molar volumesϕ_V was determined form the solutions densities using the following equation and the values are given in Table 3.

$$\phi_V = M/\rho \; - 1000(\rho - \rho_o)/m\rho\rho_o \qquad (2)$$

where M is the molar mass of the salt, m is the molarity of the solution, ρ and ρ_0 are the density of the solution and aq. FA mixture respectively.

Table 3 show that the value of ϕ_V are large and positive for all the systems, suggesting strong solute-solvent interactions. The apparent molar volumes ϕ_V were found to decrease with increasing molarity (m) of amino acid in aqueous FA for all the amino acids under study. It is also found that the value increases linearly with increase in size of the alkyl chain of the amino acid and with increase in the mass fraction (w_1) of FA in solution. It indicates that the solute–solvent (cosolute) interactions increase with increasing concentration (w_1) of FA, size of the alkyl side chain of amino acids. The limiting apparent molar volumes ϕ_V^0 were obtained by a least-square treatment to the plots of ϕ_V versus $\sqrt{m}$ using the Masson equation [15].

$$\phi_V = \phi_V^0 + S_V^* \cdot \sqrt{m} \qquad (3)$$

where ϕ_V^0 ($= \bar{V}_2^0$) is the apparent molar volume at infinite dilution and S_V^* is the experimental slope. The ϕ_V^0values has been determined by fitting the dilute data (m

< 0.1 mol·kg^{-1}) to eq 3. The values of ϕ_V^0 and S_V^* are reported in Table 4. The plots of ϕ_V against $\sqrt{m}$ were found to be linear with negative slopes. At infinite dilution, each monomer of solute is surrounded only by the solvent molecules, and being infinite distant with other ones. It follows, therefore, that ϕ_V^0 is unaffected by solute-solute interaction and it is a measure only of the solute-solvent interaction [16,17]. The ϕ_V^0 data are often embeded with important information of solute hydrophobic, hydration properties and solute–solvent interactions [18,19] occured in aqueous FA solution.

A perusal of Table 4 and Fig 1 shows that the values of ϕ_V^0 are large and positive for all the amino acids at the investigated temperature, suggesting the presence of strong solute–solvent interaction [20]. Furthermore, the values of ϕ_V^0 increase with increasing number of carbon atoms (or size of alkyl group) from Gly to Val. A similar increase in ϕ_V^0 with increasing number of carbon atoms for amino acids in aqueous glycerol, at 298.15 K, was also reported by Banipal et al [21]. The behaviour of ϕ_V^0 for the present systems can be explained employing the co-sphere model, proposed by Friedman and Krishnan [22], according to which the effect of overlap of hydration co spheres is destructive. Mishra et al. [23] using this model observed that an overlap of co spheres of two ionic species causes an increase in volume, whereas an overlap of hydrophobic–hydrophobic groups and ion–hydrophobic groups results in a net decrease in volume. Since, the observed positive ϕ_V^0 values (Table 4), is due to the effect of ion–hydrophilic interactions (between zwitterionic centres of the amino acids and the –OH, -NH_2, -NH, -COO^- or ketonic =O groups of FA) which predominate over ion–hydrophobic interactions (between zwitterionic centres and non-polar parts of FA) and hydrophobic–hydrophobic interactions (between non-polar parts of the amino acids and FA) and increase in the following order

glycine < L-alanine < L-valine

at investigated temperature. The increase ϕ_V^0 may be attributed to the release of some solvation molecules from the loose solvation layers of the solutes in solution.

The values of ϕ_V^0 and S_V^* for the amino acids in pure water is adopted from the literature [24,25]. The parameter S_V^* is the volumetric viral coefficient, and it characterizes the pair wise interaction of solute species in solution [26,27]. S_V^* is found to be negative under investigations, which suggest that the pair wise (solute-solute) interaction is restricted by the interaction of the charged functional group one molecule to side chain of the other amino acid molecules. From Table 4, a quantitative comparison between ϕ_V^0 and S_V^* values show that, the magnitude of ϕ_V^0 values is higher than S_V^*, suggesting that the solute-solvent (co solute) interaction dominate over the cosolute-cosolute interactions in all studied solution.

4.3.2 Contributions of the zwitterionic end group, CH_2 groups and other alkyl chains of the amino acids to ϕ_V^0

The ϕ_V^0 value for the homologous series varies linearly with the number of carbon atoms in the alkyl chain (R) of the amino acids. Similar correlations have been reported earlier by a number of Workers [24,25] and this linear variation can be represented as follows:

$$\phi_V^0 = \phi_V^0(NH_3^+, COO^-) + n_c\phi_V^0(CH_2) \quad (4)$$

where n_c is the number of carbon atoms in the alkyl chain of the amino acid, $\phi_V^0(NH_3^+, COO^-)$ and $\phi_V^0(CH_2)$ are the zwitterionic end group and methylene group contribution to ϕ_V^0 respectively. The values of $\phi_V^0(NH_3^+, COO^-)$ and $\phi_V^0(CH_2)$, calculated by a least-square regression analysis and was listed in Table 5, where those values in pure water are also provided from the literature. It is well described in the literature [28] that $\phi_V^0(CH_2)$ obtained by this scheme characterizes the mean contribution of the ϕ_V^0 (CH) and ϕ_V^0 (CH_3) values of the amino acids

$$\phi_V^0(CH) = 0.5\phi_V^0(CH_2) \quad (5)$$

$$\phi_V^0(CH_3) = 1.5\phi_V^0(CH_2) \quad (6)$$

and are listed in Table 5. The table show that the contribution of (NH_3^+, COO^-) to ϕ_V^0 is larger than that of the CH_2- group and increases with the increase in the mass

fraction (w_1) of the co solute FA, which indicates that the interactions between the co solute and charged end groups (NH_3^+, COO^-) of amino acids are much greater than those between the co solute and CH_2- group. Similar results were also reported [29] for some α-amino acids in aqueous sodium caprylate solutions.

4.3.3 Standard Transfer Volume

The standard transfer volume for the homologus series of amino acid, $\Delta\phi_V^0$, from pure water to aqueous FA solutions is defined by

$$\Delta\phi_V^0(\text{amino acid}) = \phi_V^0(\text{amino acis} + \text{aqueous FA}) - \phi_V^0(\text{water}) \quad (7)$$

The results are illustrated in Table 6. The value of $\Delta\phi_V^0$ is, by definition, free from solute-solute interactions and therefore provided information regarding solute-solvent interactions [20]. This agreement among the amino acids can be explained by the co-sphere model, as developed by Friedman and Krishnan [22], according to which the effect of overlap of the hydration co-spheres is constrctive. The overlap of hydration co-spheres of two ionic species results in an increase in volume, but that of hydration co-spheres of hydrophobic-hydrophobic groups and ion-hydrophobic groups results in a net volume decrease. Since amino acids exist predominantly as zwitterions in pure water and there is an overall decrease in volume of water due to electrostriction, the observed increasing positive volumes of transfer, indicate that in the ternary solutions (amino acid +aq. FA), indicates that the amino acids have the ion-hydrophilic and hydrophilic-hydrophilic group interactions predominate over the ion-hydrophobic and hydrophobic-hydrophobic groups interactions, and the contribution increases with increasing the molarity of FA in solutions. The observed trend can also be explained on the basis of the following equation [30, 31].

$$\phi_v^0 = \phi_{vw} + \phi_v - \phi_s \quad (8)$$

where ϕ_{vw} is the van der Waals volume; ϕ_v is the volume associated with voids or empty space; and ϕ_s is the shrinkage volume due to electrostriction. Assuming the ϕ_{vw} and ϕ_v have the same magnitudes in water and in aqueous FA solutions for the same class of solutes [32], the observed positive $\Delta\phi_V^0$ values ascribed to the decrease in the volume of shrinkage. Banipal and co-workers [21] also reported a

decrease in the $\Delta\phi_V^0$ value with increasing size of the non-polar side chain of amino acids in aqueous glycerol. The introduction of a CH_3- group in L-alanine provides an additional tendency for hydrophobic-hydrophilic and hydrophobic-hydrophobic group interactions, and as a result, greater electrostriction of water is produced leading to smaller changes of $\Delta\phi_V^0$. Similarly, when the H-atom of glycine is replaced by the (CH_3CH_2CH-) group in L-valine, the additional propensity for hydrophobic-hydrophilic group interactions increases further and thus leads to change in $\Delta\phi_V^0$ values. This is in good agreement with the conclusion drawn by Li et al. [33] in a study of Glycine, L-Alanine and L-Serine in glycerol-water mixture at 298.15 K.

The contribution of the other alkyl chain groups of the amino acids have been calculated from the difference between the limiting apparent molar volumes (ϕ_V^0) values of each amino acid and that of glycine using the following scheme

$$\Delta\phi_V^0(\mathrm{R}) = \phi_V^0(\text{amino acid}) - \phi_V^0(\text{glycine}) \quad (9)$$

where $\Delta\phi_V^0(\mathrm{R})$ defines the side chain transfer contribution to ϕ_V^0 of the respective amino acid relative to the H-atom of glycine. In this scheme, it is assumed that the volume contribution of the H-atom in glycine is negligible. The results are listed in Table 5. The table shows that the $\Delta\phi_V^0(\mathrm{R})$ values for L-alanine (CH_3CH-) and L-valine (CH_3CH_2CH-) is positive, which suggests the contribution of alkyl chain is greater than relative to the H-atom of glycine in solute-solvent interaction in solution.

4.3.4 Hydration Number estimated from apparent molar volume

The number of water molecules (n_H) hydrated to the amino acids can be estimated from the value of measured standard partial molar volume. The values of ϕ_V^0 of the studied amino acids can be expressed as [24]

$$\phi_V^0(\text{amino acid}) = \phi_V^0(\text{int}) + \phi_V^0(\text{elect}) \quad (10)$$

where $\phi_V^0(\text{int})$ is the intrinsic partial molar volumes of the amino acids and $\phi_V^0(\text{elect})$ is the electrostriction partial molar volume as a result of hydration of the amino acids. The $\phi_V^0(\text{int})$ consists of two terms: the van der Waals volume and the volume

due to packing effects. The values of ϕ_V^0(int) for the amino acids were calculated from their crystal molar volume by [24] using the following relationship,

$$\phi_V^0(\text{int}) = (0.7 / 0.634)\ \phi_V^0(\text{cryst}) \qquad (11)$$

where, 0.7 is the packing density in an organic crystal and 0.634 is the packing density of randomly packed spheres. The molar volume of crystals ϕ_V^0(cryst) was calculated using the crystal densities of the amino acids represented by Berlin and Pallansch (1968) [34,35] at 298.15 K. The ϕ_V^0(elect) values can be calculated [36] from the intrinsic partial molar volumes of the amino acids ϕ_V^0(int), and experimentally determined ϕ_V^0 values. Thus number of water molecules hydrated to the amino acids due to electrostriction causes decrease in volume can be related to the hydration numbers [24] is estimated using the following relation

$$n_H = \frac{\phi_V^0(\text{elect})}{(V_e^0 - V_b^0)} \qquad (12)$$

where V_e^0 is the molar volume of the electrostricted water and V_b^0 is the molar volume of bulk water. This model implies that for every water molecules taken from the bulk phase to the surroundings of amino acid, the volume is decreased by $(V_e^0 - V_b^0)$. The value of $(V_e^0 - V_b^0)$ is calculated [24] to be -3.0 or -3.3 $cm^3\ mol^{-1}$ at 298.15K respectively. The obtained n_H values are listed in Table 6, where n_H varies with the solvent composition, showing a tendency to decrease with an increase in the mass fraction (w_1) of FA, for all the amino acids under investigation. The observed decreasing tendency of n_H supports the view [37] that the FA has a dehydration effect on these amino acids in aqueous FA solutions.

The positive sign of the transfer volumes can be ascribed mainly to the fact that the hydration number n_H of the amino acids is reduced by the addition of FA; i.e., the electrostriction effect which brings about the shrinking in the volume of the solvent caused by the electric field of the dipolar solutes is reduced in the mixture as compared with that in pure water.

The schematic representation of solute-solvent interaction, for the studied amino acids in aqueous folic acids binary mixtures, in view of various derived parameters is depicted in Scheme 2.

4.3.5 Viscosity

The experimental viscosity data for the studied systems are listed in Table 2. The relative viscosity (η_r) has been analyzed using the Jones-Dole equation [38]

$$(\eta/\eta_o - 1)/\sqrt{m} = (\eta_r - 1)/\sqrt{m} = A + B\sqrt{m} \quad (13)$$

where $\eta_r=\eta/\eta_o$, η and η_0 are the relative viscosities, the viscosities of the ternary solutions (amino acid + aq. FA) and binary aqueous FA mixture and m is the molarity of the ternary solutions. A and B are empirical constants known as viscosity A- and B-coefficients, which are specify to solute-solute and solute-solvent interactions, respectively. The values of A and B-coefficients are estimated by least-square method by plotting $(\eta_r - 1)/\sqrt{m}$ against $\sqrt{m}$, and reported in Table 4. The values of the A-coefficient are found to increases slightly with the increase in mass of FA in the solvent mixture. These results indicate the presence of very weak solute-solute interactions. These results are in excellent agreement with those obtained from S_V^* values.

The extent of solute-solvent interaction in the solution estimated from the viscosity B-coefficient [16] gives valuable information concerning the solvation of the solvated solutes and their effects on the structure of the solvent in the local vicinity of the solute molecules in the solutions. From Table 4 and Fig 2, it is evidence that the values of the B-coefficient are positive and much higher than A-coefficient, thereby suggesting the solute-solvent interactions are dominant over the solute-solute interactions. The higher B-coefficient values for higher viscosity values is due to the solvated solutes molecule associated by the solvent molecules all round to the formation of associated molecule by solute-solvent interaction, would present greater resistance, and this type of interactions are strengthen with increase of mass fraction (w_1) of FA in the solvent mixtures. These results are in good agreement with those obtained from ϕ_V^0 values discussed earlier in apparent molar volume section.

The Table 4 also shows that B-coefficients for all the amino acids are increase with the increase of the size of the side chains. The B-coefficients reflect the net structural effects of the charged groups and the hydrophobic CH_2- groups of the amino acids. As B-coefficients vary linearly with the number of carbon atoms of the alkyl chain (n_c), these two effects can be resolved as follows

$$B = B(NH_3^+, COO^-) + n_c B(CH_2) \quad (14)$$

The regression parameters, i.e., the zwitterionic group contribution B (NH_3^+, COO^-), and the methylene group contribution B (CH_2), to B-coefficients are listed in Table 7. It shows that both the $B(NH_3^+, COO^-)$ and $B(CH_2)$ values increases with increasing concentration (w_1) of FA in ternary solutions, indicated that the zwitterionic and CH_2-group enhances the structure to solute-solvent interaction in the aqueous salt solutions. The side chain contributions to B-coefficients, $B(R)$, has also been derived using the same scheme as that of ϕ_V^0(R)and are listed in Table 7, which shows that $B(R)$ values are positive and greater for L-valine than L-alanine in all of the concentrations of studied solutions. This order is due to the greater structure making tendency and these findings are in line with our volumetric results discuss earlier.

Moreover, it is interesting to note that the B-coefficients of the studied amino acids show a linear correlation with the limiting partial molar volumes ϕ_V^0 for the amino acids in aqueous FA solution. This means:

$$B = A_1 + A_2\phi_V^0 \qquad (15)$$

The coefficients A_1 and A_2 are included in Table 8. This correlation is not unexpected, as both the viscosity B-coefficient and the partial molar volume reflect the solute–solvent interactions in the solutions. The positive slope (or A_2) shows the linear variation of B-coefficient with limiting apparent molar volumes ϕ_V^0. A similar correlation was also used for amino acids in different solvents [27,39].

4.3.6 *Refractive index*

The refractive index measurement is also a convenient method for investigating the ion-solvent interaction of salts in solution. The values of n_D are reported in Table 2. The molar refraction, R_M can be evaluated from the Lorentz-Lorenz relation [40]

$$R_M = \left\{(n_D^2-1)/(n_D^2+2)\right\}(M/\rho) \qquad (16)$$

where R_M, n_D, M and ρ are the molar refraction, the refractive index, the molar mass and the density of solution respectively. The refractive index of a substance is defined as the ratio c_o/c, where c is the speed of light in the medium and c_o the speed of light in vacuum. Stated more simply, the refractive index of a compound describes

its ability to refract light as it moves from one medium to another and thus, the higher the refractive index of a compound, the more the light is refracted [41]. As stated by Deetlefs et al. [42] the refractive index of a substance is higher when its molecules are more tightly packed or in general when the compound is denser. The refractive index is directly proportional to molecular polarizability. Hence, a perusal of Table 2 and 3 we found that the refractive index (n_D) and the molar refraction (R_M) respectively are increases linearly with an increasing concentration of the solution and homologues series of amino acids.

The Limiting molar refraction (R_M^0) estimated from the following

$$R_M = R_M^0 + R_S \sqrt{m} \tag{17}$$

Accordingly, we found that the higher refractive index, and R_M^0 (Table 4) values indicating the fact that the salts are more tightly packed and more solvated in solution. This is also in good agreement with the results obtained from density and viscosity parameters discussed above.

4.3.7 Ultrasonic Speed

Apparent molar isentropic compressibility:

The adiabatic compressibility, defined by the thermodynamic relation:

$$\beta_s = -\frac{1}{V}\left(\frac{\partial V}{\partial P}\right)_S \tag{18}$$

where *V* is volume, *P* is pressure and *S* is entropy, is related to the solution density ρ, and the ultrasonic speed *(u)*, by the Newton-Laplace's equation:

$$\beta_s = 1 / u^2 \rho \tag{19}$$

providing the relation between thermodynamics and acoustics. The apparent molar adiabatic compressibility (ϕ_K), of the solutions was determined from the following relation,

$$\phi_K = M\beta_s / \rho + 1000\left(\beta_s \rho_o - \beta_o \rho\right) / m\rho\rho_o \tag{20}$$

where β_0, β_s are the adiabatic compressibility of the binary mixture and ternary solution respectively and *m* is the molarity of the ternary solution. The values of ϕ_K

are reported in Table 3. Limiting apparent molar adiabatic compressibility (ϕ_K^0) or apparent molar adiabatic compressibility at infinite dilution and experimental slopes (S_K^*), were obtained by fitting ϕ_K against the square root of concentration ($\sqrt{m}$) using the least squares method [43].

$$\phi_K = \phi_K^0 + S_K^* \cdot \sqrt{m} \tag{21}$$

The values of ϕ_K^0 and S_K^* are presented in Table 4. The values of ϕ_K^0 and S_K^* are important parameter providing information about the extent of solute-solvent and solute-solute interaction respectively. The behaviour is useful in characteristic of solvation and electrostriction (the contraction of the solvent around the solute) of salt in solutions.

From Table 4 and Fig. 1, it is observed that the value of limiting apparent molar isentropic compressibility ϕ_K^0 are positive and increases with the increase in concentration (w_1) of FA for all the studied solution, and shows the stronger solute-solvent interaction. The result is good agreement with the ϕ_V^0 value discussed earlier. It is also observed that the values of ϕ_K^0 for the studied amino acids follow the order:

Glycine < L-alanine < L-valine

Since the contribution of methylene group to the apparent compressibility is positive, it implies that the ions having the larger hydrophobic group may have more positive values for the partial molar expansibilities. Hence, L-valine may have largest hydrophobic group resulting higher values of ϕ_K^0.

Hydration number from apparent molar isentropic compressibility:

The limiting partial molar adiabatic compressibility of the amino acids also can be expressed by a simple model: [24]

$$\phi_K^0 = \phi_K^0(\text{int}) + \phi_K^0(\text{elect}) \tag{22}$$

where $\phi_K^0(\text{int})$ is the intrinsic partial molar adiabatic compressibility of the amino acid and $\phi_K^0(\text{elect})$ is the electrostriction partial molar adiabatic compressibility due to the hydration of the amino acid. As has been noted by Millero et. al. as a first approximation, one can assume that $\phi_K^0(\text{int}) \approx 0$, since one would expect $\phi_K^0(\text{int})$ to very small [24]. Thus ϕ_K^0 may be thought to represent $\phi_K^0(\text{elect})$. The ϕ_K^0 values of the

amino acids in water are all positive; this must come from the hydration of the charged centres of the amino acids, as the hydrated water molecules are already compressed and than that in the bulk. For the amino acids, the order of increasing ϕ_K^0 values as well as hydration number n_H in aqueous FA is:

glycine < L-alanine < L-valine

and reported in Table 6. As has been noted by Mathieson and Conway, ions which a slight hydrogen-bond with water have unusual compressibility [44]. This corresponds to the order of increasing absolute values of ϕ_K^0 in aqueous FA, which answers to the order of increasing hydration numbers. Thus, the less hydrated amino acids in water has the lower compressibility ratio in the mixed solvent and then loses hydrated water molecules more easily in the transfer from water to the mixed solvent.

4.3.8 Structural effect of the Cosolute Folic Acid

The interaction strength depends on the factors such as the size of the guest molecule, the van der Waals interactions, the release of water molecules, hydrogen bonding, charge transfer interactions, hydrophobic interactions, and the release of conformational strain, etc [45]. With considering the above factors, FA are proposed in such a way that the interaction with amino acids, the solute-solvent interaction is higher for L-valine than L-alanine which is also turn higher than glycine, this is also due to the +I effect. +I effect increases as alkyl chain group increases from glycine to L-valine, are more favourably interact, with retention of configuration of FA itself.

4.4 Conclusion

Physico-chemical and thermodynamic properties of simple amino acids in aqueous FA binary mixture were done. It is evident that the association of the investigated amino acids, the L-valine is greater than L-alanine which is, in turn, greater than that glycine. The reliable values of derivative obtained from the studies properties suggest that the solute-solvent interaction is dominant over the solute-solute interaction in solutions. The structural effect of folic acid gives the favourable support in the molecular interaction. Above all this study demands a novelty of some amino acids prevailing in the aqueous solutions of folic acid.

Tables:

Table 1. Values of density (ρ), viscosity (η), refractive index (n_D) and ultrasonic speed (u) in different mass fraction (w_1) of aqueous Folic Acid at 298.15K

Mass fraction of	$\rho \cdot 10^{-3}$/kg m^{-3}		η /mPa s		n_D		u /m s^{-1}	
aq. β-CD (w_1)	Expt	Lit	Expt	Lit	Expt	Lit	Expt	Lit
$w_1 = 0.0001$	0.99708	-	0.812	-	1.3331	-	1493.5	-
$w_1 = 0.0003$	0.99717	-	0.823	-	1.3332	-	1495.3	-
$w_1 = 0.0005$	0.99726	-	0.840	-	1.3333	-	1497.2	-

Table 2. Experimental values of density (ρ), viscosity (η), refractive index (n_D) and ultrasonic speed (u) of amino acids in different mass fraction (w_1) of aqueous Folic Acid (FA) at 298.15K

M/mol kg^{-1}	$\rho \cdot 10^{-3}$/kg m^{-3}	η/mPa s	n_D	u/m s^{-1}
$w_1 = 0.0001$				
Glycine + aq. FA				
0.0100	0.99742	0.82	1.3333	1493.8
0.0251	0.99797	0.83	1.3336	1496.9
0.0402	0.99855	0.84	1.3339	1502.0
0.0553	0.99915	0.85	1.3342	1508.7
0.0704	0.99978	0.86	1.3345	1517.0
0.0855	1.00042	0.87	1.3348	1526.6
Alanine + aq. FA				
0.0100	0.99741	0.82	1.3334	1494.2
0.0251	0.99797	0.84	1.3337	1498.6
0.0402	0.99858	0.85	1.3340	1505.7
0.0553	0.99924	0.86	1.3343	1514.8
0.0704	0.99992	0.87	1.3346	1526.2
0.0856	1.00064	0.89	1.3349	1539.3

Valine + aq. FA				
0.0100	0.99740	0.83	1.3333	1494.7
0.0251	0.99797	0.84	1.3337	1500.9
0.0402	0.99859	0.86	1.3341	1510.6
0.0554	0.99926	0.87	1.3344	1523.2
0.0706	0.99998	0.89	1.3348	1538.1
0.0858	1.00074	0.90	1.3352	1555.3
$w_1 = 0.0003$				
Glycine + aq. FA				
0.0100	0.99750	0.84	1.3335	1495.7
0.0251	0.99803	0.85	1.3338	1499.1
0.0402	0.99859	0.86	1.3341	1504.8
0.0553	0.99918	0.87	1.3344	1512.1
0.0704	0.99979	0.88	1.3347	1521.3
0.0855	1.00041	0.89	1.3350	1532.2
Alanine + aq. FA				
0.0100	0.99748	0.84	1.3336	1496.1
0.0251	0.99801	0.85	1.3339	1500.9
0.0402	0.99858	0.87	1.3342	1508.7
0.0553	0.99919	0.88	1.3345	1518.8
0.0705	0.99982	0.89	1.3348	1530.9
0.0856	1.00050	0.91	1.3351	1545.6
Valine + aq. FA				
0.0100	0.99747	0.84	1.3337	1496.6
0.0251	0.99800	0.86	1.3340	1503.1
0.0402	0.99859	0.88	1.3343	1513.2
0.0554	0.99921	0.89	1.3346	1526.4
0.0706	0.99987	0.91	1.3349	1542.8
0.0858	1.00058	0.93	1.3352	1561.3
$w_1 = 0.0005$				
Glycine + aq. FA				
0.0100	0.99757	0.85	1.3336	1497.7

0.0251	0.99807	0.86	1.3339	1501.6
0.0402	0.99860	0.87	1.3342	1507.7
0.0553	0.99915	0.89	1.3345	1516.0
0.0704	0.99972	0.90	1.3348	1526.3
0.0855	1.00031	0.91	1.3351	1538.3
Alanine + aq. FA				
0.0100	0.99755	0.85	1.3338	1498.2
0.0251	0.99805	0.87	1.3341	1503.6
0.0402	0.99859	0.88	1.3344	1512.5
0.0553	0.99917	0.90	1.3347	1524.0
0.0705	0.99977	0.91	1.3350	1537.6
0.0856	1.00040	0.93	1.3352	1554.2
Valine + aq. FA				
0.0100	0.99754	0.86	1.3339	1498.6
0.0251	0.99802	0.88	1.3342	1505.9
0.0402	0.99856	0.90	1.3345	1517.5
0.0554	0.99913	0.91	1.3348	1532.0
0.0706	0.99975	0.93	1.3351	1549.6
0.0858	1.00039	0.95	1.3353	1569.9

Table 3. Molarity (m), apparent molar volume (ϕ_V), $(\eta_r-1)/\sqrt{m}$, molar refraction (R_m) and apparent molar adiabatic compressibility (ϕ_K) of amino acids in different mass fraction of aqueous FA (w_1) at 298.15K

m /mol·kg^{-1}	$\phi_V \cdot 10^6$ /m^3·mol^{-1}	$(\eta_r-1)/\sqrt{m}$ /kg$^{1/2}$·mol$^{-1/2}$	R_m	$\phi_K \cdot 10^{10}$ /m^3·mol^{-1}·Pa^{-1}
$w_1 = 0.0001$				
Glycine + aq. FA				
0.0100	41.19	0.074	15.49	-0.149
0.0251	39.59	0.124	15.50	-0.798
0.0402	38.43	0.166	15.50	-1.260
0.0553	37.54	0.194	15.51	-1.636
0.0704	36.61	0.218	15.51	-1.978

0.0855	35.88	0.240	15.51	-2.276
		Alanine + aq. FA		
0.0100	56.25	0.123	18.39	-0.317
0.0251	53.65	0.179	18.40	-1.141
0.0402	51.74	0.215	18.40	-1.748
0.0553	49.96	0.251	18.40	-2.230
0.0704	48.66	0.283	18.41	-2.678
0.0856	47.35	0.311	18.41	-3.063
		Valine + aq. FA		
0.0100	85.40	0.172	24.18	-0.482
0.0251	81.79	0.233	24.19	-1.561
0.0402	79.63	0.276	24.20	-2.339
0.0554	77.74	0.314	24.21	-2.978
0.0706	75.94	0.347	24.22	-3.503
0.0858	74.31	0.378	24.22	-3.960
		$w_1 = 0.0003$		
		Glycine + aq. FA		
0.0100	42.19	0.084	15.50	-0.200
0.0251	40.79	0.137	15.51	-0.882
0.0402	39.68	0.175	15.51	-1.394
0.0553	38.63	0.206	15.51	-1.792
0.0704	37.75	0.232	15.52	-2.167
0.0855	37.06	0.260	15.52	-2.510
		Alanine + aq. FA		
0.0100	58.25	0.133	18.40	-0.359
0.0251	55.65	0.191	18.41	-1.239
0.0402	53.99	0.229	18.41	-1.900
0.0553	52.51	0.262	18.42	-2.432
0.0705	51.38	0.296	18.42	-2.880
0.0856	50.06	0.330	18.42	-3.321
		Valine + aq. FA		
0.0100	87.40	0.193	24.20	-0.524

0.0251	84.19	0.252	24.21	-1.631
0.0402	81.88	0.295	24.22	-2.429
0.0554	80.29	0.334	24.22	-3.092
0.0706	78.80	0.373	24.23	-3.696
0.0858	77.25	0.408	24.23	-4.186
		$w_1 = 0.0005$		
		Glycine + aq. FA		
0.0100	44.19	0.107	15.50	-0.242
0.0251	42.79	0.158	15.51	-1.004
0.0402	41.68	0.196	15.51	-1.519
0.0553	40.82	0.233	15.52	-1.978
0.0704	40.04	0.260	15.52	-2.392
0.0855	39.30	0.285	15.53	-2.757
		Alanine + aq. FA		
0.0100	60.26	0.155	18.41	-0.460
0.0251	57.65	0.211	18.42	-1.408
0.0402	55.99	0.255	18.42	-2.153
0.0553	54.51	0.294	18.43	-2.748
0.0705	53.38	0.323	18.43	-3.234
0.0856	52.29	0.354	18.43	-3.715
		Valine + aq. FA		
0.0100	89.39	0.214	24.22	-0.564
0.0251	86.99	0.278	24.22	-1.814
0.0402	84.88	0.327	24.23	-2.742
0.0554	83.38	0.364	24.24	-3.434
0.0706	81.80	0.403	24.24	-4.039
0.0858	80.55	0.435	24.24	-4.558

Table 4. Limiting apparent molar volumes (ϕ_V^0), experimental slopes (S_V^*), viscosity A, *B*-coefficients, limiting partial molar adiabatic compressibilities (ϕ_K^0), and experimental slopes (S_K^*) of amino acids in different mass fraction of aqueous FA (w_1) at 298.15K

Mass fraction of aq. FA (w_1)	$\phi_V^0 \cdot 10^6$ /$m^3 \cdot mol^{-1}$	$S_V^* \cdot 10^6$ /$m^3 \cdot mol^{-3/2} \cdot kg^{1/2}$	*B* /$kg^{1/2} \cdot mol^{-1/2}$	*A* /$kg \cdot mol^{-1}$	R_m^0	$\phi_K^0 \cdot 10^{10}$ /$m^3 \cdot mol^{-1} \cdot Pa^{-1}$	$S_K^* \cdot 10^{10}$ /$m^3 \cdot mol^{-3/2} \cdot Pa^{-1} \cdot kg^{1/2}$
Glycine + aq. FA							
w_1 = 0.0001	43.96	-27.60	0.869	-0.012	15.48	0.955	-11.04
w_1 = 0.0003	48.99	-27.07	0.905	-0.006	15.44	1.009	-11.99
w_1 = 0.0005	46.78	-25.45	0.935	0.011	15.49	1.070	-13.05
Alanine + FA							
w_1 = 0.0001	60.96	-46.48	0.975	0.023	18.38	1.115	-14.27
w_1 = 0.0003	65.40	-41.99	1.047	0.029	18.49	1.284	-15.36
w_1 = 0.0005	64.28	-41.20	1.038	0.048	18.40	1.248	-16.95
Valine + aq. FA							
w_1 = 0.0001	90.99	-56.77	1.065	0.064	24.15	1.324	-18.09
w_1 = 0.0003	92.50	-52.02	1.189	0.177	24.19	1.316	-19.06
w_1 = 0.0005	94.16	-46.25	1.145	0.097	24.20	1.498	-20.78

Table 5. Contributions of zwitter ionic group (NH_3^+, COO^-), CH_2 group, and the other alkyl chains to the limiting apparent molar volume, ϕ_V^0, for amino acids in different mass fraction of aqueous FA (w_1) at 298.15K

	$\phi_V^0 \cdot 10^6$ /m³·mol⁻¹				$\Delta\phi_V^0(R) \cdot 10^6$ /m³·mol⁻¹		
w_1 / groups	0.0000	0.0001	0.0003	0.0005	0.0001	0.0003	0.0005
NH_3^+, COO^-	27.98	27.79	28.77	30.67	-	-	-
(CH)	7.61	8.09	8.11	8.06	-	-	-
Gly (CH_2)	15.22	16.17	16.22	16.11	-	-	-
(CH_3)	22.83	24.26	24.33	24.17	-	-	-
Ala (CH_3CH-)	32.51	33.17	33.63	33.61	17.00	17.41	17.50
Val (CH_3CH_2CH-)	63.00	63.20	63.73	63.49	47.03	47.51	47.38

Table 6. Values of $\Delta\phi_V^0$, ϕ_V^0(elect), ϕ_K^0(elect), and hydration number (n_H) for amino acids in different mass fraction of aqueous FA (w_1) at 298.15 K

Mass fraction of aq. FA (w_1)	$\Delta\phi_V^0 \cdot 10^6$ /m³·mol⁻¹	$\phi_V^0 \cdot 10^6$(elect) /m³·mol⁻¹	$\phi_K^0 \cdot 10^{10}$(elect) /m³·mol⁻¹·Pa⁻¹	n_H	
				From volume	From compressibility
	Glycine				
0.0001	0.76	-7.89	0.96	2.63	3.18
0.0003	1.79	-6.86	1.01	2.29	3.36
0.0005	3.58	-5.07	1.07	1.69	3.57
	Alanine				
0.0001	0.47	-10.79	1.12	3.60	3.72
0.0003	1.91	-9.35	1.18	3.12	3.95
0.0005	3.79	-7.47	1.25	2.49	4.16
	Valine				
0.0001	0.01	-11.10	1.31	3.70	4.37
0.0003	1.52	-9.59	1.39	3.20	4.62
0.0005	3.18	-7.93	1.48	2.64	4.93

Table 7. Contributions of zwitter ionic group (NH_3^+, COO^-), CH_2 group, and the other alkyl chains to the *B*-coefficient in different mass fraction of aqueous FA (w_1) at 298.15 K

B / $kg^{1/2}·mol^{-1/2}$				B(R) / $kg^{1/2}·mol^{-1/2}$		
w_1 / groups	0.0001	0.0003	0.0005	0.0001	0.0003	0.0005
NH_3^+, COO^-	0.852	0.957	1.044	-	-	-
(CH)	0.009	0.009	0.011	-	-	-
Gly (CH_2)	0.017	0.018	0.021	-	-	-
(CH_3)	0.026	0.027	0.031	-	-	-
Ala (CH_3CH-)	0.053	0.050	0.065	0.036	0.032	0.044
Val (CH_3CH_2CH-)	0.083	0.081	0.101	0.066	0.063	0.080

Table 8. Values of A_1, and A_2 coefficient for the amino acids in different mass fraction of aqueous FA at 298.15 K

Solute	A_1	A_2
Glycine	-0.122	0.022
Alanine	-0.172	0.018
Valine	-1.223	0.025

Figures:

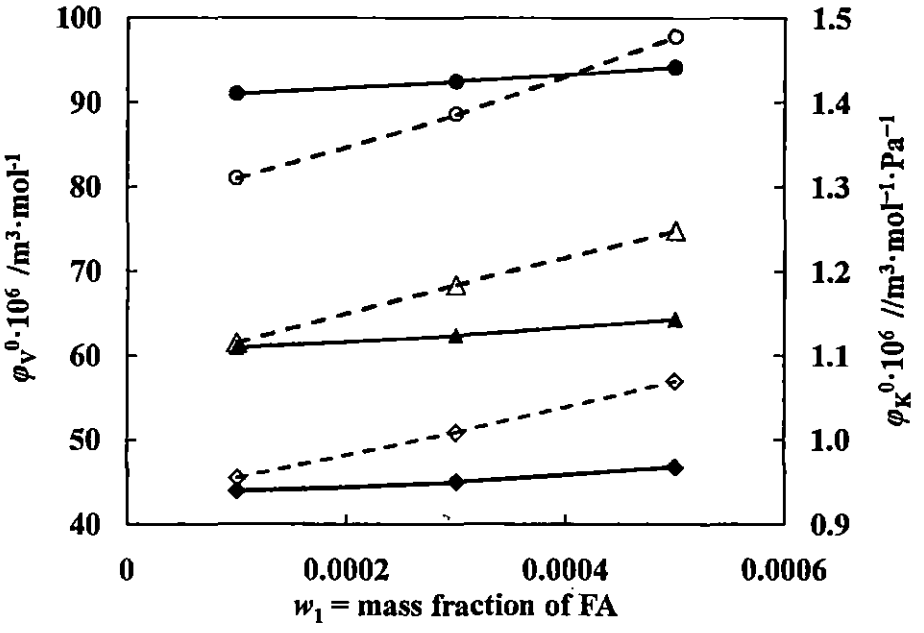


Figure 1. Plot of limiting apparent molar volume (ϕ_V^0) for glycine (♦), alanine (▲), valine (●), and limiting molar isentropic compressibility (ϕ_K^0) for glycine (◊), alanine (Δ), valine (○), against mass fraction of aq. FA (w_1) respectively

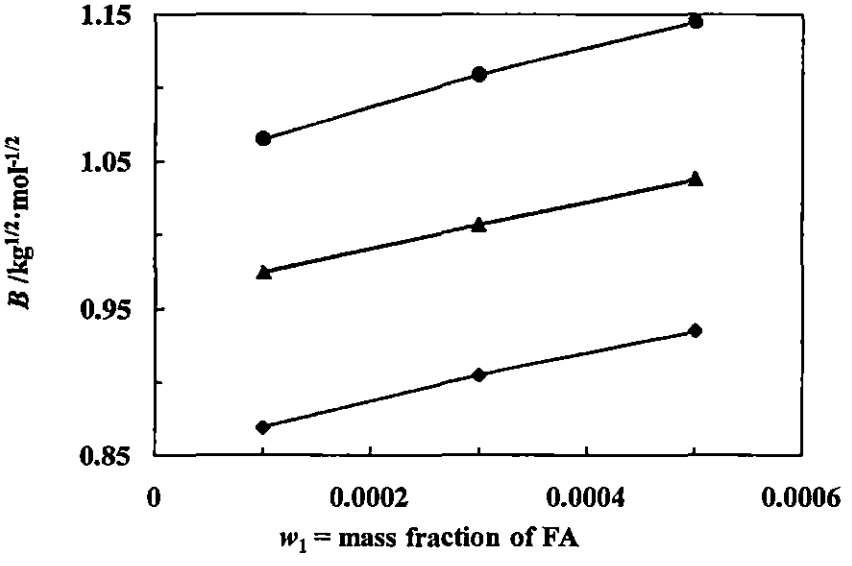
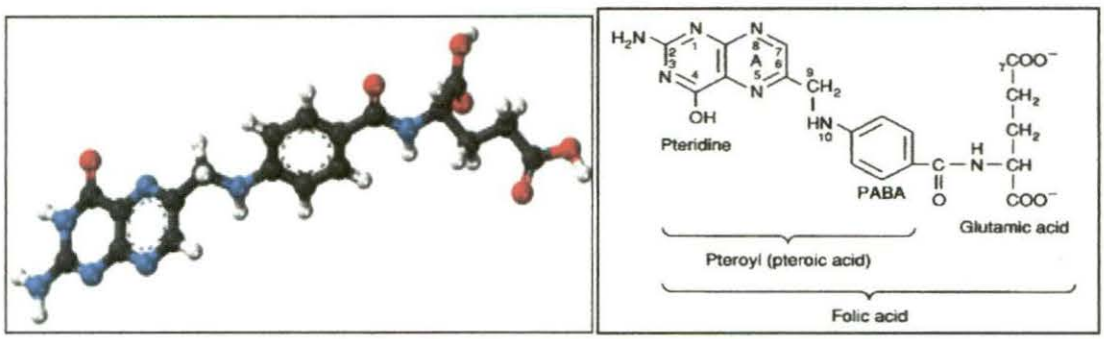
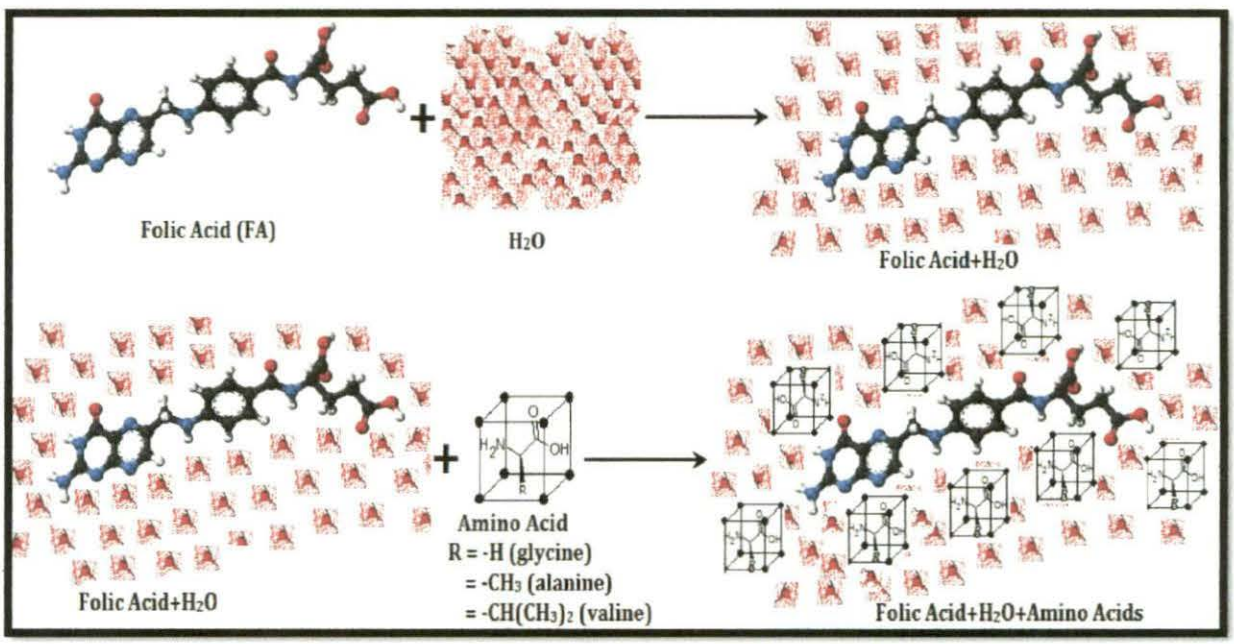


Figure 2. Plot of Viscosity *B*-coefficient for glycine (♦), alanine (▲) and valine (●) vs mass fraction of aq. FA (w_1)

Schemes:



Scheme 1: The molecular structure of Folic Acid



Scheme 2. The schematic representation of solute-solvent interaction, for the studied amino acids in aqueous folic acids binary mixtures