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Thermodynamic investigations of interactions in aqueous solutions of glycine and some small peptides with caffeine and 1,3-dimethyluracil at 298.15 K

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Abstract

Enthalpies of dilution for ternary aqueous solutions of glycine, glycyl-glycine, triglycine, and L-α-alanyl-L-α-alanine with caffeine, as well as of glycyl-glycine, and L-α-alanyl-L-α-alanine with 1,3-dimethyluracil have been determined. The results have been analysed in terms of the McMillan–Mayer formalism in order to obtain the enthalpic virial coefficients. Weak interactions were observed. The densities and molar volumes at infinite dilution were determined for the systems mentioned, as well as for aqueous solutions of glycine, glycyl-glycine and triglycine.

Keywords: Caffeine; Dimethyluracil; Heat of dilution; Partial molar volume; Peptide

1. Introduction

It is commonly recognised that liquid water by the nature of its structure plays a unique role in sustaining the folding of biological molecules into functional conformations and in the determination of interactions with specific molecules, due to which their biological functions are performed. Knowledge of the hydration properties of the pertinent classes of molecules and of the interactions between

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them are prerequisites for understanding the various intra- and intermolecular interactions involved in complex folding, binding and aggregation processes.

In order to characterise the hydration processes better we undertook systematic, thermodynamic studies of nucleic acid bases and their alkyl derivatives [1–9].

The present work is devoted to the observation of the effect of interactions occurring in aqueous solutions between glycine, glycyl-glycine, triglycine, or L-α-alanyl-L-α-alanine and caffeine, as well as between glycyl-glycine or L-α-alanyl-L-α-alanine and 1,3-dimethyluracil. Two experimental techniques were applied in these studies. The densities and enthalpies of dilution were measured. From the determined densities, the apparent molar volumes of transfer of the studied compounds from water to aqueous solutions of glycine and the mentioned oligopeptides were calculated. The measured enthalpies of dilution of the ternary solutions were analysed in terms of McMillan–Mayer formalism, in order to obtain the enthalpic virial coefficients.

2. Experimental

1,3-Dimethyluracil was synthesised in the Laboratory of M. Dramiński at the Military Medicine University of Łódź (Poland). It was thoroughly purified by repeated crystallisation and repeated vacuum sublimation and then carefully dried for several days before use. Caffeine, glycine, glycyl-glycine, triglycine and L-α-alanyl-L-α-ananine were purchased from Sigma. All the chemicals were of the highest available purity and were used after drying in a vacuum for 72 h. The solutions were prepared by weight using degassed and deionised distilled water.

The measurements of the dilution enthalpies of the aqueous solutions were performed using a batch LKB 10700-2 microcalorimeter at 298.15 ± 0.01 K. The method of measurement was described elsewhere [9].

The densities of the solutions relative to the density of pure water were measured using an Anton Paar DMA 60/602 digital densimeter. The measurements were carried out at 298.15 ± 0.01 K. The temperature stability achieved using a Hetoterm Model C8-7 Thermostat was ±0.002 K. The calibration constant of the densimeter was determined daily using the known densities of water (0.997045 g cm^{-3} at 298.15 K) [10] and dry air [11]. Under these conditions the uncertainties were equal to 5×10^{-6} g cm^{-3}. The accuracy of the densimeter was controlled by determining the known densities of standard liquids (toluene, cyclohexane, chlorobenzene and isooctane) obtained from the Polish Committee of Standards, Warsaw.

3. Results and discussion

The results of calorimetric measurements of the dilution enthalpies are presented in Tables 1 and 2. The measured densities are collected in Tables 3 and 4.

The dilution enthalpies of caffeine and 1,3-dimethyluracil have already been reported in our earlier paper [12] and in papers of other authors, for example, Ref.

Table 1
Enthalpies of dilution from concentration m^i to m^f of caffeine and 1,3-dimethyluracil in water at 298.15 K

m^i/ (mol kg^{-1})	m^f/ (mol kg^{-1})	ΔH_{dil}/ (J mol^{-1})	m^i/ (mol kg^{-1})	m^f/ (mol kg^{-1})	ΔH_{dil}/ (J mol^{-1})
Caffeine			1,3-Dimethyluracil (cont.)		
0.08290	0.05528	810.2	0.69847	0.49513	42.42
0.06904	0.04541	820.2	0.68664	0.49538	40.54
0.06245	0.04114	798.4	0.49538	0.35935	44.27
0.05528	0.03701	753.7	0.49513	0.35882	43.06
0.04939	0.03253	764.6	0.48192	0.31540	56.30
0.04541	0.03057	718.9	0.35935	0.26027	42.81
0.04114	0.02757	691.1	0.35882	0.25931	43.94
0.03701	0.02479	699.8	0.31540	0.20751	51.60
0.03253	0.02182	660.4	0.26027	0.19040	38.88
0.03057	0.02054	641.3	0.25931	0.18949	37.53
0.02757	0.01848	631.7	0.20989	0.13826	42.80
0.02182	0.01470	531.3	0.20751	0.13749	41.98
1,3-Dimethyluracil			0.18949	0.13938	31.66
0.98428	0.69847	31.45	0.13826	0.09167	30.26
0.96000	0.68664	32.15	0.13938	0.10314	26.79
0.74325	0.48192	53.18	0.13749	0.09160	29.27
0.72377	0.48510	48.81	0.09167	0.06090	23.67

[13]. The values obtained in this work agree well with the literature data. Highly endothermic effects of dilution suggest that quite strong solute–solute interactions occur in the solution. The results of investigations reported in the literature clearly indicate that caffeine has a strong association tendency in aqueous solution [14]. This is also reflected in the high positive values of the Kirkwood–Buff G_{cc} integral [15]. Recently, Lilley et al. [12] showed that the highly non-ideal caffeine solutions in water can be described by self-association, applying the isodesmic model of solute association. It can be supposed that the reasons for the high negative H^e values for 1,3-dimethyluracil in aqueous solution are similar.

The measured densities of the caffeine solutions (Table 3) are in very good agreement with the analogous literature data [15].

3.1. Treatment of the experimental data

The treatment of the data obtained from the dilution experiments involving ternary aqueous solutions is based on the excess enthalpy expression [16–18]

$$H^e(m_x,m_y) = H(m_x,m_y) - H^*_w - m_x H^\ominus_x - m_y H^\ominus_y \tag{1}$$

where $H^e(m_x,m_y)$ and $H(m_x,m_y)$ are referred to the amount of the solution containing 1 kg of water; m_x and m_y are the molalities of the solutes x and y, respectively; $H(m_x,m_y)$ is the enthalpy of the solution, H^*_w, the standard enthalpy of 1 kg of water, and $H^\ominus$ the standard partial molal enthalpy of each solute species.

Table 2
Enthalpies of dilution from concentration m^i to m^f of caffeine and 1,3-dimethyluracil (x) with aqueous solutions of peptides (y)

m_x^i/(mol kg^{-1})	m_x^f/(mol kg^{-1})	m_y^i/(mol kg^{-1})	m_y^f/(mol kg^{-1})	ΔH_{dil}/(J kg^{-1})	ΔH^{**}/(J kg^{-1})
Caffeine + glycine					
0.07229	0.04741	0.06897	0.04523	38.17	1.292
0.04741	0.03177	0.04523	0.03031	22.76	0.421
0.06152	0.04054	0.06108	0.04025	33.18	1.195
0.05887	0.03886	0.05283	0.03487	27.90	0.593
0.03886	0.02600	0.03487	0.02333	16.09	0.029
0.06868	0.04510	0.06599	0.04333	36.14	1.131
0.04510	0.03007	0.04333	0.02889	21.66	0.307
0.05822	0.03819	0.06887	0.04517	37.90	1.270
0.03819	0.02549	0.04517	0.03015	22.52	0.171
0.05152	0.03390	0.04934	0.03246	25.63	0.281
0.03390	0.02276	0.03246	0.02179	14.36	−0.107
Caffeine + glycyl-glycine					
0.06725	0.04433	0.06613	0.04359	36.64	1.401
0.06934	0.04575	0.06824	0.04503	38.16	1.692
0.05038	0.03324	0.05076	0.03349	26.84	0.586
0.05191	0.03464	0.05223	0.03486	27.30	0.604
0.05256	0.03463	0.04850	0.03196	25.62	0.586
0.04433	0.02974	0.04359	0.02925	21.83	0.365
0.03962	0.02653	0.03788	0.02536	18.25	0.213
0.03324	0.02229	0.03349	0.02246	15.33	0.118
Caffeine + triglycine					
0.07114	0.04645	0.07063	0.04612	41.51	1.939
0.06879	0.04525	0.06899	0.04538	39.75	1.674
0.06590	0.04389	0.06548	0.04361	36.58	1.315
0.06084	0.03991	0.06018	0.03948	33.89	1.134
0.05664	0.03710	0.05652	0.03702	31.40	0.812
0.05301	0.03466	0.05207	0.03405	28.64	0.662
0.04551	0.03030	0.04601	0.03063	23.97	0.380
0.03991	0.02666	0.03948	0.02637	19.65	0.234
Caffeine + L-α-alanyl-L-α-alanine					
0.06989	0.04603	0.06894	0.04541	34.75	−0.388
0.03082	0.02070	0.03040	0.02042	12.48	−0.394
0.05137	0.03426	0.05159	0.03441	24.94	−0.419
0.05953	0.03906	0.06050	0.03970	30.42	−0.328
0.05600	0.03742	0.05551	0.03709	27.03	−0.320
0.03742	0.02513	0.03709	0.02491	16.58	−0.341
0.04916	0.03283	0.04885	0.03263	23.26	−0.551
0.03283	0.02199	0.03263	0.02186	13.88	−0.403
0.06238	0.04103	0.06010	0.03953	29.80	−0.558
0.04430	0.02913	0.04583	0.03014	22.15	−0.482
0.02913	0.01953	0.03014	0.02021	12.44	−0.321
1,3-Dimethyluracil + glycyl-glycine					
0.1946	0.1287	0.1971	0.1304	13.07	1.426
0.1287	0.0857	0.1304	0.0868	6.24	0.825

Table 2 (continued)

m_x^i/(mol kg^{-1})	m_x^f/(mol kg^{-1})	m_y^i/(mol kg^{-1})	m_y^f/(mol kg^{-1})	ΔH_{dil}/(J kg^{-1})	ΔH^{**}/(J kg^{-1})
0.0857	0.0573	0.0868	0.0580	2.92	0.419
0.1578	0.1040	0.1578	0.1040	8.89	0.992
0.1040	00696	0.1040	0.0696	4.16	0.602
0.0696	0.0467	0.0696	0.0467	1.95	0.301
0.1988	0.1307	0.1015	0.0667	9.01	0.730
0.0873	0.0584	0.0445	0.0298	1.87	0.220
0.2434	0.1609	0.1243	0.0822	13.13	1.046
0.1609	0.1074	0.0822	0.0549	5.91	0.470
0.1248	0.0822	0.2482	0.1635	11.61	1.185
0.0822	0.0547	0.1635	0.1088	5.68	0.645
0.0547	0.0366	0.1088	0.0728	2.71	0.317
0.1011	0.0667	0.1979	0.1306	7.80	0.638
1,3-Dimethyluracil + L-α-alanyl-L-α-alanine					
0.2437	0.1609	0.2432	0.1605	−21.94	−18.300
0.1609	0.1069	0.1605	0.1067	−9.57	−8.326
0.1069	0.0714	0.1067	0.0713	−3.97	−3.529
0.1976	0.1304	0.1970	0.1299	−14.29	−12.160
0.1304	0.0868	0.1299	0.0865	−5.94	−4.950
0.0868	0.0580	0.0865	0.0578	−2.74	−2.469
0.2451	0.1613	0.1226	0.0807	−18.74	−9.812
0.1613	0.1082	0.0807	0.0541	−7.87	−3.962
0.1931	0.1280	0.0982	0.0651	−11.47	−5.917
0.1280	0.0851	0.0651	0.0433	−4.94	−2.453
0.1256	0.0826	0.2498	0.1643	−4.95	−9.666
0.0826	0.0550	0.1643	0.1094	−1.64	−4.176
0.0550	0.0367	0.1094	0.0729	−0.62	−1.903
0.1749	0.1157	0.1785	0.1180	−11.13	−9.756
0.1157	0.0772	0.1180	0.0788	−5.01	−4.557

Using the McMillan–Mayer approach [19], the excess enthalpy of a ternary solution per 1 kg of water can be expressed as a power series in the molalities [16,17,20]

$$H^e(m_x,m_y) = h_{xx}m_x^2 + 2h_{xy}m_xm_y + h_{yy}m_y^2 + h_{xxx}m_x^3 + 3h_{xxy}m_x^2m_y + 3h_{xyy}m_xm_y^2 + h_{yyy}m_y^3 + \cdots \qquad (2)$$

where h_{ij}, h_{ijk} are the enthalpic virial coefficients characterising the pair and triplet interactions of the solvated species. For the binary solution, only the terms relating to solute x remain in Eq. (2).

The experimental dilution enthalpy of the ternary solution can be expressed as a sum of three terms. In order to calculate the coefficients in Eq. (2) on the basis of the experimental dilution data, an auxiliary function has been introduced [20]

$$\Delta H^{**} = \Delta H_{dil}[(m_x^i,m_y^i) \rightarrow (m_x^f,m_y^f)] - \Delta H_{dil}(m_x^i \rightarrow m_x^f) - \Delta H_{dil}(m_y^i \rightarrow m_y^f) \qquad (3)$$

Table 3
Densities and apparent molar volumes of aqueous solutions of caffeine

No.	m/ (mol kg^{-1})	d/ (g cm^{-3})	V_ϕ/ (cm^3 mol^{-1})	No.	m/ (mol kg^{-1})	d/ (g cm^{-3})	V_ϕ/ (cm^3 mol^{-1})
1	0.03045	0.998581	143.74	9	0.08005	1.001176	142.25
2	0.05021	0.999611	142.96	10	0.08045	1.001199	142.22
3	0.05148	0.999674	142.99	11	0.08701	1.001516	142.42
4	0.05546	0.999877	142.96	12	0.09029	1.001691	142.33
5	0.06416	1.000337	142.66	13	0.09047	1.001701	142.32
6	0.06960	1.000627	142.46	14	0.10016	1.002210	142.14
7	0.07063	1.000671	142.58	15	0.10016	1.002212	142.12
8	0.07563	1.000945	142.32				

where the first term is the enthalpy of dilution of the ternary solution, and the second and third are the enthalpies of dilution of the corresponding binary solutions per 1 kg of water; m^{i} and m^{f} are the initial and final molalities of the solutes, respectively. The enthalpy of dilution of the binary solution is defined as

$$\Delta H_{\mathrm{dil}}(m_x^{\mathrm{i}} \to m_x^{\mathrm{f}}) = h_{xx} m_x^{\mathrm{f}}(m_x^{\mathrm{f}} - m_x^{\mathrm{i}}) + h_{xxx} m_x^{\mathrm{f}}(m_x^{\mathrm{f}} - m_x^{\mathrm{i}})(m_x^{\mathrm{f}} + m_x^{\mathrm{i}}) + \cdots \tag{4}$$

The equation for the cross interaction coefficients can be evaluated from the following combination of Eqs. (3) and (4)

$$\begin{aligned}\Delta H^{**} &= 2h_{xy} m_y^{\mathrm{f}}(m_x^{\mathrm{f}} - m_x^{\mathrm{i}}) + 3h_{xxy} m_y^{\mathrm{f}}(m_x^{\mathrm{f}} - m_x^{\mathrm{i}})(m_x^{\mathrm{f}} + m_x^{\mathrm{i}}) \\ &\quad + 3h_{xyy} m_y^{\mathrm{f}}(m_x^{\mathrm{f}} - m_x^{\mathrm{i}})(m_y^{\mathrm{f}} + m_y^{\mathrm{i}}) + \cdots \end{aligned} \tag{5}$$

The values of ΔH^{**} were obtained from the experimental values of the dilution enthalpies in binary and ternary solutions, presented in Tables 1 and 2. The dilution enthalpies of the aqueous binary solutions of the peptides were obtained previously [17,21].

The apparent molar volumes V_ϕ were calculated from the measured densities of the solutions (Tables 3 and 4) according to the relation

$$V_\phi = M/d - 1000(d - d_0)/mdd_0 \tag{6}$$

where M is the molar mass of the solute, d and d_0 are the densities of the solution and solvent, respectively, and m is the molality of the solution. In the case of the three-component systems, caffeine/1,3-dimethyluracil–oligopeptide–water, d_0 denotes the oligopeptide solution density and m is the molality of caffeine or 1,3-dimethyluracil, expressed in moles per kg of the oligopeptide solution. The V_ϕ were fitted to an equation of the form

$$V_\phi = V_\phi^0 + A_{\mathrm{v}} m \tag{7}$$

where V_ϕ^0 is the apparent molar volume at infinite dilution and A_{v} is the experimental slope coefficient. The values of V_ϕ^0 are assumed to be identical to the partial molar volumes of the solute $\bar{V}_2^0$. Neglecting the error in the solution molality and

Table 4
Densities and apparent molar volumes of aqueous solutions of caffeine(1) and 1,3-dimethyluracil(1) with peptides(2)

No.	$m/(\mathrm{mol\ kg^{-1}})$	$d/(\mathrm{g\ cm^{-3}})$	$V_\phi/(\mathrm{cm^3\ mol^{-1}})$	No.	$m/(\mathrm{mol\ kg^{-1}})$	$d/(\mathrm{g\ cm^{-3}})$	$V_\phi/(\mathrm{cm^3\ mol^{-1}})$
Caffeine(1) + glycine(2)							
	$m_0(2) = 0.47240$;	$d_0(2) = 1.011735$					
1	0.04020	1.013699	143.92	3	0.06321	1.014859	143.21
2	0.05043	1.014234	143.17	4	0.06888	1.015089	143.89
	$m_0(2) = 0.47804$;	$d_0(2) = 1.011793$					
5	0.02591	1.013067	143.71	6	0.04313	1.013934	143.15
	$m_0(2) = 0.47804$;	$d_0(2) = 1.011902$					
7	0.07377	1.015502	143.74				
	$m_0(2) = 0.47859$;	$d_0(2) = 1.011830$					
8	0.03836	1.013733	143.21	9	0.05108	1.014341	143.56
	$m_0(2) = 0.47235$;	$d_0(2) = 1.011630$					
10	0.02006	1.012621	143.59	11	0.04189	1.013703	143.32
	$m_0(2) = 0.47801$;	$d_0(2) = 1.011841$					
12	0.05515	1.014550	143.55	14	0.06086	1.014820	143.68
13	0.05518	1.014534	143.86				
Caffeine(1) + glycyl-glycine(2)							
	$m_0(2) = 0.50014$;	$d_0(2) = 1.023266$					
1	0.05904	1.026154	142.67				
	$m_0(2) = 0.50014$;	$d_0(2) = 1.023286$					
2	0.03398	1.024967	142.30	3	0.08888	1.027630	142.50
	$m_0(2) = 0.49955$;	$d_0(2) = 1.023314$					
4	0.04946	1.025732	142.75	5	0.06911	1.026688	142.68
	$m_0(2) = 0.49999$;	$d_0(2) = 1.023359$					
6	0.03869	1.025268	142.39	9	0.06473	1.026531	142.53
7	0.05400	1.026019	142.36	10	0.07529	1.027048	142.47
8	0.06034	1.026325	142.42				
	$m_0(2) = 0.49961$;	$d_0(2) = 1.023333$					
11	0.07820	1.027133	142.84				
Caffeine(1) + triglycine(2)							
	$m_0(2) = 0.24922$;	$d_0(2) = 1.015578$					
1	0.03510	1.017296	143.52	4	0.05996	1.018535	142.99
2	0.04531	1.017808	143.18	5	0.06566	1.018839	142.89
3	0.05545	1.018314	142.99	6	0.06892	1.018992	142.70
	$m_0(2) = 0.24566$;	$d_0(2) = 1.015330$					
7	0.03080	1.016840	143.49	9	0.05022	1.017809	143.02
8	0.04031	1.017311	143.30				
	$m_0(2) = 0.23738$;	$d_0(2) = 1.014723$					
10	0.03118	1.016263	143.21	12	0.06617	1.017994	142.92
11	0.06121	1.017745	143.00	13	0.07138	1.018271	142.61
Caffeine(1) + L-α-alanyl-L-α-alanine(2)							
	$m_0(2) = 0.50000$;	$d_0(2) = 1.020513$					
1	0.03855	1.022414	142.68	4	0.06898	1.023960	141.84
2	0.04767	1.022882	142.25	5	0.07871	1.024421	142.08
3	0.06042	1.023530	142.25	6	0.08815	1.024858	142.36
	$m_0(2) = 0.50000$;	$d_0(2) = 1.020524$					
7	0.05481	1.023228	142.55	9	0.08381	1.024681	142.09

Table 4 (continued)

No.	m/(mol kg^{-1})	d/(g cm^{-3})	V_ϕ/(cm^3 mol^{-1})	No.	m/(mol kg^{-1})	d/(g cm^{-3})	V_ϕ/(cm^3 mol^{-1})
8	0.06539	1.023801	141.72				
	$m_0(2) = 0.50029$;	$d_0(2) = 1.020791$					
10	0.06922	1.024269	141.54				
	$m_0(2) = 0.50029$;	$d_0(2) = 1.020800$					
11	0.07933	1.024750	141.91	12	0.08849	1.025246	141.41
1,3-Dimethyluracil(1) + glycyl-glycine(2)							
	$m_0(2) = 0.50001$;	$d_0(2) = 1.023285$					
1	0.13887	1.027285	109.00	2	0.19965	1.028995	109.01
	$m_0(2) = 0.50001$;	$d_0(2) = 1.023275$					
3	0.29757	1.031723	108.92				
	$m_0(2) = 0.50025$;	$d_0(2) = 1.023408$					
4	0.10030	1.026298	109.10	6	0.28782	1.031550	109.04
5	0.19531	1.028967	109.15				
	$m_0(2) = 0.50025$;	$d_0(2) = 1.023436$					
7	0.24545	1.030411	109.04	10	0.36546	1.033723	108.95
8	0.27164	1.031141	109.01	11	0.40091	1.034700	108.89
9	0.32817	1.032729	108.89				
1,3-Dimethyluracil(1) + L-α-alanyl-L-α-alanine(2)							
	$m_0(2) = 0.50010$;	$d_0(2) = 1.020836$					
1	0.17212	1.025868	108.67	6	0.30109	1.029514	108.68
2	0.19547	1.026529	108.71	7	0.32866	1.030285	108.67
3	0.22864	1.027475	108.69	8	0.34455	1.030723	108.67
4	0.24833	1.028031	108.69	9	0.36556	1.031319	108.63
5	0.28095	1.028934	108.74	10	0.40409	1.032362	108.66

considering only the error in density determination, the uncertainty δV_ϕ was calculated using

$$\delta V_\phi = -(m + 1000/m)\delta d/d^2 \tag{8}$$

where δd is the uncertainty in the solution density (5×10^{-6} g cm^{-3}). Weighting factors, inversely proportional to δV_ϕ^2, were included in the least-squares analysis using Eq. (4). Values of $\bar{V}_2^0$ and A_v, together with their standard deviations, are given in Table 4.

4. Discussion

4.1. Enthalpic pair interaction coefficients

The calculated values of the enthalpic pair interaction coefficients h_{xy} for the pairs of caffeine with glycine, glycyl-glycine, triglycine and L-α-alanyl-L-α-alanine, as well as for 1,3-dimethyluracil with glycyl-glycine and with L-α-alanyl-L-α-alanine in water are presented in Table 5. As can be seen from these data, the interactions

Table 5
The enthalpic pair interaction coefficients h_{xy} for the pairs of caffeine, 1,3-dimethyluracil and urea with glycine and zwitterionic oligopeptides

Peptide	h_{xy}/(J kg mol^{-2})		
	Caffeine	1,3-Dimethyluracil	Urea
Glycine	668(133)		−480 [a]
Glycyl-glycine	267(61)	−160(6)	−778 [b], −638 [c]
Triglycine	315(61)		
L-α-Alanyl-L-α-alanine	1504(59)	713(18)	−354 [b]

[a] Ref. [22]. [b] Ref. [23]. [c] Ref. [24].

between caffeine and glycine or the investigated zwitterionic oligopeptides in aqueous solution are thermochemically unfavourable. This observation seems to be surprising with regard to the properties and structure of the interacting species. The caffeine and the other cosolutes investigated here contain in their molecules functional groups that can interact by means of both dipole–dipole interaction and hydrogen bond formation. Moreover, the presence of two charged ionic groups in the molecules of glycine and the oligopeptides also enables ion–dipole interactions. These interactions, however, seem to be relatively weak due to the strong hydration of the solute molecules or the steric hindrance. As a consequence, the endothermic effect of dehydration dominates over caffeine–cosolute attraction forces, which results in the observed positive h_{xy} values.

The dependence of h_{xy} on the size of the investigated oligopeptides is not clear. The interaction coefficients become more positive, as expected, when the size of the side apolar group in the molecular increases. The addition of the second molecule of aminoacid to the glycine molecule decreases the h_{xy} value. These variations, however, are not regular. As can be seen from the data, the interaction coefficients for caffeine with glycyl-glycine and with triglycine are almost the same. It is probable that some endo- and exothermic effects of the interaction compensate each other or that, in both these systems, the caffeine molecule interacts with the same fragment of the oligopeptide particle, independently of the whole particle dimension.

The small amount of experimental data that refer to the systems containing 1,3-dimethyluracil does not allow h_{xy} values for 1,3-dimethyluracil–oligopeptide pairs to be analysed in a similar way. It can be noted, however, that in this case the interactions of the 1,3-dimethyluracil with glycyl-glycine are thermochemically favourable. Similarly to the systems analysed previously, an increase in the size of the apolar group in the oligopeptide molecule provides a significant positive contribution to the h_{xy} value.

It seemed interesting to compare the thermochemical properties of the systems investigated here with other analogous systems described in the literature. Table 5 contains the enthalpic pair interaction coefficients that refer to the interactions of urea with glycine [22], glycyl-glycine [22,23], and L-α-analyl-L-α-alanine [23] in water. In contrast to the pairs containing caffeine, the h_{xy} values for the urea–oligopeptide pairs are negative. It is possible that the urea molecule, which is not only a proton-acceptor (like caffeine of 1,3-dimethyluracil) but also a proton-donor, is able to interact more strongly than the nucleic acid bases with the oligopeptides under question. Nevertheless, the tendencies in the h_{xy} variation in the systems containing urea and caffeine are similar, although, in the case of pairs containing caffeine the differences in the h_{xy} values are larger. This means that caffeine differentiates the interactions with the oligopeptide zwitterions to a larger extent that urea does.

In order to get some additional information about the influence of different functional groups in the peptide molecule on the h_{xy} (caffeine–oligopeptide) values, the well-known Savage and Wood group additivity model (SWAG) [16] was applied. Due to the small amount of experimental data, the caffeine molecule was assumed to be the one functional group. For the glycine and the oligopeptide molecules, the CH_2 group and the peptide group (CONH, denoted "Pep") were distinguished. In addition, the terminal charged groups (NH_3^+ and COO^-) were assumed to be the one defined (denoted "Ion"). Applying the multiple linear regression method, the group contributions presented in Table 6 were calculated. For the sake of comparison, the literature data that refer to the interactions of urea with the same zwitterionic oligopeptides were analysed analogously (Table 6). As can be seen from the table, the interactions of both caffeine and urea with the apolar CH_2 group are positive, as expected. They represent a thermochemical repulsion between the solvated polar molecule (caffeine or urea) and the solvated apolar group. The group coefficients that describe the interactions with a strongly polar peptide group are negative, which suggests attraction forces of dipole–dipole type, or even hydrogen bond formation. The most surprising result was obtained in the case of caffeine–ionic group interaction. The group coefficient for this contribution is very small (statistically negligible) which can mean that the caffeine molecule does not interact with the charged terminal groups in the zwitterionic oligopeptides, due to the very strong and stable hydration shields of the ionic groups. Another explanation of this observation may be an energetic compensation of endothermic (dehydration) and exothermic (ion–dipole attraction) effects connected with this type of interaction.

Table 6
Group additivity coefficients h_{xj} (in J kg mol^{-2}) in water

Group coefficient	X is caffeine	X is 1,3-dimethyluracil	X is urea
X–CH_2	544(106)	436	190
X–"Pep"	−720(167)	[a]	−444
X–"Ion"	50(224)	[a]	−670

[a] The sum of the (X–"Pep") and (X–"Ion") contributions, 1030 J kg mol^{-2}.

In the case of pairs containing 1,3-dimethyluracil, it was not possible to determine the group coefficients. From the comparison of the h_{xy} data for 1,3-dimethyluracil + glycyl-glycine and 1,3-dimethyluracil + L-α-alanyl-L-α-alanine pairs, it can be only stated that the introduction of the CH_2 group into the oligopeptide molecule causes an increase in the h_{xy} value and that this growth is smaller than that in the case of caffeine. The sum of the effects of the 1,3-dimethyluracil interactions with the peptide group and with the terminal ionic groups is strongly negative (Table 6).

Because the group coefficients result from the energetic effect of a partial dehydration of the interacting species (groups or molecules) and the attraction forces, the data from Table 6 suggest that the strength of hydration cospheres of the analysed compounds decreases in the order caffeine > 1,3-dimethyluracil > urea.

Some additional information about the solute–cosolute interactions can be obtained from the analysis of volumetric properties of the systems under question.

4.2. Molar volumes

The apparent molar volumes for both caffeine and 1,3-dimethyluracil in aqueous solution decrease along with the increase in the solute molality (Table 4). As is known, the negative slope of the $V_\phi = f(m)$ function can be attributed to a reduction in the hydrophobic hydration through an overlap of the cospheres [24,25]. Therefore, these results confirm the opinion presented earlier on the strong solute–solute interactions in the solution of caffeine. The addition of glycine or glycyl-glycine into the caffeine aqueous solution seems to disturb the caffeine self-association process. The slope A_v of the $V_\phi = f(m_{caf})$ function becomes positive (Table 7).

The partial molar volumes for both caffeine and 1,3-dimethyluracil in the infinitely diluted aqueous solution do not depend on the presence of the examined oligopeptides.

Table 7
Partial molar volumes of caffeine and 1,3-dimethyluracil in binary and ternary aqueous solutions

	$V_2^0/(cm^3\ mol^{-1})$	$A_v/(cm^3\ kg\ mol^{-2})$
Caffeine	143.7(0.3)	−16.3
Caffeine + glycine	143.4(0.3)	2.9
Caffeine + glycyl-glycine	142.4(0.3)	2.4
Caffeine + triglycine	144.0(0.1)	−17.3
Caffeine + L-α-alanyl-L-α-alanine	142.7(0.3)	−9.1
1,3-Dimethyluracil	109.2(0.2) [a]	−1.3 [a]
1,3-Dimethyluracil + glycyl-glycine	109.2(0.1)	−0.8
1,3-Dimethyluracil + L-α-alanyl-L-α-alanine	108.8(0.1)	−0.2

[a] From Ref. [3].

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