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THE AROMATIC AMINO ACID BEHAVIOUR IN AQUEOUS AMIDE SOLUTIONS
The temperature dependence of the *L*-phenylalanine-urea interaction

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The enthalpies of solution of L-phenylalanine in the mixtures of water with the protein denaturant urea have been measured in the temperature range of 288.15–318.15 K. Using the results of the present research and literature data of free energies, the standard thermodynamic functions of the solute transfer from water to aqueous urea solutions have been estimated in a wide temperature range. The enthalpic, heat capacity, entropic and free energy parameters of the solute-urea pair and triplet interactions have been computed. The amino acid – amide pair interaction was found to be attractive in the temperature range studied due to the favourable enthalpic term. The triplet interaction being slightly repulsive reveals the enthalpic origin also. The examination of the Savage and Wood additivity-of-groups approach does indicate the inapplicability of this scheme to enthalpies and entropies of interaction. It has been found for the first time that the heat capacity of interaction changes its sign at 303 K, i.e. the temperature dependence of enthalpic and entropic parameters passes through the pronounced extrema near the temperature of the minimum of the heat capacity of pure water.

Keywords: *amino acids, denaturant aqueous solutions, enthalpy of solution, L-phenylalanine–urea interaction*

Introduction

The amino acids are an important group of biologically active compounds being single blocks of protein macromolecules. The urea addition to a protein aqueous solution causes the denaturing effect – the transition from the native folded state to the unfolded conformation in which the hydrophobic core of a protein molecule becomes exposed to aqueous media [1–3]. The mechanism of the urea action is still to a great extent a mystery [1–6]. Urea may act directly by binding to polar groups of a protein weakening internal H-bonds [4] and indirectly through the change in the properties of the H-bond network of liquid water near apolar groups of protein, thereby increasing their solubility in an aqueous phase and weakening the hydrophobic interaction [1–3, 5]. It is obvious that a relative contribution of both mechanisms should depend on a protein structure, temperature, urea concentration, etc. It is also worth remembering that the denaturation requires an addition of a sufficiently large amount of urea to water and, although urea slightly influences its structure [6, 7], the solvent medium is no longer water, but it is rather a mixed solvent, where the protein – urea interaction is similar, but not the same, as in a dilute aqueous solution. It is evident that both hydration of amino acids and related compounds and the interaction of these solutes with urea aqueous solutions are of particular interest for biophysical chemistry.

There have been a large number of experimental (mainly calorimetric) studies of the interaction of biologically active solutes with each other or with urea in water at 298.15 K (see, for instance, [1–3, 8–11] and references therein). However, most of these detailed efforts have been directed towards the systems in which the solutes have been highly soluble amides, amino acids or peptides. Moreover, it is not quite clear whether the results obtained at 298 K may be used to explain the processes occurring in vivo, i.e. at 310 K.

The principle objective of the present research is to obtain the temperature dependence of the interaction between the slightly soluble aromatic *L*-phenylalanine amino acid and urea in water in a wide temperature range.

Experimental

Materials

L-phenylalanine (Carl Roth, f.d. Biochemie) was dried under reduced pressure at 343 K and used without further purification. Urea (Harnstoff, >99.5 mass%) was used as supplied. Karl Fischer titration indicated the presence of 0.15 mass% water in urea, which was taken into account while preparing the solutions. The water used was doubly distilled.

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Apparatus and methods

The calorimetric measurements were carried out with a new automated isoperibol calorimeter fitted with a 70 cm^3 titanium vessel [12, 13]. The vessel is equipped with a calibration heater, a titanium stirrer and a thermistor. A glass ampoule containing a solute is attached to the stirrer and crushed against the vessel bottom to initiate the dissolution process. Thermistor resistance is measured directly by the digital Standard Temperature Measuring Instrument. The signal of the instrument is converted to the degrees of The International Temperature Scale of 1990. The detection limit of the apparatus is 10 μK. The temperature instability in the bath is 1 mK in the temperature range of 278–333 K. The calorimeter was tested by measuring the enthalpies of solution $\Delta_{sol}H^m$ of potassium chloride and 1-propanol in water at 298.15 K [13] according to recommendations given elsewhere [14].

Results and discussion

In the previous papers [12, 13, 15] we have shown that the experimental enthalpies of solution ($\Delta_{sol}H^m$) of aromatic amino acids in water do not depend on the solute concentration below ~0.1 mol kg^{-1}. It allows to calculate the standard enthalpies of solution ($\Delta_{sol}H^0$) as average values in the range of the experimental data, so that one can write $\Delta_{sol}H^m = \Delta_{sol}H^0$ for a single experiment. We use the same procedure in the present research. The $\Delta_{sol}H^m = \Delta_{sol}H^0$ values listed in Table 1 represent the result of a single measurement in the mixed solvent in the range of the amino acid molality of 0.003–0.01 mol kg^{-1}. The $\Delta_{sol}H^0$–$f(X_2)$ curves have been fitted to the second order polynomials:

$$\Delta_{sol}H^0 = A_0 + A_1X_2 + A_2X_2^2 \qquad (1)$$

except for the data at 288.15 K, where the linear fit is found to be quite sufficient. The coefficients obtained in a least-squares fitting routine are listed in the footnote of Table 1.

Figure 1 compares the enthalpies of transfer Δ_tH^0 ($\Delta_tH^0 = \Delta_{sol}H^0_{(mixture)} - \Delta_{sol}H^0_{(water)}$) of different solutes from water to the water-urea mixtures at 298.15 K. Our experimental results are seen to be in a good agreement with the literature Δ_tH^0 values [16]. Both polar and aliphatic amino acids reveal the similar behaviour in urea solutions, i.e. their enthalpies of transfer are negative, while the Δ_tH^0 values for apolar benzene [17] are positive. The *L*-phenylalanine behaviour is rather surprising because its transfer is more exothermic than that for *L*-alanine or *L*-threonine despite the presence of the apolar aromatic side chain in the molecule. We will return to this point later when the solute-urea interactions will be analysed but at the moment only comment that

Table 1 Standard enthalpies of solution ($\Delta_{sol}H^0$, kJ mol^{-1}) of *L*-phenylalanine in the water-urea mixtures at 288.15–318.15 K

X_2[a]	$\Delta_{sol}H^0$	X_2	$\Delta_{sol}H^0$
288.15 K		293.15 K	
0	6.36±0.07 [15]	0	7.24±0.08 [15]
0.01365	5.99	0.01941	6.53
0.02418	5.73	0.03726	6.17
0.03212	5.32	0.03452	6.15
0.05276	4.89	0.05186	5.62
0.06682	4.55	0.08538	4.77
0.07970	4.10	0.1011	4.33
0.1046	5.53	0.1186	3.99
0.1180	3.11		
298.15 K		306.15 K	
0	8.23±0.07 [13]	0	9.67±0.07 [15]
0.006873	7.85	0.02147	8.79
0.02404	7.15	0.04149	8.12
0.03229	6.89	0.05254	7.88
0.04048	6.75	0.06871	7.39
0.04945	6.38	0.08883	7.00
0.06833	5.99	0.1151	6.58
0.07956	5.69		
0.1020	4.99		
0.1202	4.74		
313.15 K		318.15 K	
0	10.93±0.03 [15]	0	11.97±0.04 [15]
0.01767	10.31	0.01578	11.52
0.02966	10.00	0.02362	11.25
0.03667	9.74	0.04965	10.50
0.04661	9.52	0.05832	10.18
0.07013	8.82	0.07358	9.98
0.1001	8.31	0.1013	9.35
		0.1219	8.78
		0.1232	8.75

[a]a urea mol fraction. The coefficients of Eq. (1) obtained from the least-squares routine are:
288.15 K – A_0=6.34(0.03), A_1= –27.36(0.49), s_f=0.06 kJ mol^{-1}; 293.15 K – A_0=7.22(0.03), A_1= –33.46(1.38), A_2=52.09(10.90), s_f=0.04 kJ mol^{-1},
298.15 K – A_0=8.14(0.07), A_1= –38.95(2.67), A_2=88.59(21.64), s_f=0.09 kJ mol^{-1};
306.15 K – A_0=9.65(0.03), A_1= –41.59(1.27), A_2=129.52(10.52), s_f=0.04 kJ mol^{-1};
313.15 K – A_0=10.94(0.04), A_1= –35.72(2.04), A_2=92.60(19.10), s_f=0.05 kJ mol^{-1};
318.15 K – A_0=11.97(0.06), A_1= –31.23(2.15), A_2=43.95(16.00), s_f=0.07 kJ mol^{-1}

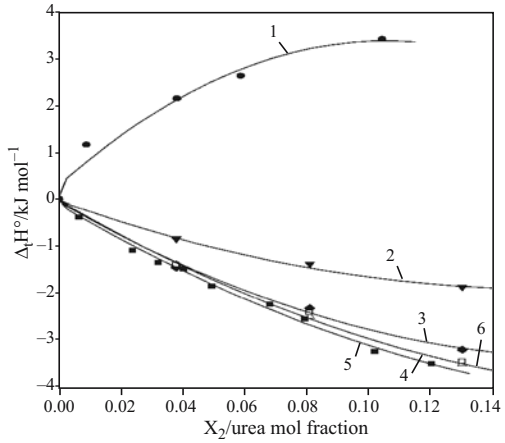

Fig. 1 Enthalpies of transfer of benzene (1) [17], *L*-alanine (2) [16], *L*-threonine (3) [16], glycine (4) [16] and *L*-phenylalanine (5, 6) from water to the water-urea mixtures 298.15 K. (5, dark symbols) – this work, (6, light symbols) – ref. [16]. Lines – the second order polynomial description

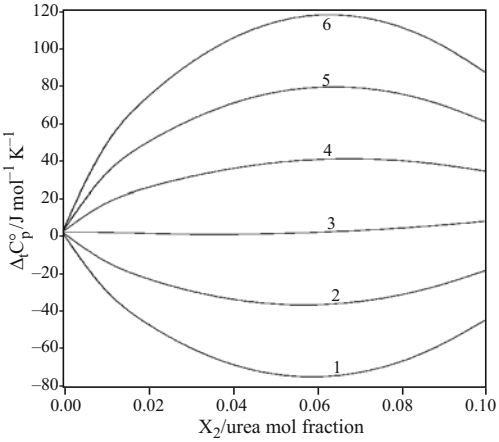

Fig. 2 Calculated heat capacities of transfer of *L*-phenylalanine from water to the water-urea mixtures at 1 – 283.15, 2 – 293.15, 3 – 303.15, 4 – 313.15, 5 – 323.15 and 6 – 333.15 K

the enthalpies of glycine [16] and *L*-phenylalanine transfer are almost identical.

The temperature dependence of the $\Delta_t H^0$ values has been analysed with the second-order polynomial equation [12, 15]:

$$\Delta_t H^0(T) = \Delta_t H^0(\Theta) + \Delta_t C_p^0(\Theta)(T-\Theta) + \\ + 0.5(\partial \Delta_t C_p^0/\partial T)[(T-\Theta)^2] \quad (2)$$

where $\Delta_t H^0$ (T) and T (current temperature, K) are variables, $\Delta_t H^0$ (Θ) and $\Delta_t C_p^0$ (Θ) are the enthalpy and heat capacity parameters desired at a reference temperature Θ (K), respectively. The equation parameters, thus, have a clear physical meaning.

Figure 2 illustrates the calculated curves $\Delta_t C_p^0$ *vs.* X_2 in a wide temperature range. At low temperatures the heat capacities of transfer are negative, and the curve $\Delta_t C_p^0$ *vs.* X_2 passes through the minimum at X_2=0.06 urea mol fraction. As the temperature is increased the heat capacities of transfer become positive, the maximum being reached at higher temperatures. One notable feature is that the $\Delta_t C_p^0$ values equal to zero near 303 K indicating that the amino acid transfer from water to the aqueous urea solution is not accompanied by any heat capacity changes. This unusual *L*-phenylalanine behaviour seems rather surprising but there are, at least, two experimental facts dealing with the phenomenon observed. First, the heat capacity of benzene and toluene transfer also reaches the maximum in the water-urea system [17] which occurs, however, at lower urea concentrations. Secondly and generally, the heat capacity of *L*-phenylalanine transfer changes its sign at approximately the same temperature as the standard heat capacity of urea solution, while the heat capacity of pure water at constant pressure reaches the minimum [18].

Nozaki and Tanford [1] reported free energies of transfer (mol fractions scale) of various amino acids in water-urea mixtures at 298.15 K. Combination of these values with our calorimetric data allows to compute for the first time the temperature dependence of all thermodynamic functions accompanying the *L*-phenylalanine transfer from water to aqueous urea solutions. Gibbs–Helmholtz equation can be written in the form:

$$\frac{\Delta_t G^0(T)}{T} - \frac{\Delta_t G^0(298.15)}{298.15} = -\int_{T_1}^{T_2} \frac{\Delta_t H^0}{T^2} dT \quad (3)$$

Thus, using the temperature dependence of the $\Delta_t H^0$ values and the free energy data at 298.15 K [1], we are able to calculate standard free energies of transfer and then the entropic contribution into the $\Delta_t G^0$ values:

$$(-T\Delta_t S^0) = \Delta_t G^0 - \Delta_t H^0 \quad (4)$$

The free energy of *L*-phenylalanine transfer is negative in the temperature and concentration range studied, slightly depending on the temperature (not shown). The enthalpic $\Delta_t H^0$ and entropic ($-T\Delta_t S^0$) terms in the free energies of transfer are plotted at fixed urea mol fractions in Figs 3, 4. Both curves pass through the extrema at approximately 303 K, which is just what one would expect from the heat capacity values illustrated in Fig. 2. The $\Delta_t G^0$<0 is mainly the result of the enthalpic contribution favouring the solute transfer. Thus, the spontaneous solute transfer in the physiological temperature range has the enthalpic origin, however, it reveals the entropic origin at higher temperatures.

The formally exact theory of solutions developed by McMillan and Mayer [19] and then adapted by Kauzmann [20] and Friedman [21] represents thermodynamic properties of a dilute solution by means a virial

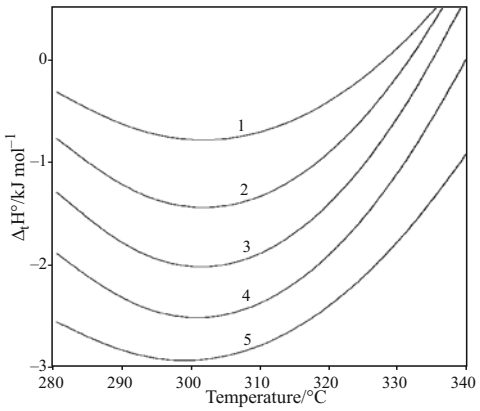

Fig. 3 Enthalpic contribution into the free energy of *L*-phenylalanine transfer from water to the water-urea mixtures at 1 – 0.02, 2 – 0.04, 3 – 0.06, 4 – 0.08 and 5 – 0.1 urea mol fractions

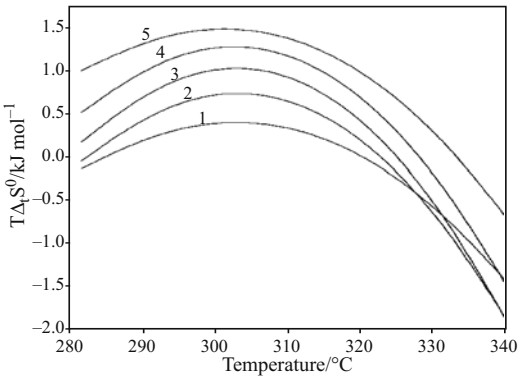

Fig. 4 Entropic contribution into the free energy of *L*-phenylalanine transfer from water to the water-urea mixtures at 1 – 0.02, 2 – 0.04, 3 – 0.06, 4 – 0.08 and 5 – 0.1 urea mol fractions

expansion, the coefficients of which reflect the interactions between pairs, triplets and a high number of solute molecules. The enthalpic urea (2)-*L*-phenylalanine (3) pair (h_{23}) and triplet (h_{223}) interaction parameters are computed as in our previous papers [22–24] using the coefficients of Eq. (1):

$$h_{23} = A_1 M_{H_2O}/2;\ h_{223} = (A_2 - A_1) M_{H_2O}{}^2/3 \quad (5)$$

The temperature dependence of the energetics of the urea-*L*-phenylalanine pair and triplet interactions is expressed by the same type relationship which has been applied for the enthalpies of transfer (Eq. (2)):

$$h_{23} = -349\ (6) - 5.87\ (0.60)(T - 298.15) + \\ + 0.5\ 0.95(0.09)\ [(T - 298.15)^2],\ s_f = 9\ \text{J kg mol}^{-2} \quad (6)$$

$$h_{223} = 15.0\ (0.8) - 0.75\ (0.08)(T - 298.15) + \\ + 0.5\ 0.11(0.01)[(T - 298.15)^2],\ s_f = 1\ \text{J kg}^2\ \text{mol}^{-3} \quad (7)$$

where values in brackets from here represent the standard deviation of the coefficients obtained. The free energy g_{23} and entropic ($-Ts_{23}$) interaction parameters have been computed by the same way as the $\Delta_t G^0$ and ($-T\Delta_t S^0$) values (Eqs (3), (4)). The $g_{23} = -192$ (8) J kg mol^{-2} value at 298.15 K has been evaluated from the free energy data [1].

The results are illustrated in Figs 5, 6. The h_{23} parameters, representing the enthalpy change, when a solvated urea molecule interacts with the solvated amino acid molecule in liquid water are negative at 275–335 K, which means that such interactions contribute in an attractive sense to the total interaction which is monitored by the free energy. The entropic term is positive and contributes in a repulsive sense to the total interaction. The free energy of interaction is seen to reveal the enthalpic origin in the physiological temperature range. At low and high temperatures, however, the entropic term becomes favourable and overcomes the unfavourable enthalpic one. The triplet interaction between the amino acid molecule and two urea molecules is noticeably weaker than the pair one and shows the opposite behaviour (Fig. 6). It does indicate a fast convergence of the virial expansion and applicability of the theory to the systems studied [3]. The triplet interaction is slightly repulsive ($g_{223}>0$) in the physiological temperature range due to the unfavourable enthalpic term. At low and high temperatures the unfavourable entropic term induces the repulsion. Both the g_{23} and g_{223} values vary slightly with the temperature due to the enthalpic-entropic compensation. This effect, however, is expressed weaker than in the case of tetraalkylammonium bromide-amide interaction [22, 23].

It is useful to compare the interaction of different amino acids with urea in water. However, as it has

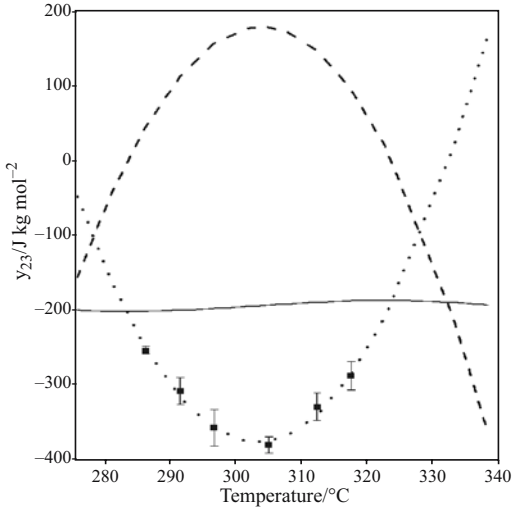

Fig. 5 Enthalpic (h_{23}, dotted line), entropic (($-Ts_{23}$) dash line) and free energy (g_{23}, solid line) parameters of *L*-phenylalanine-urea pair interaction. Points – experimental values calculated according to Eq. (5)

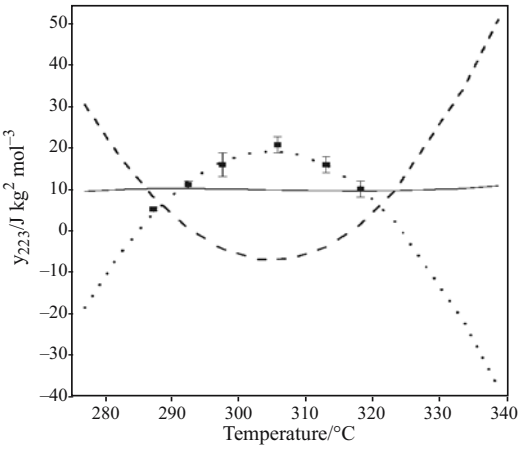

Fig. 6 Enthalpic (h_{223}, dotted line), entropic (($-Ts_{23}$), dash line) and free energy (g_{223}, solid line) parameters of *L*-phenylalanine-urea triplet interaction. Points – experimental values calculated according to Eq. (5)

been mentioned above, at the moment the available information is restricted by the standard temperature, i.e. 298.15 K. The treatment of the data [16] in terms of Eqs (1), (5) gives the enthalpic parameters of the glycine-urea and *L*-alanine-urea pair interaction equal to –346(28) and –202(20) J mol kg^{-1}, respectively. The former value is identical to the *L*-phenylalanine-urea parameter, the latter one is smaller but it is the same order of magnitude. This result seems rather surprising because our estimation of the benzene-urea enthalpic interaction parameter results in the h_{23} value being 513 (153) J mol kg^{-1}. If group additivity for the interactions between solvated solutes, as formulated by Savage and Wood [25], occurs in the systems studied, we would expect that the sum of the *L*-alanine-urea and benzene-urea interaction parameters should provide a reasonable estimation of the h_{23} value for *L*-phenylalanine. However, the estimated h_{23} parameter is seen to be positive. It gives $\Delta_t H^0 \geq 0$, which disagrees with the experimental data (Fig. 1). The same result is obtained if one would use the h_{23} values for toluene and glycine.

It is intuitively clear that all solute-solute distances and possible orientations contribute to the y_{23} values. The introduction of a new functional group into one solute molecule may change the interaction of the remaining groups with second molecule, especially if a new group induces the preferential orientations between interacting species [3]. The addition of the alanine residue to the benzene ring appears to induce the preferential orientation between the head group and an urea molecule, since the enthalpy of interaction between urea and different amino acids is almost independent of the nature of the side chain. Therefore, the interaction of the benzene ring with urea is unlikely to be equal to the benzene-urea interaction. If so, that benzene and toluene are not appropriate models for the *L*-phenylalanine side chain in water-urea mixtures.

Figures 5, 6 illustrate some more interesting features which are worthy of note. First, this seems rather surprising but is, at least, approximately true that the interaction parameters at 298 and 310 K are almost identical. This result shows that the measurements performed at a room temperature provide a reasonable estimation of the *L*-phenylalanine behaviour at 310 K. Secondly, the temperature dependences of the enthalpic and entropic parameters pass through pronounced extrema near 303 K. It indicates that aqueous medium influences strongly the *L*-phenylalanine – urea pair and triplet interactions since the temperature dependence of the heat capacity of pure water has the minimum at approximately the same temperature. Finally, the *L*-phenylalanine – urea pair interaction should become thermochemically unfavourable at higher temperatures. To check this prediction we have measured the enthalpies of *L*-phenylalanine solution in pure water and X_2=0.02004 urea mol fraction at 328.15 K. The $\Delta_{sol}H^0$ values are 13.94±0.04 and 13.65±0.02 kJ mol^{-1}, respectively. It allows to estimate the enthalpic interaction parameter as follows: $h_{23} \approx (13.65–13.94)\ 0.009/0.02004 = –130$ J kg mol^{-2}. This value is seen to be in a good agreement with the data plotted in Fig. 5.

Conclusions

In conclusion, from the investigation that has been performed, we may state that the temperature dependence of the enthalpic and entropic parameters reveals unusual behaviour near 303 K indicating that water influences strongly *L*-phenylalanine – denaturant interaction. We believe that additional thermodynamic studies for the aromatic amino acid – amide interaction may shed some light on this problem.

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References

1 Y. Nozaki and C. Tanford, J. Biol. Chem., 238 (1963) 4074.
2 C. Tanford, Adv. Protein Chem., 24 (1970) 1.
3 Yu. M. Kessler and A. M. Zaitsev, Solvophobic effects, Ellis Horwood, Chichester, 1994.
4 J. A. Schellman, Biopolymers, 26 (1987) 549.
5 N. Muller, J. Phys. Chem., 94 (1990) 3856.

6 F. Vanzi, B. Madan and K. Sharp, J. Am. Chem. Soc., 120 (1998) 10748.
7 P. Astrand, A. Wallqvist and G. Karlstrom, J. Phys. Chem., 98 (1994) 8224.
8 B. Palecz, J. Therm. Anal. Cal., 54 (1998) 257.
9 P. Cheek and T. H. Lilley, J. Chem. Soc., Faraday Trans., 1 84 (1988) 1927.
10 J. Fernández and T. H. Lilley, J. Chem. Soc., Faraday Trans., 88 (1992) 2503.
11 B. Palecz, J. Therm. Anal. Cal., 54 (1998) 265.
12 A. V. Kustov and V. P. Korolev, Thermochim. Acta, 447 (2006) 212.
13 A. V. Kustov, A. A. Emel`yanov, A. F. Syschenko, M. A. Krest`yaninov, N. I. Zheleznyak and V. P. Korolev, Russ. J. Phys. Chem., 80 (2006) 1724.
14 I. Wadsö and R. N. Goldberg, Pure Appl. Chem., 73 (2001) 1625.
15 A. V. Kustov and V. P. Korolev, Russ. J. Phys. Chem., 81 (2007) 193.
16 M. Abu-Hamdiyyah and A. Shehabuddin, J. Chem. Eng. Data, 27 (1982) 74.
17 Š. Hovorka, V. Dohnal, E. Carrillo-Nava and M. Costas, J. Chem. Thermodyn., 32 (2000) 1683.
18 E. V. Ivanov and V. K. Abrossimov, in: Biologically active compounds in solutions: structure, thermodynamics, reactivity, Ed. A. M. Kutepov, Modern aspects of structural and volumetric studies of urea aqueous solutions, Moscow, Nauka 2001, p. 110 (in Russian).
19 W. G. McMillan Jr. and J. E. Mayer, J. Chem. Phys., 13 (1945) 276.
20 J. J. Kozak, W. S. Knight and W. Kauzmann, J. Chem. Phys., 48 (1968) 675.
21 H. L. Friedman and C. V. Krishnan, J. Solution Chem., 2 (1973) 119.
22 A. V. Kustov and V. P. Korolev, Thermochim. Acta, 437 (2005) 190.
23 V. P. Korolev, N. L. Smirnova and A. V. Kustov, Thermochim. Acta, 427 (2005) 43.
24 A. V. Kustov, O. A. Antonova and V. P. Korolev, Russ. J. Inorg. Chem., 49 (2004) 944.
25 J. J. Savage and R. H. Wood, J. Solution Chem., 5 (1976) 733.

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