

Thermodynamic solvation of a series of homologous α -amino acids in non-aqueous mixture of ethylene-glycol and *N,N*-dimethyl formamide

Kalachand Mahali¹, Sanjay Roy², Bijoy Krishna Dolui^{1*}

¹Department of Chemistry, Visva-Bharati, Santiniketan, India; *Corresponding Author: bijoy_dolui@yahoo.co.in

²Department of Chemistry, Shibpur Dinabundhoo Institution (College), Howrah, India.

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ABSTRACT

Standard free energies ($\Delta G_i^0(i)$) and entropies ($\Delta S_i^0(i)$) of transfer of some homologous α -amino acids viz. glycine (gly), DL-alanine (ala), DL- α -amino butyric acid (aba) and DL-nor-valine (nor-val) from protic ethylene glycol (EG) to dipolar aprotic *N,N*-dimethyl formamide (DMF) have been evaluated from solubility measurements at five equidistant temperatures *i.e.* from 15°C to 35°C. The observed $\Delta G_i^0(i)$ and $T\Delta S_i^0(i)$ vs composition profiles are complicated because of the various interaction effects. The chemical effects of the transfer Gibbs energies ($\Delta G_{t,ch}^0(i)$) and entropies of transfer ($T\Delta S_{t,ch}^0(i)$) have been obtained after elimination of cavity effect, estimated by the scaled particle theory and dipole-dipole interaction effects, estimated by the use of Keesom-orientation expression. The chemical contributions of transfer energetics of homologous α -amino acids are guided by the composite effects of increased dispersion interaction, basicity and decreased acidity, hydrogen bonding effects and solvophobic solvation of ethylene glycol and *N,N*-dimethyl formamide mixed solvent as compared to that of reference solvent (ethylene glycol).

Keywords: Non-aqueous Solvent System; Transfer Energetic; Zwitterions; α -Amino Acids; Solvophobic Solvation

1. INTRODUCTION

It is well known that amino acids are fundamental structural units of proteins. The native state of a protein is determined by the nature and sequence of its constituent's amino acids as well as by the solvent environment.

Much attention had been paid [1-5] to determine the various thermodynamic properties of amino acids in aqua-organic mixed solvent system.

The purpose of such studies is to gain the various aspects of protein folding and unfolding processes and protein hydration [6,7]. In this regard Tanford, Nozaki and other authors [8,9] reported free energies of some amino acids from water to urea from solubility measurements. Transfer free energies and entropies data of some amino acids, dipeptides, tripeptides, and other biomolecules in aqueous ethylene glycol and glycerol are also available [10-13].

All these experiments tried to give an idea about the relative stabilization of those amino acids and other biomolecules in aqua-organic media with respect to water and the complex solute-solvent and solvent-solvent interactions therein.

In fact, the environment in which the different biological processes occur may be much more "amide like" than "water like". Therefore relevant data in amide solvents like *N,N*-dimethyl formamide in particular, are likely to be very much useful to understand biological processes better [14].

Also if we want to understand the role of the highly complex aqueous chemistry in the context of stabilizations of proteins and other biomolecules composed of amino acids and the involved structural "eccentricities" of water we have to first realize the chemistry of much similar non-aqueous solvents as a baseline "normal behavior", there by a better understanding of solute-solvent interactions will be possible in aqua-organic solvents.

With that end in view, in the present paper we are reporting the transfer free energies ($\Delta G_i^0(i)$) and entropies ($\Delta S_i^0(i)$) of a series of homologous α -amino acids, namely glycine (gly), DL-alanine (ala), DL- α -amino butyric acids (aba) and DL-Nor-valine (val) from ethylene glycol (EG) to non-aqueous mixture of protic ethylene-glycol and dipolar aprotic *N,N*-dimethyl formamide

(ethylene glycol and *N,N*-dimethyl formamide) at 25°C, as determined from solubility measurements using “formal titrimetry” at five equidistant temperatures ranging from 15°C - 35°C.

After eliminating effects due to cavity formation and dipole-dipole interactions and neglecting dipole-induced dipole interactions the results have been discussed in terms of dispersion interaction, acidity-basicity, solvophilic and solvophobic solvation and in the case of transfer entropies in terms of relative structuredness as well.

2. MATERIALS AND METHODS

2.1. Materials

α -amino acids like glycine (gly) (E Merck) and DL-alanine (ala), amino butyric acid (aba) and nor-valine (n-val) were used after drying as described earlier [15].

Ethylene glycol (LR, BDH) was purified by the usual method [16]. Ethylene glycol (LR, BDH) was refluxed with 2% - 3% NaOH (Merck) for 3 - 4 hours and then distilled; the distilled glycol was then dried over freshly baked anhydrous Na_2SO_4 (Merck) for 4 - 5 days, then decanted off and fractionally distilled through a 2/3 m long Vigreux column, rejecting the head and tail portions.

N,N-dimethyl formamide (DMF) (LR, BDH) was purified [16] first by distilling under reduced pressure in N_2 atmosphere and preserving the distillate over dry K_2CO_3 (Merck) for a week or so. The solvent was then decanted off and treated with pure P_2O_5 (Riedel) and finally distilled under reduced pressure.

The water content of the solvents were determined by Karl-Fisher titration and found to be less than 0.02-mol dm^{-3} in each case.

Non-aqueous mixtures of co-solvent (ethylene glycol and *N,N*-dimethyl formamide) that have been used were 20, 40, 60, 80 and 100 wt% and were protected by storing in desiccators when not in use.

2.2. Methods

The solubility of these four amino acids were measured by the formol titrimetric method as described in our previous paper [12,17]. These measurements were taken at 15°C, 20°C, 25°C, 30°C and 35°C temperatures. The low-cum-high temperature thermostat used for all measurements was capable of registering temperatures having an accuracy of $\pm 0.1^\circ\text{C}$. Three sets of measurements were made for all the solutes by equilibrating the solutions from both above and below the required temperatures and at least two sets of measurements were made for all the solvents and the solubilities were found to agree to within $\pm 1\%$ to 1.5%.

3. RESULTS

3.1. Computation of Total Transfer Free Energy and Entropy

The solvent parameters are listed in **Table 1**. The measured solubility (m) of the amino acids (on molal scale) is listed in **Table 2**.

As in the previous studies by Bates and coworkers on Tris [18] and by Kundu and coworkers [18,19] on non-electrolyte like para-nitroaniline, benzoic acid and amino acids [15], glycine (G), diglycine (DG), and triglycine (TG), the Gibbs energies of solutions (ΔG_s^0) of these amino acids on molal scale were calculated for each solvent using **Eq.1**.

$$\Delta G_s^0 = -RT \ln Cy = -RT \ln C = -RT \ln m \quad (1)$$

where y is the molar activity coefficient of the solutes but taken tentatively to be unity in each solvent. True, since these amino acids are likely to be mostly in zwitterionic forms as in non-aqueous solvent mixtures [20, 21], the involved activity coefficient factor $-RT \ln y$ in ΔG_s^0 arising from interactions of dipolar solutes with large dipole moments may not be that small. But as there is neither the required experimental data nor any appropriate theoretical correlations for computing the same, these have been tacitly taken to be negligibly small, as is usually done for non-electrolytes [11]. This is because the effective contribution of activity coefficient factor $-RT \ln y_s/y_R$ in the transfer free energetics $\Delta G_t^0(i) = \Delta G_s^0(i) - \Delta G_R^0(i)$ in particular which is our main concern likely to be hardly significant.

The free energies, ΔG_s^0 at different temperatures are fitted by the method of least squares to an equation of the form;

$$\Delta G_s^0 = a + bT + cT \ln T \quad (2)$$

where T is the temperature in Kelvin scale. The values of the coefficients a , b , c are presented in **Table 3**. These are found to reproduce the experimental data within $\pm 0.04 \text{ kJ}\cdot\text{mol}^{-1}$. Transfer Gibbs energies, ΔG_t^0 and entropies, ΔS_t^0 of the amino acids from ethylene-glycol to *N,N*-dimethylformamide mixtures were calculated at 25°C on mole fraction scale by using the following **Eqs.3 & 4**:

$$\Delta G_t^0(i) = {}_s\Delta G_s^0(i) - {}_R\Delta G_s^0(i)$$

i.e.

$$\Delta G_t^0(i) = (a_s - a_R) + (b_s - b_R)T + (c_s - c_R)T \ln T - RT \ln(M_s/M_R) \quad (3)$$

and

$$\Delta S_t^0(i) = (b_R - b_s) + (c_R - c_s)(1 + \ln T) + R \ln(M_s/M_R) \quad (4)$$

Table 1. Solvent parameters *i.e.* values of mean molecular weight (M_s), density (d_s), dielectric constant (ϵ_s) of the ethylene glycol and *N,N*-dimethyl formamide system at different temperatures.

wt% DMF	x_{DMF}	M_s (kg·m ⁻³)	$10^3 d_s$	ϵ_s
15°C				
0	0	62.07	1.1159	42.64
20	0.175	63.92	1.0815	41.92
40	0.362	65.98	1.0497	41.14
60	0.561	68.17	1.0152	40.32
80	0.744	70.51	0.9849	39.44
100	1.000	73.14	0.9500	37.64
20°C				
0	0	62.07	1.1150	41.67
20	0.175	63.92	1.0750	40.96
40	0.362	65.98	1.0450	40.20
60	0.561	68.17	1.0125	39.39
80	0.744	70.51	0.9740	37.50
100	1.000	73.14	0.9500	37.64
25°C				
0	0	62.07	1.1099 (1.1100)	40.70
20	0.175	63.92	1.0750	39.90
40	0.362	65.98	1.0410	39.10
60	0.561	68.17	1.0070	38.30
80	0.744	70.51	0.9740	37.50
100	1.000	73.14	0.9443 (0.9440)	36.70
30°C				
0	0	62.07	1.1050	39.73
20	0.175	63.92	1.0700	39.04
40	0.362	65.98	1.0350	38.32
60	0.561	68.17	1.0050	37.53
80	0.744	70.51	0.9750	36.69
100	1.000	73.14	0.9400	35.01
35°C				
0	0	62.07	1.1015	38.76
20	0.175	63.92	1.0647	38.08
40	0.362	65.98	1.0332	37.38
60	0.561	68.17	1.0001	36.60
80	0.744	70.51	0.9685	35.78
100	1.000	73.14	0.9343	34.92

Values in parenthesis were taken from ref. [16].

here the subscript “*s*” and “*R*” refer to the co-solvent (ethylene glycol and *N,N*-dimethyl formamide) mixtures and reference solvent (ethylene glycol) respectively and M is the molar mass of the pure and mixed solvent. $\Delta G_t^0(i)$ and $T\Delta S_t^0(i)$ values of amino acids thus obtained and presented in the **Table 3**. The estimated values shows an uncertainties in $\Delta G_t^0(i)$ and $\Delta S_t^0(i)$ are about ± 0.05 kJ·mol⁻¹ and 2 kJ⁻¹·mol⁻¹, respectively.

3.2. Computation of Chemical Part of Transfer Free Energy and Entropy

Now $\Delta X_t^0(i)$ (where $X = G$ or S) may be ascribed as the sum of the following terms (assuming dipole induced dipole term to be negligibly small). *i.e.*

$$\Delta X_t^0(i) = \Delta X_{t,cav}^0(i) + \Delta X_{t,dd}^0(i) + \Delta X_{t,ch}^0(i) \quad (5)$$

here, $\Delta X_{t,cav}^0(i)$ means for the transfer energy contribution of the cavity effect which is involved due to creation of cavities for the species in ethylene glycol and ethylene glycol and *N,N*-dimethyl formamide mixed solvent system and $\Delta X_{t,dd}^0(i)$ stands for the dipole-dipole interaction effect involving interaction between dipolar-zwitter-ionic amino acids and the solvent molecules, on the other hand, $\Delta X_{t,ch}^0(i)$ includes that for all other effects such as those arising from acid-base or short-range dispersion interaction, solvophilic or solvophobic solvation and structural effects etc. Here $\Delta X_{t,cav}^0(i)$ values were computed by using Scaled particle theory (SPT) [17], assuming the solutes and solvent molecules as equivalent to hard-sphere models as dictated by their respective diameter (Vide **Table 4**).

$$\Delta G_{t,dd}^0(i) = \left({}_s \Delta G_{dd}^0(i) - {}_R \Delta G_{dd}^0(i) \right)$$

and

$$\Delta S_{t,dd}^0(i) = \left({}_s \Delta S_{dd}^0(i) - {}_R \Delta S_{dd}^0(i) \right)$$

were calculated by means of the Keesom-orientation expression [22] for ${}_s \Delta G_{dd}^0(i)$ in a solvent S , as given below

$${}_s \Delta G_{dd}^0(i) = -(8\pi/9) N^2 \mu_s^2 \mu_x^2 \sigma_{sx}^{-3} (kT)^{-1} v_s^{-1} = A/TV_s \quad (6)$$

where

$$A = -(8\pi/9) N^2 \mu_s^2 \mu_x^2 \sigma_{sx}^{-3} (k)^{-1}$$

and

$$V_s = M_s / d_s$$

and that of $\Delta S_{t,dd}^0(i)$ as follows:

$${}_s \Delta S_{t,dd}^0(i) = \left\{ \delta {}_s \Delta G_{dd}^0(i) / \delta T \right\}_p \quad (7)$$

i.e. $T {}_s \Delta S_{t,dd}^0(i) = {}_s \Delta G_{dd}^0(i) [1 + T\alpha]$, where N stands for Avogadro's number, μ_s, μ_x are the dipole moment of

Table 2. Solubilities (m) of Glycine, DL-alanine, DL- α -amino butyric acid and DL-nor-valine in binary mixtures of ethylene glycol and *N,N*-dimethyl formamide at different temperatures.

wt% solvent	Glycine					DL-alanine				
	15°	20°	25°	30°	35°	15°	20°	25°	30°	35°
100%EG	0.143	0.16	0.176	0.184	0.190	0.130	0.145	0.150	0.159	0.162
	(0.143) ^a		(0.170) ^a		(0.186) ^a	(0.126) ^a		(0.140) ^a		(0.160) ^a
20%DMF	0.098	0.11	0.130	0.140	0.146	0.097	0.105	0.113	0.120	0.128
40%DMF	0.069	0.077	0.084	0.093	0.101	0.078	0.084	0.090	0.096	0.102
60%DMF	0.064	0.074	0.08	0.087	0.094	0.0603	0.064	0.0694	0.0738	0.078
80%DMF	0.061	0.069	0.078	0.083	0.09	0.049	0.054	0.059	0.064	0.069
100%DMF	0.038	0.042	0.046	0.049	0.052	0.041	0.044	0.048	0.0518	0.055
wt% solvent	DL-amino butyric acid					DL-nor-valine				
	15°	20°	25°	30°	35°	15°	20°	25°	30°	35°
100%EG	0.200	0.212	0.224	0.240	0.254	0.118	0.128	0.139	0.156	0.160
	(0.190) ^a		(0.220) ^a		(0.255) ^a	(0.120) ^a		(0.130) ^a		(0.138) ^a
20%DMF	0.145	0.155	0.169	0.180	0.189	0.094	0.105	0.118	0.128	0.139
40%DMF	0.102	0.110	0.120	0.126	0.1345	0.068	0.074	0.083	0.091	0.099
60%DMF	0.079	0.086	0.094	0.099	0.104	0.039	0.048	0.052	0.057	0.062
80%DMF	0.040	0.044	0.049	0.055	0.061	0.026	0.030	0.035	0.041	0.046
100%DMF	0.036	0.040	0.045	0.050	0.056	0.022	0.026	0.032	0.036	0.042

^aRef. [17].

solvents and amino acid molecules respectively (**Table 4**).

σ_{sx} is the distance at which the attractive and repulsive interactions between the solvent and solute molecules are equal and is generally equal to $1/2(\sigma_s + \sigma_x)$ where σ_s and σ_x are the hard sphere diameter of solvent and solute molecules respectively (**Table 4**) and α is the isothermal expansibility of the solvent and given by $(\delta \ln V_s / \delta T)_p = -(\delta \ln d_s / \delta T)$. Following Marcus [22] and Kim *et al.* [23] in order to get these $\Delta X_{t,dd}^0(i)$ term on mole fraction scale the quantity was again multiplied by the term X_{s1} .

$$X_{s1} = X_s (\mu_s / \sigma_s^3) / (\mu_R / \sigma_R^3) \quad (8)$$

which is the real mole fraction contribution due to dipole-dipole interaction [22]. Subtraction of $\Delta X_{t,cav}^0(i)$ and $\Delta X_{t,dd}^0(i)$ from the total we can get $\Delta X_{t,ch}^0(i)$ of amino acids. The values of $\Delta X_{t,cav}^0(i)$, $\Delta X_{t,dd}^0(i)$ and $\Delta X_{t,ch}^0(i)$ are presented in **Table 4**.

4. DISCUSSION

4.1. Type of Interactions of Amino Acids with Solvent Mixture

The solubilities data reveals that the solubility of amino

acids increases with the increase in temperature. DL- α -amino butyric acid is somewhat more soluble in the ethylene glycol and *N,N*-dimethyl formamide solvent mixtures than DL-alanine, which is contrary to the prediction based on the hydrophobic nature of these two compounds. This may be due to the relative law crystal lattice energy of DL- α -amino butyric acid.

Figure 1 presents the variation of $\Delta G_t^0(i)$ of amino acids with mole% *N,N*-dimethyl formamide in ethylene glycol and *N,N*-dimethyl formamide mixtures. $\Delta G_t^0(i)$ Values of four amino acids (*i.e.* Glycine, DL-alanine, DL- α -amino butyric acid and DL-nor-valine) indicate their more or less gradual destabilizations with gradual increased concentration of *N,N*-dimethyl formamide in ethylene glycol and *N,N*-dimethyl formamide mixtures. As $\Delta G_t^0(i)$ is composed of $\Delta G_{t,cav}^0(i)$, $\Delta G_{t,dd}^0(i)$ and $\Delta G_{t,ch}^0(i)$ or others so their collective contribution to $\Delta G_t^0(i)$ show such little complex nature of variation with mole% of *N,N*-dimethyl formamide.

The upward trends of $\Delta G_{t,ch}^0(i)$ profiles of all amino acids (**Figure 2**) indicate their relative destabilization with increased concentration of *N,N*-dimethyl formamide. The order of stability with respect to chemical contribution of solute-solvent interaction is DL-val. > DL-Aba. > DL-Ala. > Gly.

Table 3. Coefficients a, b and c in Glycine, DL-alanine, DL- α -amino butyric acid and DL-nor-valine and Gibbs energies ΔG_i^0 and entropies $T\Delta S_i^0$ of transfer of the acids (on mole fraction scale) in $\text{kJ}\cdot\text{mol}^{-1}$ from Ethylene Glycol to Ethylene Glycol and N,N -dimethyl formamide mixtures at 25°C.

Solvents	a ($\text{kJ}\cdot\text{mol}^{-1}$)	b ($\text{kJ}\cdot\text{mol}^{-1}\cdot\text{K}^{-1}$)	c ($\text{kJ}\cdot\text{mol}^{-1}\cdot\text{K}^{-1}$)	ΔG_i^0 ($\text{kJ}\cdot\text{mol}^{-1}$)	$T\Delta S_i^0$ ($\text{kJ}\cdot\text{mol}^{-1}$)
Glycine					
100%EG	255.67	-5.5316	0.82289	0	0
20%DMF	290.02	-6.2071	0.92171	0.746	4.141
40%DMF	35.87	-0.5172	0.07326	1.667	2.036
60%DMF	147.78	-3.0374	0.44978	1.707	1.646
80%DMF	167.03	-3.4608	0.51282	1.725	2.082
100%DMF	124.76	-2.5575	0.37993	2.938	-1.779
DL-alanine					
100EG	101.00	-2.1190	0.31519	0	0
20%DMF	32.66	-0.5185	0.07496	0.688	2.596
40%DMF	36.01	-0.6055	0.08859	1.174	1.396
60%DMF	26.46	-0.3869	0.05622	1.731	0.941
80%DMF	51.20	-0.8859	0.12948	2.060	3.509
100%DMF	20.34	-0.2177	0.03067	2.481	1.689
DL-α-aminobutyric acid					
100%EG	-36.37	0.9983	-0.15163	0	0
20%DMF	64.87	-1.2511	0.18400	0.656	0.516
40%DMF	63.49	-1.2145	0.17889	1.429	-0.114
60%DMF	184.26	-3.9182	0.58267	1.927	-0.217
80%DMF	-36.58	1.1475	-0.17548	3.443	3.458
100%DMF	-10.72	0.5762	-0.0903	3.578	3.787
DL-nor-valine					
100%EG	-100.62	2.5476	-0.38504	0	0
20%DMF	100.79	-1.970	0.28963	0.383	-0.126
40%DMF	15.67	-0.0609	0.00511	1.177	-1.210
60%DMF	239.59	-5.0502	0.74963	2.201	-0.293
80%DMF	27.54	-0.1811	0.02045	3.103	4.160
100%DMF	96.06	-1.6717	0.24193	3.787	6.402

The size of N,N -dimethyl formamide (0.498 Å) is greater than ethylene glycol (0.437 Å). Thus free energy change due to cavity formation is also more negative in N,N -dimethyl formamide relative to ethylene glycol. $\Delta G_{i,cav}^0(i)$ is more negative for the higher homologue among four amino acids having larger hard-sphere di-

ameter (**Table 4**). On the other hand the dipole moment of N,N -dimethyl formamide (3.82D) is also greater than ethylene glycol (2.28D). Therefore, $\Delta G_{i,dd}^0(i)$ values are more negative in higher concentration of N,N -dimethyl formamide in this ethylene glycol and N,N -dimethyl formamide mixed solvent system. The order of $\Delta G_{i,dd}^0(i)$ is

Table 4. Gibbs energies of transfer $\Delta G_t^0(i)$, $\Delta G_{t, cav}^0(i)$, $\Delta G_{t, dd}^0(i)$, $\Delta G_{t, ch}^0(i)$ and enthalpy of transfer, $\Delta H_{t, cav}^0(i)$ and entropies of transfer $T\Delta S_t^0(i)$, $T\Delta S_{t, cav}^0(i)$, $T\Delta S_{t, dd}^0(i)$ and $T\Delta S_{t, ch}^0(i)$ of Glycine, DI-alanine, DI- α -amino-butyric acid and DI-nor-valine from Ethylene Glycol to Ethylene Glycol and *N,N*-dimethyl formamide mixtures at 25°C (on mole fraction scale in kJ·mol⁻¹).

Solvents	$\Delta G_t^0(i)$	$\Delta G_{t, cav}^0(i)$	$\Delta G_{t, dd}^0(i)$	$\Delta G_{t, ch}^0(i)$	$T\Delta S_t^0(i)$	$\Delta H_{t, cav}^0(i)$	$T\Delta S_{t, cav}^0(i)$	$T\Delta S_{t, dd}^0(i)$	$T\Delta S_{t, ch}^0(i)$
Glycine									
100%EG	0	0	0	0	0	0	0	0	0
20%DMF	0.746	-0.495	-1.13	2.371	4.141	-0.324	0.171	-1.53	5.50
40%DMF	1.667	-0.925	-4.95	7.542	2.036	-0.112	0.813	-6.80	8.023
60%DMF	1.707	-1.291	-11.60	14.598	1.646	0.754	2.045	-16.30	15.901
80%DMF	1.725	-1.578	-21.80	25.103	2.082	2.597	4.175	-31.30	29.207
100%DMF	2.938	-1.762	-35.50	40.20	-1.779	6.062	7.824	-52.10	42.522
Alanine									
100%EG	0	0	0	0	0	0	0	0	0
20%DMF	0.688	-0.523	-1.01	2.221	2.596	-0.379	0.144	-1.36	3.812
40%DMF	1.174	-0.969	-4.42	6.563	1.396	-0.140	0.829	-6.07	6.637
60%DMF	1.731	-1.343	-10.30	13.363	0.746	0.851	2.194	-14.40	12.952
80%DMF	2.060	-1.627	-19.60	23.287	3.503	2.966	4.593	-28.01	26.92
100%DMF	2.481	-1.794	-31.90	36.135	1.106	6.944	8.738	-46.80	39.751
Amino-butyric acid									
100%EG	0	0	0	0	0	0	0	0	0
20%DMF	0.656	-0.546	-0.937	2.139	0.516	-0.427	0.119	-1.26	1.657
40%DMF	1.429	-1.008	-4.01	6.447	-0.114	-0.165	0.843	-5.51	4.533
60%DMF	1.927	-1.387	-9.44	12.754	-0.217	0.934	2.321	-13.2	10.662
80%DMF	3.443	-1.668	-18.0	23.111	3.458	3.282	4.950	-25.7	24.208
100%DMF	3.578	-1.821	-29.30	34.699	3.787	7.700	9.521	-42.80	37.066
Nor-valine									
100%EG	0	0	0	0	0	0	0	0	0
20%DMF	0.383	-0.566	-0.860	1.809	-0.126	0.680	1.246	-1.16	0.212
40%DMF	1.177	-1.040	-3.69	5.907	-1.210	0.961	2.001	-5.06	1.849
60%DMF	2.201	-1.424	-8.69	12.332	-0.293	2.150	3.574	-12.20	8.333
80%DMF	3.103	-1.703	-16.50	20.745	4.160	4.696	6.399	-23.60	21.361
100%DMF	3.347	-1.845	-27.00	31.89	6.402	9.487	11.332	-39.50	34.57

The required diameter and other solvent parameters of ethylene glycol and *N,N*-dimethyl formamide mixtures are taken from Ref. [22]. The required diameter of glycine, alanine, amino butyric acid and nor-valine are 5.64, 6.16, 6.58 and 6.92 Å, respectively, as given in Ref. [24]. Dipole-moment values of α -amino acids are 15.7D for glycine, 15.9D for alanine and 16D for amino butyric acid and nor-valine [25].

Gly. > DI-Ala. > DI-Aba. > DI-nor-val. As $\Delta G_{t, dd}^0(i)$ values are guided by dipole moment and hard-sphere diameter of both solutes (here amino acid) as well as solvent, the above order is well supported from **Table 4**.

$\Delta G_{t, ch}^0(i)$ values of the four amino acid represent the

free energy change in the ethylene glycol and *N,N*-dimethyl formamide mixed solvent system due to different short range chemical interactions *i.e.* acid-base, dispersion, hard-soft, H-bonding, solvophilic/solvophobic interaction etc.

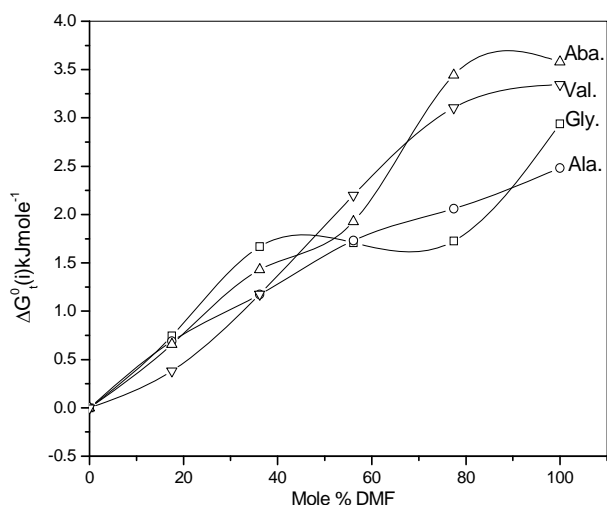


Figure 1. Variation of $\Delta G_t(i)$ $\text{kJ}\cdot\text{mol}^{-1}$ of glycine, DL-alanine, DL-alpha amino butyric acid and DL-nor-valine in non-aqueous ethylene glycol and N,N -dimethyl formamide mixtures.

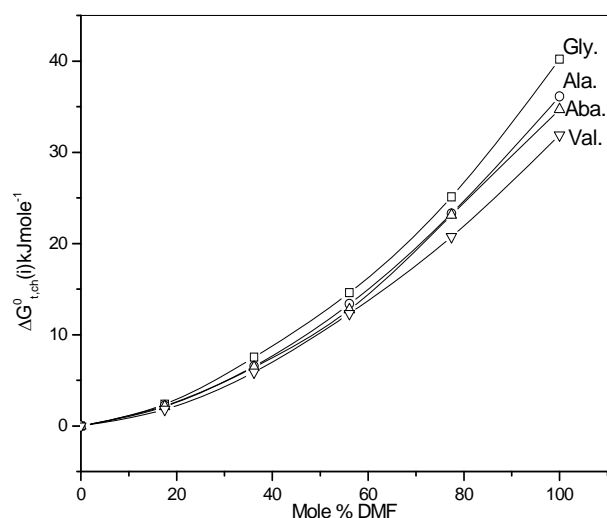


Figure 2. Variation of $\Delta G_{t,ch}^0(i)$ $\text{kJ}\cdot\text{mol}^{-1}$ of glycine, DL-alanine, DL-alpha amino butyric acid and DL-nor-valine in non-aqueous ethylene glycol and N,N -dimethyl formamide mixture.

As the proportion of N,N -dimethyl formamide in the mixed solvent system will be gradually increased the solvent character may undergoes a gradual but material change in respect to the above types of chemical interactions.

While ethylene glycol is a good Bronsted acid, N,N -dimethyl formamide is not. Thereby anionic part (COO^-) of these four amino acids can be more solvated in ethylene glycol than N,N -dimethyl formamide due to acid-base interaction. Therefore with the increased concentration of N,N -dimethyl formamide $\Delta G_{t,ch}^0(i)$ values

become more and more positive. On the other hand the cationic part (NH_3^+) of the zwitterionic form of amino acids will be more solvated with the increased concentration of N,N -dimethyl formamide as it possess stronger lewis basicity and cationophilicity than ethylene glycol. Furthermore, in respect of H-bonding capacity ethylene glycol is more potential than N,N -dimethyl formamide. So amino acids will be less solvated with increased concentration of N,N -dimethyl formamide in ethylene glycol and N,N -dimethyl formamide mixtures.

It should be noted that N,N -dimethyl formamide (0.498 Å) is more polarisable than ethylene glycol (0.437 Å). Therefore N,N -dimethyl formamide, here undergo more stronger soft-soft and dispersion interactions with larger amino acids (*i.e.* α -aba & n-val). These phenomenon is well evidenced in the stability order *i.e.* $\Delta G_{t,ch}^0(i)$ (n-val.) $>$ $\Delta G_{t,ch}^0(i)$ (α -aba.) $>$ $\Delta G_{t,ch}^0(i)$ (ala.) $>$ $\Delta G_{t,ch}^0(i)$ (gly.).

For larger amino acids like DL-alanine, DL- α -amino butyric acid and DL-nor-valine along with soft-soft, dispersion and specific charge transfer interactions and another indirect and hence secondary interaction, namely solvophilic solvation (S_bS) is likely to be significant. Ethylene glycol, like H_2O , being extensively capable of intermolecular hydrogen bond formation, organizes a cage-like structure around organic moiety [here $-\text{CH}_3$ (Alanine), $-\text{CH}_3-\text{CH}_3$ (α -amino butyric acid), $-\text{CH}_3-\text{CH}_3-\text{CH}_3$ (non-valine)] by being induced by the latter, causing 'solvophilic solvation' which is similar to 'hydrophobic hydration' (H_bH) in aqueous solution.

Thus for larger amino acids although dispersion interaction is significant and tends to decrease $\Delta G_{t,ch}^0(i)$ values, the latter "solvophilic solvation" (S_bS) still decrease and tends to increases $\Delta G_{t,ch}^0(i)$ values with increase concentration of DMF as compared to those in pure ethylene glycol.

Thus the chemical contribution of transfer free energies, $\Delta G_{t,ch}^0(i)$ of these four homologous α -amino acids are guided by the composite effects of increased dispersion interaction, basicity and decreased acidity, hydrogen bonding effects and solvophobic solvation of ethylene glycol and N,N -dimethyl formamide mixtures as compared to that of reference solvent (ethylene glycol).

4.2. Role of Amino Acids for Controlling Solvent-Solvent Interactions

Figure 3 represents the $T\Delta S_t^0(i)$ change of these amino acids with ethylene glycol and N,N -dimethyl formamide mixtures. Here for all four amino acids roller-coaster type behavior are found. Like $\Delta G_t^0(i)$, $T\Delta S_t^0(i)$ can be taken to be composed of cavity, dipole-dipole and chemical interaction effects *i.e.*

$$T\Delta S_t^0(i) = T\Delta S_{t,cav}^0(i) + T\Delta S_{t,dd}^0(i) + T\Delta S_{t,ch}^0(i)$$

where $T\Delta S_{t,cav}^0(i)$ represents the difference of entropy change involved in creating appropriate cavities for accommodating the amino acids molecule in the reference solvent ethylene glycol and ethylene glycol and *N,N*-dimethyl formamide mixed co-solvent system in the present study.

$T\Delta S_{t,dd}^0(i)$ stands for the dipole-dipole interactions originated due to dipolar amino acid molecule and dipolar mixed solvent system. $T\Delta S_{t,ch}^0(i)$ term referred to as the chemical effect, stands for the structural interaction effect that appears due to the change of solvent structure induced by the amino acid molecules, if any, apart from that involved in the cavity effect. Now combined effect in $T\Delta S_t^0(i)$ values may represents such behavior as shown in **Figure 3**.

In **Figure 4** $T\Delta S_{t,ch}^0(i)$ Vs ethylene glycol and *N,N*-dimethyl formamide mixed solvent composition profiles is illustrated. For all amino acids regular increase of $T\Delta S_{t,ch}^0(i)$ values with increase concentration of of *N,N*-dimethyl formamide is reflected. The $T\Delta S_{t,ch}^0(i)$ value varies as DI-n val. < DI- α -aba. < DI-ala. < gly. Now in order to understand the variation of $T\Delta S_{t,ch}^0(i)$ for all four amino acids with increased DMF concentration in ethylene glycol and *N,N*-dimethyl formamide mixed solvent systems; one must note that solvation here occurs mainly through solvophobic solvation (S_bS), a phenomenon similar to hydrophobic hydration (H_bH) as in aqua-organic systems.

Solvation by this effect, solphobic solvation (S_bS) significantly decreases the entropy in ethylene glycol. Gradual desolvation with increased concentration of *N,N*-dimethyl formamide in ethylene glycol and *N,N*-dimethyl formamide mixtures for these amino acids is also guided by the increased hard-sphere diameter (DI-n val. > DI- α -aba. > DI-ala. > gly.) of the amino acids.

Therefore as reflected in **Figure 4**, DI-nor-valine will be more desolvated (*i.e.* lower $T\Delta S_{t,ch}^0$) than other and glycine will be least desolvated (*i.e.* higher $T\Delta S_{t,ch}^0$).

On the other hand observed monotonic increase of $T\Delta S_{t,ch}^0$ for these amino acids along with other interactions, is also guided by dispersion interaction. But solvophobic solvation being larger in the reference solvent, ethylene glycol than in the mixed solvents, it is quite likely that transfer of amino acids will disrupt the solvent structure, and hence make $T\Delta S_{t,ch}^0$ (amino acids) increasingly positive, as observed in **Figure 4**.

5. CONCLUSIONS

From the overall observations it may be concluded that the stability of the four homologous α -amino acids are guided by superimposed effects of increased cavity

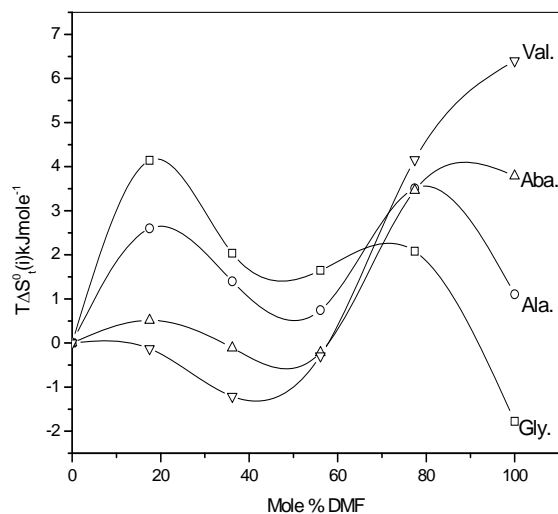


Figure 3. Variation of $T\Delta S_t^0(i)$ of glycine, DI-alanine, DI-alpha amino butyric acid and DI-nor-valine in non aqueous ethylene glycol and *N,N*-dimethyl formamide mixtures.

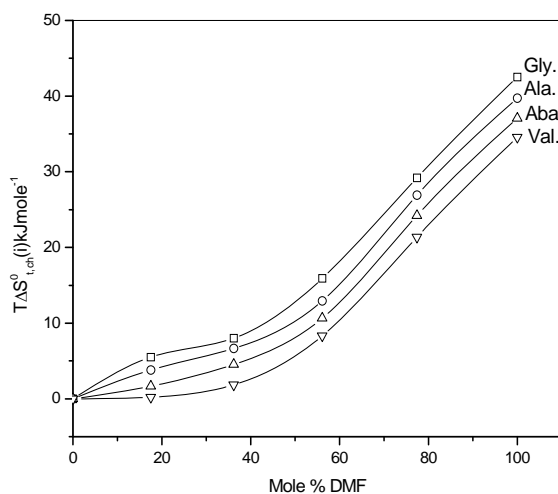


Figure 4. Variation of $T\Delta S_{t,ch}^0(i)$ of glycine, DI-alanine, DI-alpha amino butyric acid and DI-nor-valine in non-aqueous ethylene glycol and *N,N*-dimethyl formamide mixtures.

effect, dipole-dipole interactions, dispersion interactions, basicity effect and decreased acidity effect, solvophobic solvation with increased concentrations of *N,N*-dimethyl formamide in ethylene glycol and *N,N*-dimethyl formamide mixtures. Also it is transpiring that ethylene glycol, having protic character will be good stabilizer of amino acids, proteins as well as dipolar biomolecules. On the other hand, dipolar aprotic *N,N*-dimethyl formamide solvent will be good stabilizer for heavier amino acids having larger apolar moieties. Structural eccentricities of H_2O solvent may also be indirectly reestablished here by quite different type of solute-solvent interactional behaviour in our present solvent system.

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