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THERMODYNAMIC INVESTIGATION OF SALTING EFFECT OF OVALBUMIN IN VARIOUS SALTS SOLUTIONS

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Calorimetric and volumetric investigations of ovalbumin salting by sodium chloride, lithium chloride, potassium chloride, lithium sulfate and ammonium sulfate buffered solutions in the range of concentrations of about 0.2–1.2 M of salts are reported. The evolution of enthalpies and apparent molar volumes *vs.* salts concentration was investigated using ITC MicroCal titration microcalorimeter and Anton Paar DMA 60/602 digital densimeter. It was found that the changes in enthalpies of salting and apparent molar volumes follow a similar trend. Conclusions about the ability of salts: to precipitate ovalbumin solution are presented.

Keywords: *apparent molar volume, enthalpy of salting, ovalbumin*

Introduction

The mechanism of the salting processes has been the subject of investigations for well over 50 years [1-6]. However, only few investigations were based on the determination of the enthalpies changes accompanying the salting processes. The development of the high-sensitive conduction and titration microcalorimetry as well as scanning calorimetry enable this kind of research. In previous works [7–11] we have reported results on the changes of the enthalpies and apparent molar volumes of lysozyme and bovine albumin *vs.* salt concentrations. It was found that the enthalpies of salting of proteins could be represented as a function of the salt concentration, however there exists a region of salt concentrations in which we can observe distortions from monotonic decrease of the enthalpic values, attributed to specific salting process of a protein. Recently [8, 9] we have also use titration calorimetry for the determination of enthalpic pairwise interaction coefficients describing the lysozyme-lysozyme aggregation upon salt addition.

The purpose of this work is to report the results of experimental determination of changes of enthalpies and apparent molar volumes of ovalbumin solutions in buffer acetate *vs.* salt concentrations. The determinations were realized for a number of salts, that is sodium, lithium and potassium chlorides and ammonium and lithium sulfates.

Experimental

Salt free ovalbumin (no. A5503), lithium chloride (no. 62476), potassium chloride (no. 60128), ammonium sulfate (no. 09978), lithium sulfate (no. 62613) were purchased from Fluka Co., sodium chloride were obtained from Merck.

Before the measurements the ovalbumin powder was dissolved in 0.1 M acetic/NaOH buffer (pH 4.26), filtered through 0.22 μm syringe filter (Roth, Germany) and degassed. The pH value of the buffer as well as of the ovalbumin stock and NaCl buffered solutions were adjusted independently (±0.05 pH) on a Mettler Toledo pH-meter (MP 220).

The calorimetric experiments were realized at 25°C with the use of ITC Microcal OMEGA ultrasensitive titration calorimeter (MicroCal Inc.).

The enthalpy of salting in each l injection was evaluated according to a relation given below:

$$\Delta_{\mathrm{sal}} H_{\mathrm{m}}(l) = \frac{\sum_{\mathrm{l}=1}^{\mathrm{l}} \Delta H_{\mathrm{m}}(l)}{\sum_{\mathrm{l}=1}^{\mathrm{l}} n(l)} \tag{1}$$

where $\Delta H_{\mathrm{m}}(l)$ is the experimental enthalpy arising from mixing a solution of ovalbumin of a given concentration and a salt solution in l-th injection, corrected by subtraction of the corresponding integrated heat effects of ovalbumin injection to the pure buffer and heat effects of buffer injection to the salt solution; $n(l)$ is the number of moles added to the cell in a l injection. The data obtained in such a manner were used

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Table 1 The enthalpies of salting $\Delta_{sal}H_m$ for chloride salts

Sodium chloride		Lithium chloride		Potassium chloride	
m_y/M	$\Delta_{sal}H_m$/kJ mol^{-1}	m_y/M	$\Delta_{sal}H_m$/kJ mol^{-1}	m_y/M	$\Delta_{sal}H_m$/kJ mol^{-1}
0.2045	–2.92	0.3129	–2.27	0.3033	–4.51
0.3026	–6.63	0.4164	–4.77	0.4140	–7.18
0.4040	–8.33	0.5071	–3.95	0.5084	–15.54
0.5125	–7.69	0.6223	–3.02	0.6043	–9.43
0.6091	–9.57	0.6578	–0.64	0.7113	2.28
0.6564	–5.20	0.8259	10.30	0.8124	3.01
0.7037	6.71	0.8583	12.14	0.9074	2.77
0.7513	4.90	0.9060	10.42		
0.9142	3.40	0.9547	9.12		

to get the dependence $\Delta_{sal}H_m(l)=f(m_y)$, where m_y is the initial concentration of salt in the cell.

Densities were measured with an Anton Paar 60/602 digital densimeter at 25°C. Temperature stability achieved by a Hetoterm Model C8–9 thermostat was better than 0.01°C. The calibration constant of the densimeter was determined daily by using the known densities of water and dry air. The concentration of ovalbumin was above 0.001 M. The apparent molar volumes of the ovalbumin were calculated from solution density d using the usual expression [12].

Table 2 The enthalpies of salting $\Delta_{sal}H_m$ for sulfate salts

Ammonium sulfate		Lithium sulfate	
m_y/M	$\Delta_{sal}H_m$/kJ mol^{-1}	m_y/M	$\Delta_{sal}H_m$/kJ mol^{-1}
0.3052	–13.08	0.2030	0.70
0.4014	–11.97	0.3078	0.89
0.5005	–13.49	0.4087	–0.68
0.6055	–20.60	0.4999	–3.65
0.7052	–30.56	0.5549	–5.73
0.8032	–24.23	0.7090	–2.57
0.9089	–20.96	0.8022	1.71
1.0031	–18.40	0.8500	4.49
	–	0.9027	41.25
		1.2019	71.57

Results and discussion

The values of the changes of enthalpies were established by several measurements for each salt at different concentrations. The concentration of ovalbumin was practically the same, about 0.00016 M. The experimental data obtained from calorimetric titration were analyzed on the basis of 16 injections. Nine or eight independent measurements over the range from 0.2 to 1 M of salt concentrations were made. The enthalpies of salting $\Delta_{sal}H_m$ of chloride and ammonium salts calculated according to Eq. (1) are presented in Tables 1 and 2. The apparent molar volumes of ovalbumin in different salts concentrations are collected in Tables 3 and 4. Similarity in the evolution of

Table 3 Apparent molar volumes $V_{\phi,3}$ of ovalbumin for chloride salts

Sodium chloride		Lithium chloride		Potassium chloride	
m_y/M	$V_{\phi,3}$/cm^3 mol^{-1}	m_y/M	$V_{\phi,3}$/cm^3 mol^{-1}	m_y/M	$V_{\phi,3}$/cm^3 mol^{-1}
0.2076	33339	0.2048	33868	0.2072	33706
0.3053	33272	0.3097	33746	0.3130	33508
0.4056	33156	0.3534	33723	0.4026	33348
0.5111	33277	0.4032	33709	0.5141	33090
0.6072	33552	0.4567	33756	0.6015	33045
0.6640	33869	0.5124	33802	0.7025	32873
0.8005	33498	0.6045	33944	0.8027	33224
1.0064	34109	0.7060	34191	0.9010	33659
1.0735	34876	0.8005	34161	1.0000	34024
		0.9084	34452	1.1010	34453
		0.9541	33409		

Table 4 Apparent molar volumes $V_{\phi,3}$ of ovalbumin for sulfate salts

Ammonium sulfate		Lithium sulfate	
m_y/M	$V_{\phi,3}$/cm^3 mol^{-1}	m_y/M	$V_{\phi,3}$/cm^3 mol^{-1}
0.3018	33499	0.2026	33073
0.4015	33499	0.3032	33683
0.5021	33616	0.4070	33936
0.6082	33925	0.5014	33936
0.7008	33935	0.5993	33140
0.8035	33941	0.7017	33242
0.9071	34218	0.7518	33425
1.0042	34757	0.8002	34166
1.1064	34644	0.9131	35000

Table 5 Minimum (m_1) and maximum (m_2) values of enthalpy changes

Salt	m_1/M	$\Delta_{sal}H_m$/kJ mol^{-1}	m_2/M	$\Delta_{sal}H_m$/kJ mol^{-1}
LiCl	0.4164	–4.77	0.8583	12.14
NaCl	0.4040	–8.33	0.7037	6.71
KCl	0.5084	–15.54	0.8124	3.01
Li_2SO_4	0.3078	0.89	0.5549	–5.73
$(NH_4)_2SO_4$	0.4014	–11.97	0.7052	–30.56

Table 6 Minimum (m_1) and maximum (m_2) values of apparent molar volumes

Salt	m_1/M	$V_{\phi,3}$/cm^3 mol^{-1}	m_2/M	$V_{\phi,3}$/cm^3 mol^{-1}
LiCl	0.4032	33709	0.9084	34453
NaCl	0.4056	33156	0.6639	33868
KCl	0.7025	32873	–	–
Li_2SO_4	0.4070	33936	0.5993	33140
$(NH_4)_2SO_4$	0.4015	33499	1.0042	34757

enthalpies changes and apparent molar volumes *vs.* salt concentration is demonstrated by Figs 1–10. From the experimental data obtained it, observed that the course of changes of enthalpies and apparent molar volumes with concentration of salts is not linear. In all the series of experiments similar distortions were observed. These variations are attributed to the binding of ions to the binding sites on ovalbumin surface at concentrations m_1 and m_2 (Tables 5 and 6). The obtained parameter δm related to the process of protein salting is defined as a difference between minimum (m_1) and maximum (m_2) values of enthalpies changes and apparent molar volumes. In the case of: lithium chloride, sodium chloride, potassium chloride

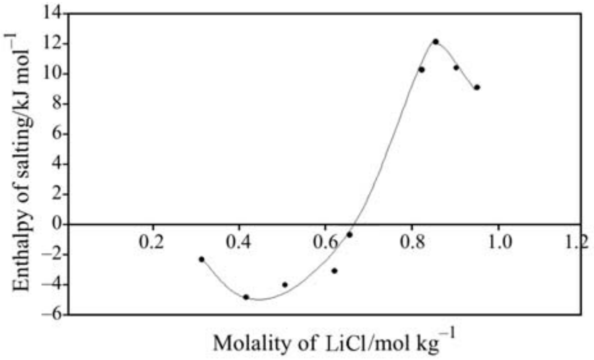

Fig. 1 The enthalpies of salting of ovalbumin solution expressed as a function of initial lithium chloride solution

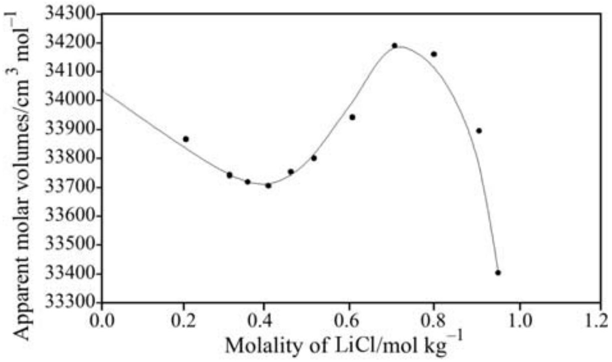

Fig. 2 Apparent molar volumes of ovalbumin solution expressed as a function of initial lithium chloride solution

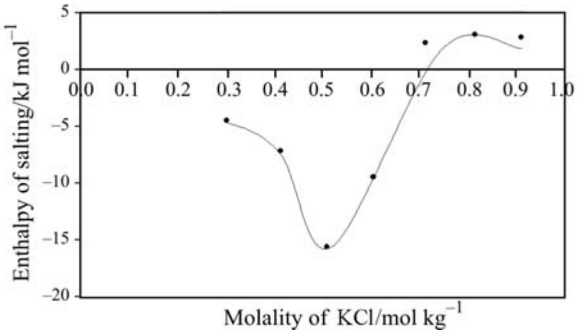

Fig. 3 The enthalpies of salting of ovalbumin solution expressed as a function of initial potassium chloride solution

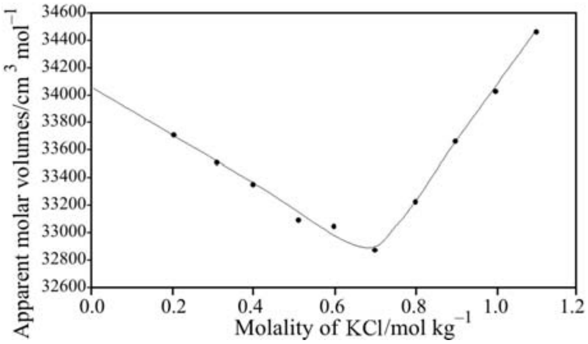

Fig. 4 Apparent molar volumes of ovalbumin solution expressed as a function of initial potassium chloride solution

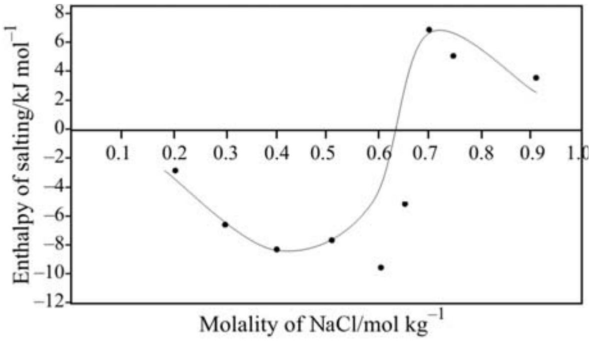

Fig. 5 The enthalpies of salting of ovalbumin solution expressed as a function of initial sodium chloride solution

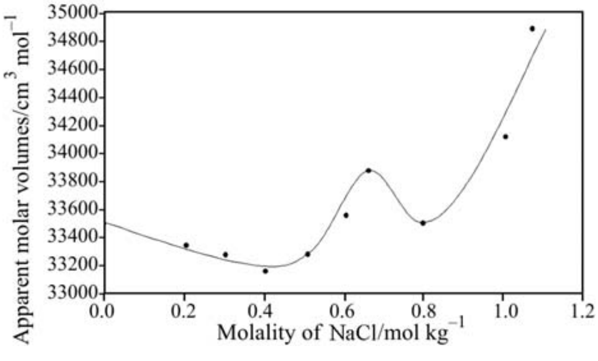

Fig. 6 Apparent molar volumes of ovalbumin solution expressed as a function of initial sodium chloride solution

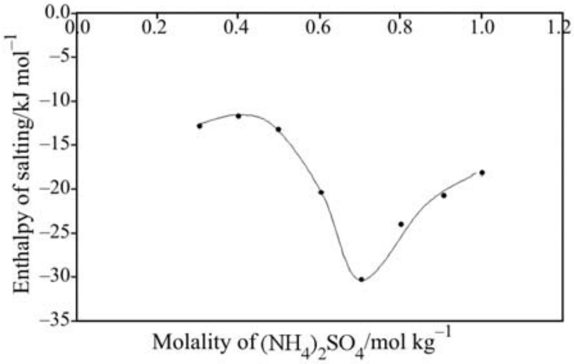

Fig. 7 The enthalpies of salting of ovalbumin solution expressed as a function of initial ammonium sulfate solution

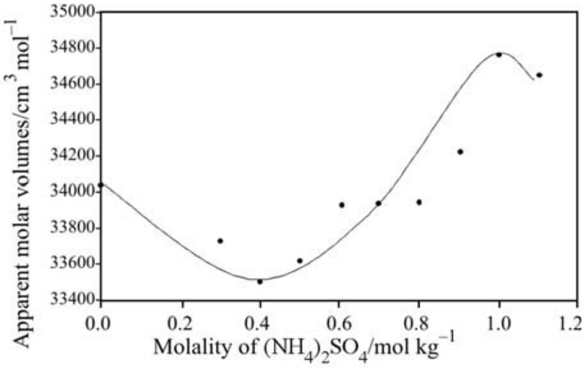

Fig. 8 Apparent molar volumes of ovalbumin solution expressed as a function of initial ammonium sulfate solution

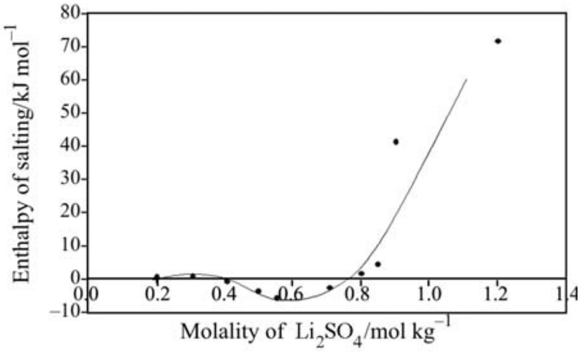

Fig. 9 The enthalpies of salting of ovalbumin solution expressed as a function of initial lithium sulfate solution

and ammonium sulfate the minimum appears at low salt concentration whereas the maximum at highest concentration. Inverse course of curves is observed in the case of lithium sulfate: concentration m_1 corresponds to a maximum in the change of the enthalpy and m_2 to a minimum.

Comparison of the obtained data with those presented elsewhere [13] demonstrates that the difference δm depends on the kind of protein and salt.

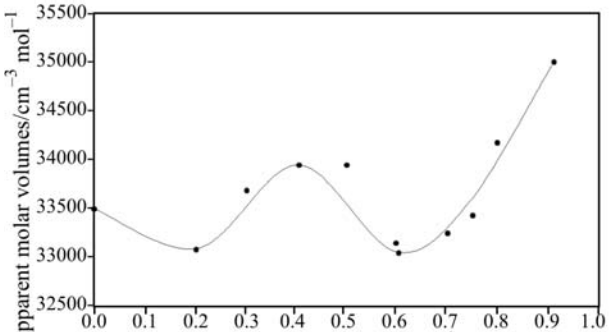

Fig. 10 Apparent molar volumes of ovalbumin solution expressed as a function of initial lithium sulfate solution

The evolution of the curves of enthalpies changes and apparent molar volumes of salting process suggests that the ability of investigated salt to precipitate ovalbumin solution changes in the following sequence: $Li^+>Na^+>K^+>NH_4^+$.

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References

1 S. N. Timasheff and G. D. Fashman, Structure and Stability of Biological Macromolecules, (1989) 417.
2 R. Giege, J. Drenght, A. F. Ducruix, A. McPherson and W. Seanger, Prog. Cryst. Growth Charact., 30 (1995) 237.
3 P. Retailleau, M. Ries-Kautt and A. Ducruix, Biophys. J., 73 (1997) 2156.
4 C. A. Schall, E. Arnold and J. M. Wiencek, J. Cryst. Growth, 165 (1996) 293.
5 L. Sibille and M. L. Pusey, Acta Cryst., D50 (1994) 396.
6 P. A. Darcy and J. C. Wiencek, Acta Cryst., D54 (1998) 1387.
7 M. Wszelaka-Rylik and W. Zielenkiewicz, J. Therm. Anal. Cal., 83 (2006) 607.
8 J. Poznański, M. Wszelaka-Rylik and W. Zielenkiewicz, Biophys. Chem., 113 (2005) 137.
9 J. Poznański, M. Wszelaka-Rylik and W. Zielenkiewicz, Thermochim. Acta, 409 (2004) 25.
10 A. Zielenkiewicz and W. Zielenkiewicz, J. Therm. Anal. Cal., 80 (2005) 407.
11 A. Zielenkiewicz and W. Zielenkiewicz, J. Mol. Liq., 121 (2005) 3.
12 A. Zielenkiewicz, K. Busseroles, G. Roux-Desranges, A. H. Roux, J-P. E. Grolier and W. Zielenkiewicz, J. Solution Chem., 24 (1995) 623.
13 A. Zielenkiewicz, J. Therm. Anal. Cal, in press.

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