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Thermodynamics of dissociation of glycine in aqueous ethanol and urea solutions

Han Gao, Xingen Hu, Ruisen Lin*

Thermodynamic & Thermochemistry Institute, Department of Chemistry, Zhejiang University, Hangzhou, 310027, China

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Abstract

The dissociation enthalpies of glycine in aqueous ethanol and urea solutions have been determined at 298.15 K by LKB-2277 BioActivity Monitor. Combined with the corresponding Gibbs free energies, the dissociation entropies have been calculated. The results have been interpreted with the point of view of structure alteration of the two solvents. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

From the studies of a wide variety of processes in aqueous solutions of simple monohydric alcohols [1–3], it is now a fairly well-established fact that small amounts of aliphatic alcohols strengthen the three-dimensional structure of liquid water. In terms of Frank and Wen's 'flickering clusters' [4], the average lifetime of such bulky ice-like clusters is extended; in other words, the equilibrium, bulky 'icebergs' ↔ monomeric water, existing in pure water is shifted to the left in aqueous alcohol solutions rich in water. It is also well-known that urea causes unfolding of native conformation of proteins. From studies of the free energies of transfer obtained from solubilities of amino acids and peptides in aqueous urea [5] and ethanol [6] solutions at 298.15 K, it is concluded that while both non-polar and polar side chains are preferentially solvated in aqueous urea solutions, the effect is much reduced in aqueous ethanol solutions. Various studies [7,8] have been made on aqueous urea solutions employing different techniques and using different substances as probes to understand the effect of urea on the structure of water. The conclusion is that the addition of urea to water causes 'statistical' structure breaking of water by shifting the bulky 'icebergs' ↔ monomeric water equilibrium toward the right. The thermodynamic quantities, especially the enthalpy and entropy, of transfer of quite a number of inert nonelectrolytes have been used to glean structure information in aquo-organic mixtures [9–12]. Studies on the effect of solvents on the dissociation constants of weak electrolytes (acid or bases) have been comparatively wide but studies on the effect of solvents on the enthalpies of dissociation of weak acids or bases are relatively few [13–17]. It has been admitted that the Gibbs free energy of transfer does not represent the complex structural contributions associated with the

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* Corresponding author.
E-mail address: zjgaohan@yahoo.com (R. Lin).

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dissociation of acids or bases due to known compensations between enthalpy and entropy of solvation. The enthalpy of dissociation of a weak acid represents the enthalpy changes due to the breaking of bonds as well as the complex structure contributions associated with the solvation of different species. The true nature of solvation is yet to be fully understood. In order to explore the roles of different solvents on the dissociation of weak acids, we have determined the dissociation enthalpies of glycine, a typical zwitterion, in aqueous ethanol and urea solutions.

2. Experimental

The calorimetric measurements were performed using an LKB-2277 BioActivity Monitor. All the measurements were carried out at 298.15 ± 0.0005 K. The reagents were pumped into the calorimeter by two LKB-2132 microperpex peristaltic pumps.

Glycine (AR grade. 99.5 ~ 100.5%, Huzhou Biochemical Factory, Shanghai, China) was recrystallized twice from low conductivity water and then dried in an infrared drier until there was no significant change in mass. Afterwards it was stored in a vacuum desiccator at room temperature for at least 48 h. Deionized water was distilled using a quartz sub-boiling purifier and stored in a CO_2-free atmosphere before use. Absolute ethanol (GR grade. assay ≥99.8%, Shanghai Zhenxing Chemical Engineering Factory, Shanghai, China) was used without further purification. Urea (AR grade. assay ≥99.0%. Ningbo Chemicals Factory, Zhejiang, China) was recrystallized twice from water and was dried at 60°C for at least 24 h. The mixtures of water with ethanol or urea were prepared by weighing. The $HClO_4$ solutions were prepared by weight by adding $HClO_4$ aqueous solution of known molarity to water–ethanol or water–urea mixtures The NaOH solutions were prepared by the same method.

The two-step dissociation process of $NH_3^+CH_2COOH$ in water–ethanol (or water–urea) mixture of x mole fraction can be expressed as

$$NH_3^+CH_2COOH \rightarrow NH_3^+CH_2COO^- + H^+ \quad \Delta H^0_{d,1} \tag{1}$$

$$NH_3^+CH_2COO^- \rightarrow NH_2CH_2COO^- + H^+ \quad \Delta H^0_{d,1} \tag{2}$$

in which $\Delta H^0_{d,1}$ and $\Delta H^0_{d,2}$ represent the first and second molar dissociation enthalpies of glycine. The two enthalpies were obtained by the following experimental measurements:

1. The molar reaction enthalpy (ΔH_1) of $NH_3^+CH_2COO^-$ with $HClO_4$ in water–ethanol (or water–urea) mixture of x mole fraction has been determined.

$$NH_3^+CH_2COO^- + H^+ + ClO_4^- \rightarrow NH_3^+CH_2COOH + ClO_4^- \quad \Delta H_1 \tag{3}$$

2. The molar reaction enthalpies (ΔH_2 and ΔH_3) of $NH_3^+CH_2COO^-$ and $HClO_4$ of the same molalities with NaOH in water–ethanol (or water–urea) mixture of x mole fraction have been determined, respectively, to obtain the difference between the two enthalpies (ΔH_2–ΔH_3).

$$NH_3^+CH_2COO^-Na^+ + OH^- \rightarrow NH_2CH_2COO^- + Na^+H_2O \quad \Delta H_2 \tag{4}$$

$$H^+ + ClO_4^- + Na^+ + OH^- \rightarrow H_2O + ClO_4^- + Na^+ \quad \Delta H_3 \tag{5}$$

During the reactions (3) and (4), both $HClO_4$ and NaOH were excess to avoid the dissociation of $NH_3^+CH_2COOH$ and the hydrolysis of $NH_2CH_2COO^-$. Since the molalities of $NH_3^+CH_2COO^-$ in reactions (3) and (4) were controlled in the range $2.5 \times 10^{-3} \sim 5.0 \times 10^{-3}$ mol kg, and the molalities of $HClO_4$ and NaOH were always ca. 0.05 mol kg^{-1} the ionic strength of the reaction mixtures were considered to be unchanged during the whole reactions, and the reactions were considered to be carried out completely at infinite dilution for glycine. Duplicate runs at five different glycine concentrations were carried out for processes (a) and (b). The experimental values of ΔH_1 and (ΔH_2–ΔH_3) were found to be independent of the molalities of $NH_3^+CH_2COO^-$. Therefore, the molar dissociation enthalpies of $NH_3^+CH_2COOH$ at infinite dilution can be obtained by the following two equations.

$$\Delta H^0_{d,1} = -\Delta H_1 \tag{6}$$

$$\Delta H^0_{d,2} = \Delta H_2 - \Delta H_3 \tag{7}$$

Table 1
Thermodynamic parameters for dissociation of glycine in ethanol–water mixtures at 298.15 K[a]

x_{EtOH}		$\Delta G_{\mathrm{d}}^{0}$/kJ mol^{-1}	$\Delta H_{\mathrm{d}}^{0}$/kJ mol^{-1}	$\Delta S_{\mathrm{d}}^{0}$/J K^{-1} mol^{-1}
0.000	(1st)	13.35	4.05 ± 0.01	−31.46
	(2nd)	55.80	44.00 ± 0.04	−39.61
0.042	(1st)	14.72	5.80 ± 0.01	−29.92
	(2nd)	55.01	46.60 ± 0.03	−28.21
0.089	(1st)	15.12	7.14 ± 0.02	−26.77
	(2nd)	54.15	46.16 ± 0.05	−26.80
0.144	(1st)	15.69	6.92 ± 0.01	−29.41
	(2nd)	53.46	44.00 ± 0.03	−31.73
0.207	(1st)	17.06	6.00 ± 0.02	−37.10
	(2nd)	53.24	42.60 ± 0.02	−35.69
0.281	(1st)	18.20	4.96 ± 0.01	−44.41
	(2nd)	53.69	41.78 ± 0.04	−39.95

[a] Note: (1st) and (2nd) in the first columns of Tables 1 and 2 represent the first and the second dissociation processes of glycine.

3. Results and discussion

The first and the second dissociation enthalpies ($\Delta H_{\mathrm{d}}^{0}$) of glycine in various ethanol–water and urea–water mixtures are collected in Tables 1 and 2, respectively. The errors in the values of $\Delta H_{\mathrm{d},1}^{0}$ and $\Delta H_{\mathrm{d},2}^{0}$ as calculated from the uncertainties associated with experimental measurements are also listed. The corresponding values of the standard Gibbs's free energy changes (ΔG_{d}^{0}) given in the two tables were calculated from the pK_{d} values reported by other researchers [18,19]. The dissociation entropies ($\Delta S_{\mathrm{d}}^{0}$) listed in the last columns were calculated from the equation

$$\Delta S_{\mathrm{d}}^{0} = \frac{(\Delta H_{\mathrm{d}}^{0} - \Delta G_{\mathrm{d}}^{0})}{T} \tag{8}$$

To illustrate the effects of solvents on the dissociation processes of glycine. it is convenient to express the dissociation enthalpy (or entropy) as the difference between the values obtained in the mixture of x mole fraction and in pure water, or in other words, the transfer dissociation enthalpy (or entropy).

Table 2
Thermodynamic parameters for dissociation of glycine in urea–water mixtures at 298.15 K

x_{EtOH}		ΔG_{d}^{0}/kJ mol^{-1}	$\Delta H_{\mathrm{d}}^{0}$/kJ mol^{-1}	$\Delta S_{\mathrm{d}}^{0}$/J K^{-1} mol^{-1}
0.000	(1st)	13.35	4.05 ± 0.01	−31.19
	(2nd)	55.80	44.00 ± 0.04	−39.58
0.018	(1st)	11.81	2.70 ± 0.02	−30.56
	(2nd)	54.18	42.92 ± 0.03	−37.77
0.037	(1st)	10.05	2.06 ± 0.03	−26.80
	(2nd)	52.07	42.52 ± 0.04	−32.03
0.051	(1st)	9.52	2.73 ± 0.02	−22.77
	(2nd)	50.93	43.00 ± 0.06	−26.60
0.071	(1st)	9.13	4.95 ± 0.02	−14.02
	(2nd)	50.01	44.59 ± 0.03	−18.18
0.082	(1st)	8.86	5.77 ± 0.01	−10.36
	(2nd)	49.93	45.96 ± 0.05	−13.32
0.097	(1st)	8.22	6.33 ± 0.02	−6.34
	(2nd)	49.30	47.24 ± 0.04	−6.91
0.112	(1st)	7.99	6.65 ± 0.01	−4.49
	(2nd)	49.01	47.90 ± 0.06	−3.72

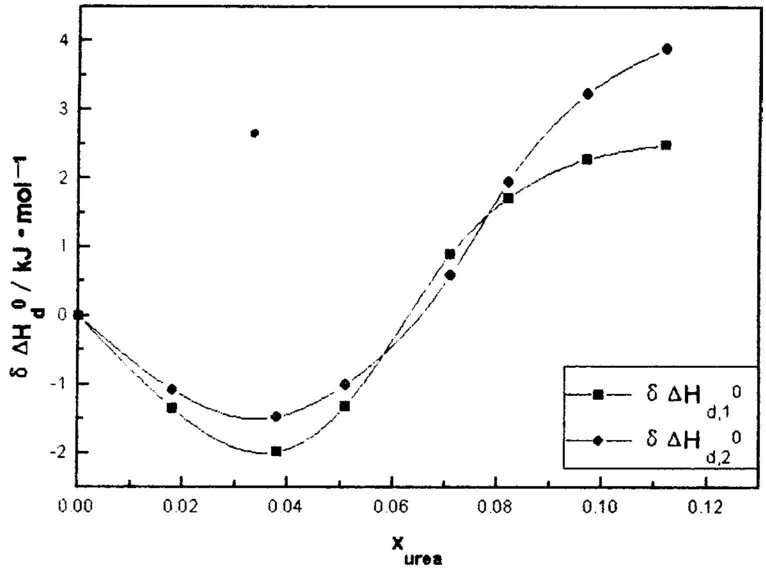

Fig. 1. Transfer dissociation enthalpies of glycine in aqueous urea solution at 298.15 K.

$$\delta \Delta H_{\mathrm{d}}^{0}=\Delta H_{\mathrm{d}}^{0}(x)-\Delta H_{\mathrm{d}}^{0}(w) \quad (9)$$

$$\delta \Delta S_{\mathrm{d}}^{0}=\Delta S_{\mathrm{d}}^{0}(x)-\Delta S_{\mathrm{d}}^{0}(w) \quad (10)$$

The values of $\delta\Delta H_{\mathrm{d}}^{0}$ and $\delta\Delta S_{\mathrm{d}}^{0}$ of glycine in aqueous urea and ethanol solutions are plotted against x_{urea} and x_{EtOH} in Figs. 1–4. In aqueous urea solutions, the $\delta\Delta H_{\mathrm{d}}^{0}$ terms ($\delta\Delta H_{\mathrm{d,1}}^{0}$ and $\delta\Delta H_{\mathrm{d,2}}^{0}$) pass through prominent minima in the region rich in water ($x_{\mathrm{urea}} \sim 0.035$) and then rise steeply (Fig. 2), while the $\delta\Delta S_{\mathrm{d}}^{0}$ terms ($\delta\Delta S_{\mathrm{d,1}}^{0}$ and $\delta\Delta S_{\mathrm{d,2}}^{0}$) go through plains in the same region and then rise (Fig. 2). Both $\delta\Delta H_{\mathrm{d}}^{0}$ and $\delta\Delta S_{\mathrm{d}}^{0}$ the terms are favourable to processes (1) and

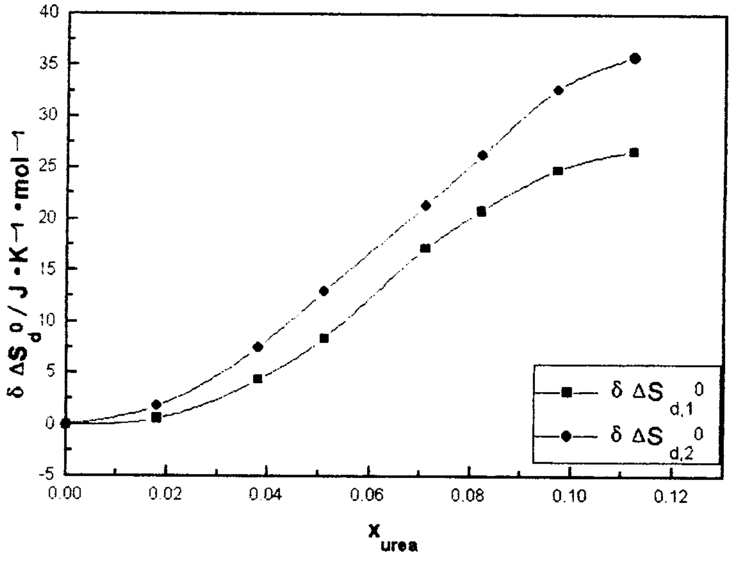

Fig. 2. Transfer dissociation entropies of glycine in aqueous urea solutions at 298.15 K.

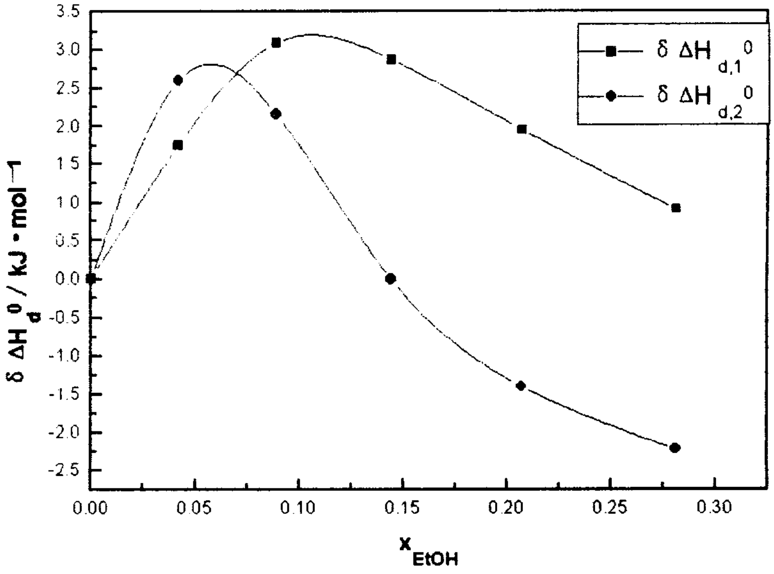

Fig. 3. Transfer dissociation enthalpies of glycine in aqueous ethanol solutions at 298.15 K.

(2) in the water-rich region. When x_{urea} exceeds ca. 0.06. the $\delta\Delta H_{\mathrm{d}}^{0}$ terms become unfavourable, but the $\delta\Delta S_{\mathrm{d}}^{0}$ terms are still favourable. In aqueous ethanol solutions, as shown in Figs. 3 and 4, both $\delta\Delta H_{\mathrm{d}}^{0}$ and $\delta\Delta S_{\mathrm{d}}^{0}$ terms go through maxima in the region rich in water ($x_{\mathrm{EtOH}} < 0.15$), but the maxima of processes (1) and (2) occur at different mole fractions. Only the $\delta\Delta S_{\mathrm{d}}^{0}$ terms are favourable to processes (1) and (2) in the region rich in water. With the increase in x_{EtOH}, the $\delta\Delta S_{\mathrm{d}}^{0}$ terms become unfavourable gradually while the $\delta\Delta H_{\mathrm{d}}^{0}$ terms become favourable. The different effects of the two solvents on the dissociation of glycine can

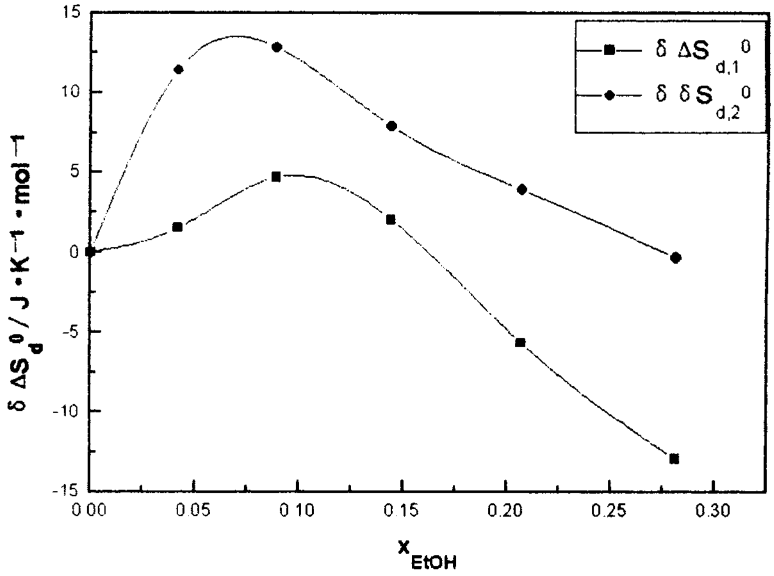

Fig. 4. Transfer dissociation entropies of glycine in aqueous ethanol solutions at 298.15 K.

be ascribed to the different structure alteration of the two solvents.

Since entropy rather than enthalpy changes are usually a better indicator of net structure implications, analysis of $\delta\Delta S_\mathrm{d}^0$ should be more rewarding. The observed maxima in the $\delta\Delta S_\mathrm{d}^0 \sim x_\mathrm{EtOH}$ profile (Fig. 4) appears to result from the effects of the initial promotion and subsequent break-down of the three-dimensional (3-D) H-bonded networks of water caused by the increasing addition of ethanol. If the maxima in the $\delta\Delta S_\mathrm{d}^0 \sim x_\mathrm{EtOH}$ profile in aqueous ethanol solutions indicate the promotion of water structure, the plains from $0 \sim 0.035$ mole fraction in the corresponding profile for aqueous urea solutions suggest that urea molecules are ineffectual in this respect. In this region, urea molecules act as structure breakers. This is possibly because urea molecules, being sterically debarred from entering the tetrahedral structure of water, occupy interstitial spaces forming a regular solution with monomeric water and thus cause the 'statistical' structure breaking of water [20]. The steady rise in $\delta\Delta S_\mathrm{d}^0$ terms beyond 0.035 mole fraction seems to result from the depletion of monomeric water and urea, probably through the formation of urea–water and possibly urea–urea aggregates [21,22]. The formation of urea–water clusters seems to be facilitated by the presence of three potential centers of H bonding on each urea molecule, and possibly due to the fact that the H bonding through carbonyl oxygen of urea is likely to be stronger than that between two water molecules. Certain important physical properties of aqueous urea solutions also indicate that the urea molecule behaves neither as a structure breaker nor as a structure maker but participates with great facility in the mobile hydrogen bonding amidst the 'flickering clusters' of water. The partial molal heat capacity of urea in water [23], the slightly positive viscosity B coefficient [24], and the enhancement of the dielectric constant of water upon the addition of urea [23] are evidence for the behavior of urea in water as an active participant in the total hydrogen bonding. The positive dilution entropy of aqueous urea solutions being quoted as evidence of structure-breaking action is not satisfying because firstly the magnitude of the dilution entropy is very small (ca. 0.034 J K^{-1} mol^{-1}), and secondly it is likely that urea may dimerize or polymerize in concentrated aqueous solutions as revealed by the nonideality of concentrated aqueous urea solutions [25]. The depolymerization would easily account for the small positive dilution entropy. Studies on autodiffusion of aqueous urea solutions [26] and the transfer thermodynamic properties of HX (X = Cl, Br, I) from water to aqueous urea solutions [27] also support the contention that urea in aqueous solutions participates in the formation of H-bonded groups of urea and water molecules. Considering the 'flickering-cluster' model [4] for water wherein each water molecule is considered to be an integral part of flickering clusters instead of belonging to monomeric or cluster species solely, the introduction of urea molecules into the heart of the flickering clusters can be achieved without prejudice to the geometry of urea. It is possible that the lifetime of the cluster may be extended and also that the dimensions of the void spaces may be changed on account of interposition of urea in the clusters. Beyond 0.112 mole fraction these urea–water clusters and possibly the aggregates of urea are expected to collapse due to the growing packing imbalance at higher concentrations of urea.

The dissimilarity in $\delta\Delta S_\mathrm{d}^0 \sim$ mole fraction profiles for aqueous solutions of urea and ethanol leads us to contend that while the addition of small amount of ethanol molecules shifts the bulky/dense equilibrium to the left by forming iceberg-like clusters around ethanol molecules in the region rich in water, the addition of urea molecules always shifts it to the right by the structure-breaking action in the region $0 \sim 0.035$ mole fraction and by the formation of urea–water clusters and possibly urea–urea aggragates in the range $0.035 \sim 0.112$ mole fraction.

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References

[1] S.K. Pal, A. Chakravorty, S.C. Lahiri, Thermochim. Acta 124 (1988) 43.

[2] Lei Qunfang, Yu Qingsen, Zong Hanxing, Lin Ruisen, J. Zhejiang University (Natural Science) 29 (1995) 172.

[3] Hu Xin-Gen, Lin Rui-Sen, Zong Han-Xing, Acta Chim. Sin. 57 (1999) 479.
[4] H.S. Frank, W.Y. Wen, Discussion Faraday Soc. 24 (1957) 133.
[5] Y. Nozaki, C. Tanford, J. Biol. Chem. 238 (1963) 4074.
[6] Y. Nozaki, C. Tanford, J. Biol. Chem. 246 (1971) 2211.
[7] A.K. Das, K.K. Kundu, J. Soln. Chem. 5 (1976) 431.
[8] H. Talukdar, S. Rudra, K.K. Kundu, Can. J. Chem. 66 (1988) 461.
[9] A. Ben-Naim, J. Phy. Chem. 71 (1967) 4002.
[10] A.K. Mishra, J.C. Ahluwalia, J. Chem. Soc. Faraday Trans. I 77 (1981) 1469.
[11] Yan Lou, Ruisen Lin, Thermochim. Acta 316 (1998) 145.
[12] Ren Xiao-Ling, Lin Rui-Sen, Hu Xin-Gen, Ni Ya-Ming, Acta Chim. Sin. 57 (1999) 875.
[13] Zhang Xin, Lin Rui-Sen, Zong Han-Xing, Yan Wen-Xing, Acta Chim. Sin. 48 (1990) 28.
[14] Hu Xingen, Lin Ruisen, Zong Hanxing, Acta Physico-Chim. Sin. 15 (1999) 838.
[15] Yan-Qing Nan, Qing-Sen Yu, Rui-Sen Lin, Thermochim. Acta 315 (1998) 101.
[16] Yan-Qing Nan, Qing-Sen Yu, Rui-Sen Lin, Thermochim. Acta 317 (1998) 201.
[17] Xingen Hu, Shuqin Li, Ruisen Lin, Hanxing Zong, Thermochim. Acta 319 (1998) 43.
[18] M.S.K. Niazi, J. Mollin, Bull. Chem. Soc. Jpn. 60 (1987) 2605.
[19] H. Talukdar, S. Rudra, K.K. Kundu, Can. J. Chem. 67 (1989) 315.
[20] H.S. Frank, F. Frank, J. Chem. Phys. 48 (1968) 4746.
[21] T.S. Sarma, J.C. Ahluwalia, Chem. Soc. Rev. 2 (1973) 203.
[22] R.L. Daniel, E.S. Vold, S.O. Owen, J. Am. Chem. Soc. 92 (1970) 6771.
[23] M. Abu-Hamidayyah, J. Phy. Chem. 69 (1965) 2720.
[24] J.A. Rupley, J. Phy. Chem. 68 (1964) 2002.
[25] R.H. Stokes, J. Phys. Chem. 70 (1966) 1199.
[26] I.V. Matyash, A.I. Toryanik, V.V. Kiselnik, J. Struct. Chem. 8 (1967) 371.
[27] K.K. Kundu, K. Mazumdar, J. Chem. Soc. Faraday Trans. I 71 (1975) 1422.