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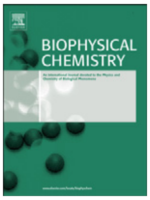

Compatible solutes: Thermodynamic properties and biological impact of ectoines and prolines

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ABSTRACT

Compatible solutes like ectoine and its derivatives are deployed by halophile organisms as osmolytes to sustain the high salt concentration in the environment. This work investigates the relation of the thermodynamic properties of compatible solutes and their impact as osmolytes. The ectoines considered in this work are ectoine, hydroxyectoine, and homoectoine. Besides solution densities (15–45 °C) and solubilities in water (3–80 °C), component activity coefficients in the aqueous solutions were determined in the temperature range between 0 and 50 °C. The latter is important for adjusting a certain water activity and therewith a respective osmotic pressure within a cell. The characteristic effect of ectoines is compared to that of prolines, as well as to that of incompatible solutes as salts and urea. The experimental results show that the influence on the activity (coefficient) of water is quite different for compatible and incompatible solutes: whereas compatible solutes cause decreasing water activity coefficients, incompatible solutes lead to an increase in water activity coefficients. Based on this quantity, the paper discusses the impact of various osmolytes on biological systems and contributes to the explanation why some osmolytes are more often and at other temperatures used than others. Moreover, it was found that the anti-stress effect of an osmolyte is weakened in the presence of a salt.

Finally, it is shown that the thermodynamic properties of compatible solutes can be modeled and even predicted using the thermodynamic model PC-SAFT (Perturbed-Chain Statistical Associating Fluid Theory).

1. Introduction

Many microorganisms are known to live under high-stress conditions. Stresses can be caused e.g. by very high or very low temperatures, by non-neutral pH values, or by extreme salt concentrations. To live in environments with extreme salt concentrations, various microorganisms produce so-called osmolytes which are organic compounds of low molecular weight. In contrast to electrolytes they do not influence the cellular metabolism, are non-toxic, and therefore also known as “compatible solutes”. One of the most-investigated compatible solutes is ectoine which is deployed e.g. by the halophilic microorganism *Halomonas elongata*.

Since 1998, ectoine is also produced at an industrial scale by so-called “bacterial milking” [1]. Due to its stabilizing effect to biological cell membranes, today ectoine is used e.g. in skin-protection and health-care applications and is an important additive of more than 200 biochemical, medical, and cosmetic products.

The influence of osmolytes like ectoine on biological solutions (e.g. protein systems) has already been earlier investigated phenomenologically. Several beneficial effects of ectoine against denaturizing stresses were observed, e.g. by Kolp et al. [2] (protection against activation of zymogens), by Schnoor et al. [3] (PCR enhancer), by Goller and Galinski [4] (protection against heat, urea, and freezing), by Galinski and Truper [5] (protection against salt stress) and others [6–8].

Concerning the protection against salt stress, there already exist some general observations: halophilic/halotolerant microorganisms (1) produce different osmolytes or (2) pump in ions from the environment to protect themselves against salt stress [7]. Moreover (3), at least one organism (*H. elongata*) even produces different osmolytes at different temperatures [7]. Moreover, it is well accepted that the ability of a molecule to act as biological osmolyte is mostly determined by the properties of the osmolyte/water binary system. However, a detailed investigation of the thermodynamic properties of those systems is so far missing.

From the thermodynamic point of view, the osmolyte reduces the chemical potential (activity) of water and therewith increases the osmotic pressure [9,10]. As these two quantities are strongly related to the water activity coefficients, this quantity will be examined (measured and modeled) throughout this work.

This paper will present new experimental data and thermodynamic modeling of osmolyte solutions and will use this approach to quantitatively estimate and explain the impact of osmolytes to

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aqueous systems. In particular the following thermodynamic properties of ectoine solutions will be investigated: solution density which is important for the design of apparatus and reservoirs, activity coefficients which reveal interactions with other system components and determine e.g. the osmotic pressure in a cell, and water solubility which is required to function in biological systems.

Fig. 1 shows the different ectoine derivatives considered within this work: hydroxyectoine, which has an additional hydroxyl group and homoectoine with an additional methylene group inserted into the ectoine ring.

As ectoine is often compared to the amino acid L-proline, it is also considered within this work. Moreover, we will compare the effect of ectoines to those of other substances (salts and urea) which are known as incompatible solutes.

2. Experimental work

2.1. Materials and reagents

Ectoine, hydroxyectoine and homoectoine were obtained from the bitop AG in Witten, Germany with high purity (>99%). They were used as obtained. For the calibration of the osmometers, solutions of sodium chloride (Merck, >99.5%) were applied. All solutions were prepared gravimetrically by weighing with an uncertainty of 0.01 mg. Water from the Millipore purification system was used for the preparation of all aqueous solutions.

2.2. Density measurements

Densities of ectoine solutions in water were determined with a vibrating-tube densimeter "DMA 602" from Anton Paar Germany GmbH (Ostfildern, Germany) at ambient pressure and temperatures between 15 and 45 °C. For the measurements, a u-tube is filled with the fluid of interest and set into oscillation by an electromagnetic field. Densities are obtained by measuring the eigenfrequency of the filled u-tube. The apparatus was calibrated with air and deionized water (data taken from [11]). According to the manufacturer, the maximum uncertainty of this apparatus is within $\pm 1.5 \cdot 10^{-6}$ g/cm³.

2.3. Measurements of water activity coefficients

The measurements were performed with a vapor-pressure osmometer at temperatures between 30 and 50 °C. Experimental activity coefficients at the freezing point were obtained by a cryoscopic osmometer.

The vapor-pressure osmometer "Osmomat O70" by Gonotec (Berlin, Germany) used in this work allows for measurements in one-solvent solutions at concentrations between 0.005 and 3.0 mol solute per kg water [mol/kg]. It was already applied earlier (Held et al. [12]) to determine solvent activity coefficients in amino-acid solutions. Measurements can be performed at temperatures up to the boiling point of the solvent. The measuring cell of the Osmomat O70 consists of two thermistors placed in a tempered water-saturated atmosphere. With the help of a syringe, a droplet is placed at the end of each of the thermistors: one droplet being water, the other one being the aqueous solution of the solute of interest. Being at the same solvent pressure, the temperature difference between the two droplets is detected. This value can be converted into the osmotic coefficient or the solvent activity coefficient [13].

In order to also determine the activity coefficients at the freezing point we used the cryoscopic osmometer "K-7400" by Knauer (Berlin, Germany). This method is based on the freezing-point depression caused by a solute dissolved in water. The first step of the measurement is to supercool the solution without freezing it. The freezing is afterward initiated by vibrating and the freezing-point temperature is measured. The freezing-point depression compared to the freezing point of pure water is a measure for the osmolality of the solution and thus for osmotic and water activity coefficients, respectively. The K-7400 allows for the measurements at maximum concentrations of 2.0 mol/kg. According to Knauer, the experimental uncertainty (relative standard deviation) is below 1%.

Before carrying out the measurements, the two osmometers had to be calibrated. This was done with sodium chloride solutions of different molalities (0.05–1.2 mol/kg for the vapor-pressure osmometer, 0–0.45 mol/kg for the freezing-point osmometer). Thereby, reference values from literature [14] were used. Experiments for the ectoines in water were performed at 0 °C, 30 °C, and 50 °C, respectively. All measurements were repeated until a constant temperature difference could be observed.

2.4. Solubility measurements

The solubility of the solutes considered in this work was determined gravimetrically as also described earlier, e.g. in [15,16]. First, the substances were filled into glass vials (20 ml) and purified water from the Millipore system was added to an extent that a supersaturated solution was obtained. These vials were placed into a rotary oven with a temperature deviation of ±0.3 K. After equilibration (48 h), a sample of 2 ml solution was withdrawn using a preheated syringe with a syringe filter (pore size 0.45 μm). The sample was weighed with an accuracy of 0.01 mg. After solvent evaporation in a drying chamber this sample was reweighed. In order to assure for total evaporation of the solvent, the sample was placed back into the drying chamber and was reweighed again after 24 h. At the point where no further weight decrease was observed the concentration of the sample (the solubility) was obtained from the weight difference of the sample before and after solvent evaporation.

3. Thermodynamic modeling

One aim of this work is the modeling of the various thermodynamic properties of aqueous ectoine solutions which may also contain electrolytes. Properties of interest are solution density, vapor-pressure depression (water activity coefficient), solute activity coefficient and solubility. For this purpose, we applied the ePC-SAFT equation of state. This model has already proven its flexibility and excellent performance in modeling complex systems containing amino acids [12,15,17], polymers [18–20], polar compounds [21,22], associating compounds [23], pharmaceuticals [24], and electrolytes [25–27].

The ePC-SAFT model is based on a perturbation theory. This type of theories uses a reference system which is easy enough to derive analytical expressions for the thermodynamic quantities (e.g. Helmholtz energy) but already covers the most-relevant properties of a molecule.

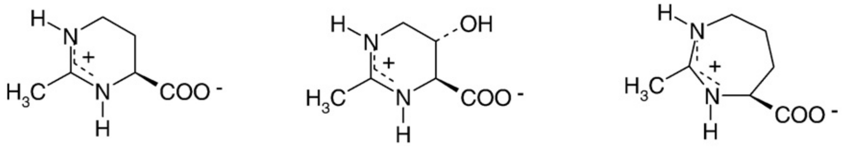

Fig. 1. The compatible solute ectoine (left) and two derivatives, the hydroxyectoine (middle) and the homoectoine (right).

As the thermodynamic behavior of a molecule is to a remarkable amount determined by its volume (which causes repulsive forces), a very often used reference for a real molecule is the so-called hard sphere. This is a spherical molecule having a fixed volume and no attractive interactions with other molecules. Deviations from that reference system, as e.g. from spherical shape or attractive interactions (e.g. due to van der Waals forces, hydrogen bonds, or charges) are usually considered as independent perturbations of the reference system and are described by additional contributions to the Helmholtz energy. ePC-SAFT considers the hard chain (a chain of spherical segments) as reference system instead of the hard-sphere system and is therefore in particular suitable for describing non-spherical and chain-like molecules as those considered in this work.

The residual Helmholtz energy a^{res} (deviation from ideal-gas state) is thus obtained as the sum of the contribution of the reference system hard chain (hc) as well as the contributions originating from the various perturbations:

$$a^{res} = a^{hc} + a^{disp} + a^{assoc} + a^{ion} \tag{1}$$

a^{disp}, a^{assoc}, and a^{ion} account for the Helmholtz energy contributions due to van der Waals (dispersive), associative, and Coulomb interactions, respectively. Whereas the expressions for a^{disp} and a^{assoc} are used as in the original PC-SAFT model [28], Cameretti et al. [25] introduced a Debye–Hückel term to account for the Helmholtz-energy contribution a^{ion} caused by charging the species (electrolyte PC-SAFT).

To describe a pure substance i, the model requires at maximum five parameters which have a physical meaning: the number of segments m_{seg}, the diameter of the segments σ_i, the van der Waals-interaction (dispersion) energy parameter between two segments of different molecules u_i/k_B. In case of associating molecules, one can define N association sites per molecule characterized by the association-energy parameter ε^{AiBi}/k_B and the association range κ^{AiBi}. For gases or liquids, these parameters are usually determined by fitting to pure-component thermodynamic properties as e.g. liquid-density or vapor pressure data. For solids, as e.g. amino acids or salts these parameters can be fitted to solution data (densities, vapor pressures, activity or osmotic coefficients).

For application to mixtures, conventional Berthelot–Lorenz — combining rules are used for two components i and j:

$$\sigma_{ij} = \frac{1}{2}\left(\sigma_i + \sigma_j\right) \tag{2}$$

$$u_{ij} = \sqrt{u_i u_j}\cdot\left(1-k_{ij}\right) \tag{3}$$

k_{ij} in Eq. (3) is a binary parameter that can be used to correct for deviations from the geometric mixing rule of the dispersion energy. This parameter (if required) is determined by fitting to the binary data, e.g. to activity coefficients or solubilities.

Eq. (3) is applied in this work to water/ion, water/ectoine, water/proline, and water/urea interactions. van der Waals interactions between two ions are neglected. Furthermore, according to our previous work [26], the k_{ij} parameter between water and an ion is set to zero.

Since amino-acid solutions could previously be described accurately using PC-SAFT without considering the charges of the zwitterions [12,15], the charge–charge interactions among the biomolecules are also neglected here for the modeling.

Once the residual Helmholtz energy a^{res} of a system is known, other properties can be derived, e.g. fugacity coefficients:

$$\ln\varphi_i = \frac{\mu_i^{res}}{k_B T} - \ln Z \tag{4}$$

with the real gas factor Z depending on the system's number density:

$$Z = 1 + \rho\left(\frac{\partial\left(a^{res}/k_B T\right)}{\partial\rho}\right) \tag{5}$$

The residual chemical potential μ^{res} of component i in Eq. (4) is obtained by:

$$\frac{\mu_i^{res}}{k_B T} = \frac{a^{res}}{k_B T} + Z - 1 + \left(\frac{\partial\left(a^{res}/k_B T\right)}{\partial x_i}\right) - \sum_{j-1}^{N} x_j\left(\frac{\partial\left(a^{res}/k_B T\right)}{\partial x_j}\right) \tag{6}$$

The fugacity coefficients obtained from Eq. (4) can be used to calculate activity coefficients. Rational activity coefficients γ^* of the solutes i reported in this work are normalized to infinite dilution and already converted into molality scale (for details see e. g. Ref. [14]). They are obtained by:

$$\gamma_i^{*,m} = \frac{\varphi_i^m(m_i)}{\varphi_i^{\infty,m}(m_i \to 0)} \tag{7}$$

where φ_i^m is the fugacity coefficient of component i in the mixture, and $\varphi_i^{\infty,m}$ ($m_i \to 0$) is the fugacity coefficient of the same component at infinite dilution. Water activity coefficients (WAC) are calculated by:

$$\gamma_w = \frac{\varphi_w(x_w)}{\varphi_{0w}(x_w = 1)} \tag{8}$$

In contrast to the rational activity coefficients γ^*, the water activity coefficients γ_w are normalized to the pure-component state, i.e. the fugacity coefficient of water φ_w in the mixture is related to the fugacity coefficient of pure water, φ_{0w}.

For the calculation of solubilities we applied the phase-equilibrium condition between the liquid and the solid phase. Assuming a pure solid phase and neglecting the influence of the difference in liquid and solid heat capacities, the mole fraction of the solute in the liquid phase (its solubility) can be calculated by [29]:

$$x_i^L = \frac{\varphi_{0i}^L}{\varphi_i^L}\cdot\exp\left\{-\frac{\Delta h_{0i}^{SL}}{RT}\left(1-\frac{T}{T_{0i}^{SL}}\right)\right\} \tag{9}$$

$\varphi_{0i}^L/\varphi_i^L$ is the ratio of the fugacity coefficients of component i (e.g. the ectoines) as pure substance and in the mixture, respectively. Δh_{0i}^{SL} is the melting enthalpy and T_{0i}^{SL} the melting temperature of the pure ectoines. As ectoines decompose before melting, these properties cannot be determined experimentally. Therefore, a group contribution method [30] was applied to estimate Δh_{0i}^{SL} and T_{0i}^{SL} as it was done earlier for solubility calculations in amino-acid systems [12,15].

4. Experimental results

4.1. Solution densities

The density of aqueous solutions of ectoine, hydroxyectoine, and homoectoine was measured at various ectoine concentrations at a minimum of two different temperatures between 15 and 45 °C and at atmospheric pressure. The results are summarized in Table 1.

4.2. Solubilities in water

The experimental determined solubilities of the considered systems are listed in Table 2. The gravimetrical method has already been proven for application in aqueous amino-acid systems at similar conditions [12,15]. Our experimental solubility data in the previous work agreed with the literature data within 3%. The solubility of the

Table 1
Experimental densities of aqueous solutions of ectoine(E), hydroxyectoine(HyE), and homoectoine(HoE) in water between 15 and 45 °C at ambient pressure.

Ectoine			Hydroxyectoine			Homoectoine		
T [K]	m_E [mol/kg]	Density [kg/m³]	T [K]	m_{HyE} [mol/kg]	Density [kg/m³]	T [K]	m_{HoE} [mol/kg]	Density [kg/m³]
288.17	0.0882	1002.63	288.17	0.0097	998.86	288.15	0.4000	1014.53
288.17	0.1757	1006.17	288.17	0.0293	999.89	288.15	0.8000	1028.44
288.17	0.3484	1013.22	288.17	0.0876	1003.23	288.15	1.2000	1041.47
288.17	0.6864	1027.21	288.17	0.2601	1012.44	288.15	1.4000	1047.59
288.17	1.3383	1055.30	288.17	0.7513	1040.64	298.15	0.4000	1011.97
288.17	2.5408	1110.84	288.17	2.0917	1121.41	298.15	0.8000	1025.63
298.24	0.0882	1000.54	298.20	0.0097	997.43	298.15	1.2000	1038.45
298.24	0.1757	1003.99	298.20	0.0293	998.47	298.15	1.4000	1044.45
298.24	0.3484	1010.94	298.20	0.0876	1001.53			
298.24	0.6864	1024.84	298.20	0.2601	1010.97			
298.24	1.3383	1052.66	298.20	0.7513	1038.09			
298.24	2.5408	1106.99	298.20	2.0917	1117.32			
318.17	0.0882	993.69	318.23	0.0097	990.64			
318.17	0.1757	997.08	318.23	0.0293	992.24			
318.17	0.3484	1003.95	318.23	0.0876	994.40			
318.17	0.6864	1017.45	318.23	0.2601	1004.07			
318.17	1.3383	1044.87	318.23	0.7513	1026.72			
318.17	2.5408	1098.37	318.23	2.0917	1110.24			

ectoines in water was measured between 3 and 80 °C. While the ectoine solubility possesses a strong temperature dependence, the solubility of hydroxyectoine in water does almost not change between 3 and 40 °C. This might be ascribed to the additional hydroxyl group within hydroxyectoine leading to stronger association forces between this solute and water, in particular at low temperatures. The associative interaction is known to decrease with increasing temperature thereby compensating for the commonly known solubility benefit at increasing temperatures.

4.3. Activity and osmotic coefficients

Microorganisms produce osmolytes to adjust the water activity (which prevents from getting lost of the water) and to compensate for the osmotic pressure in the habitat.

Often, the ideal osmotic pressure is used in literature which is defined as:

$$\pi^{\text{ideal}} = \frac{RT}{V} n_{\text{solute}} \tag{10}$$

where n_{solute} and V denote the mole number of solute molecules and the system volume at the temperature T, respectively. However, the real osmotic pressure of the system can remarkably deviate from this value which is described by the osmotic coefficient Φ:

$$\pi^{\text{real}} = \pi^{\text{ideal}} \cdot \Phi \tag{11}$$

which is defined as [31]:

$$\Phi = \frac{\ln(x_w \gamma_w)}{\ln x_w} \tag{12}$$

Here, γ_w and x_w are the activity coefficient and the mole fraction of water, respectively. However, in this work we apply a simplified expression which is commonly used in literature [13]:

$$\Phi = -\frac{\ln(x_w \gamma_w)}{M_w \sum \nu m_{\text{solute}}} \tag{13}$$

Thus, all experimental water activity coefficients presented in this work are calculated from Eq. (13). The measurement signal of the vapor-pressure osmometer stems from a boiling-point difference between the water/solute drop and the pure water drop. This quantity can be converted into the osmotic coefficient by:

$$\Phi = \frac{T_w^{\text{b,solution}} - T_{0w}^b}{K_{\text{eb}} \cdot \sum \nu_{\text{solute}} m_{\text{solute}}} \tag{14}$$

In contrast, cryoscopic measurements yield the freezing point difference between pure water and the ectoine solution (see e.g. [32]) which can again be used to obtain the osmotic coefficient:

$$\Phi = \frac{T_{0w}^f - T_w^{\text{f,solution}}}{K_{cryo} \cdot \sum \nu_{\text{solute}} m_{\text{solute}}} \tag{15}$$

In Eqs. (14) and (15) K_{eb} and K_{cryo} are the ebullioscopic (0.52 K kg mol^{-1}) and the cryoscopic (−1.86 K kg mol^{-1}) constants of water, respectively. T^f and T^b refer to freezing-point and boiling-point temperatures of pure water (0w) and the solution, respectively.

In this work the osmotic coefficients of the binary solutions water/ectoine, water/hydroxyectoine, and water/homoectoine were measured at temperatures between 0 and 50 °C and concentrations between 0 and 2 mol/kg. The osmotic coefficients determined this way could be measured within a maximum deviation of 2%.

The osmotic coefficients of the investigated ectoine systems are given in Tables 3–5. Whereas the experimental data in Table 3 was measured with the freezing-point osmometer, the data in Tables 4 (30 °C) and 5 (50 °C) was obtained by vapor-pressure osmometry. Applying Eq. (13) then allows for the determination of water activity coefficients which will be the main-focused property in the discussion part of this work.

Besides the activity coefficient of water, the rational solute activity coefficients are also of interest in many applications. Applying the Gibbs–Duhem relation allows for the conversion of osmotic-coefficient data into rational solute activity coefficients. For that

Table 2
Experimental solubilities of ectoine (E), hydroxyectoine (HyE), and homoectoine (HoE) in water between 3 and 80 °C.

T [K]	m_E [mol/kg]	T [K]	m_{HyE} [mol/kg]	T [K]	m_{HoE} [mol/kg]
276.15	5.4504	276.15	7.0348	293.15	7.0155
298.15	6.5339	298.15	7.1971	313.15	9.4802
313.15	8.1390	313.15	7.3956	333.15	10.7086
333.15	10.6181			353.15	12.0257
353.15	13.7647				

Table 3
Experimental osmotic coefficients of aqueous ectoine (E), hydroxyectoine (HyE), and homoectoine (HoE) solutions at the respective freezing-point depression ΔT^f. Solute activity coefficients were obtained by Eq. (17).

m_E [mol/kg]	Φ [−]	ΔT^f [K]	γ^*_E [−]	m_{HyE} [mol/kg]	Φ [−]	ΔT^f [K]	γ^*_{HyE} [−]	m_{HoE} [mol/kg]	Φ [−]	ΔT^f [K]	γ^*_{HoE} [−]
0.070	1.0235	−0.13	1.0291	0.207	1.0165	−0.39	1.0031	0.069	1.0000	−0.13	1.0203
0.141	1.0449	−0.27	1.0593	0.646	1.0138	−1.22	1.0097	0.138	1.0290	−0.26	1.0410
0.281	1.1017	−0.58	1.1208	0.877	1.0106	−1.65	1.0132	0.281	1.0605	−0.55	1.0852
0.352	1.1174	−0.73	1.1530	1.127	1.0067	−2.11	1.0170	0.517	1.0232	−0.98	1.1624
0.553	1.1306	−1.16	1.2476	1.395	1.0031	−2.60	1.0211	0.738	1.0759	−1.48	1.2396
0.623	1.1518	−1.33	1.2817					1.05	1.1429	−2.23	1.3574
0.755	1.1785	−1.65	1.3475					1.615	1.1926	−3.58	1.5999
1.407	1.2354	−3.23	1.6980					1.972	1.3494	−4.95	1.7751
1.583	1.2579	−3.70	1.7977								
1.759	1.2743	−4.17	1.8983								

purpose, the estimated osmotic coefficients were first approximated by a power series:

$$\Phi - 1 = \sum_{i=1}^{n} A_i m^i \tag{16}$$

and then converted into the rational solute activity coefficients by applying:

$$\ln \gamma^* = (\Phi - 1) + \int_0^m \frac{(\Phi - 1)}{m} dm \tag{17}$$

m in Eqs. (16) and (17) is the molality of the solute. In Eq. (16), the A_i values are adjustable parameters and n refers to the number of parameters needed to represent the experimental osmotic coefficients. For ectoine at 30 °C, A_1, A_2, and A_3 were found to be 0.085, 0.024, and −0.009, respectively.

5. Parameter estimation for ePC-SAFT

Within ePC-SAFT, the ectoines are considered as chains consisting of identical uncharged spheres. The azotic group and the carboxylic group were assumed to have each one association site mimicking the proton donator site (acidic group) and the proton acceptor site (azotic group, Fig. 1). The hydroxyl group of hydroxyectoine was considered as an additional association site. All association-site types were assumed to have the same energy and volume parameters (ε^{AiBi} and κ^{AiBi}). Urea was modeled having two association sites. The same model was used for water which has already been applied earlier (parameters in Ref. [25]).

In this work, the five ePC-SAFT parameters (segment number, segment diameter, dispersion-energy parameter, association-energy parameter, and association range) for the associating ectoines were determined by fitting to our own experimental liquid-density (Table 1) and osmotic-coefficient (Tables 3–5) data at 25 °C. To determine the pure-component parameters for urea, solution density and osmotic-coefficient data from the literature was applied (see Table 6).

For solubility calculations (see. Eq. (9)), the melting enthalpy and melting temperature had to be determined. In the case of urea, melting data was found in the literature which was directly taken for the solubility calculations. For amino acids, these properties are not available but they could be estimated [12,15] applying the group-contribution method of Marrero and Gani [30]. However, this method does not seem to be appropriate for ectoines. Just to give an example, due to this method the estimated melting temperatures of the considered ectoines turned out to be 380–420 K, respectively. On the other hand, differential-scanning-calorimetry measurements have shown that the decomposition point of ectoine is about 550 K, i.e. *higher* than the estimated melting point. Because of that, the melting parameters were fitted to experimental solubility data.

In order to describe all the data types with only one parameter set, a temperature-dependent binary interaction parameter k_{ij} was used with T given in Kelvin:

$$k_{ij}(T) = k_{ij,25^\circ C} + k_{ij,T} \cdot (T - 298.15K) \tag{18}$$

This procedure is commonly used (see e.g. Ref. [24]) when accurate fits for solubility data are desired. Note, that Eq. (18) is applied to all properties calculated with ePC-SAFT (densities, activity coefficients, and solubilities). The same $k_{ij,T}$ is applied for ectoine and homoectoine, whereas $k_{ij,T}$ could be set to zero for hydroxyectoine and urea. Summing up, a total of five ePC-SAFT pure-component parameters, the binary k_{ij} parameter, and the two melting properties are necessary for modeling the above-mentioned thermodynamic properties of the considered solutes in aqueous solution. This compares to our previous work dealing with amino-acid solutions [12,15,17,33].

The parameters of the ectoines and urea are summarized in Table 6. The observed deviations between modeled and measured data (absolute average deviations AAD and absolute relative deviations ARD) are also summarized in Table 6, calculated by:

$$\begin{aligned} AAD &= \frac{1}{NP} \sum_{k=1}^{NP} \left| \left(y_k^{calc} - y_k^{exp} \right) \right| \\ ARD &= 100 \cdot \frac{1}{NP} \sum_{k=1}^{NP} \left(1 - \frac{y_k^{calc}}{y_k^{exp}} \right) \end{aligned} \tag{19}$$

Solution densities, osmotic coefficients, and the solubility behavior of the considered systems can be accurately described with PC-SAFT. The absolute relative deviations ARD over all considered systems are

Table 4
Experimental osmotic coefficients of aqueous ectoine, hydroxyectoine (HyE), and homoectoine (HoE) solutions at 30 °C. Solute activity coefficients were obtained by Eq. (17).

m_E [mol/kg]	Φ [−]	ΔT^b [K]	γ^*_E [−]	m_{HyE} [mol/kg]	Φ [−]	ΔT^b [K]	γ^*_{HyE} [−]	m_{HoE} [mol/kg]	Φ [−]	ΔT^b [K]	γ^*_{HoE} [−]
0.40	0.9951	0.20	1.0753	0.50	1.0009	0.26	1.0089	0.40	0.9960	0.20	1.0412
0.80	1.0381	0.43	1.1641	1.00	1.0131	0.52	1.0236	1.18	1.0375	0.63	1.1412
1.20	1.0729	0.66	1.2630	1.50	1.0277	0.79	1.0445	1.40	1.0624	0.76	1.1677
1.60	1.1475	0.94	1.3671	2.00	1.0439	1.07	1.0723				

Table 5

Experimental osmotic coefficients of aqueous ectoine, hydroxyectoine (HyE), and homoectoine (HoE) solutions at the 50 °C. Solute activity coefficients were obtained by Eq. (17).

m_E [mol/kg]	Φ [−]	ΔT^b [K]	γ^*_E [−]	m_{HyE} [mol/kg]	Φ [−]	ΔT^b [K]	γ^*_{HyE} [−]	m_{HoE} [mol/kg]	Φ [−]	ΔT^b [K]	γ^*_{HoE} [−]
0.50	1.0123	0.26	1.0504	0.50	1.0163	0.26	1.0259	0.40	1.0089	0.21	1.0256
1.01	1.0460	0.54	1.1139	1.00	1.0229	0.52	1.0525	1.18	1.0420	0.63	1.0781
1.50	1.0945	0.84	1.1799	1.50	1.0388	0.80	1.0798	1.40	1.0434	0.75	1.0915
2.00	1.1246	1.15	1.2355	2.00	1.0515	1.08	1.1078				

very small ($ARD_{density} = 0.15\%$, $ARD_{osmotic\ coefficient} = 1.63\%$) with the highest error appearing for the solubility data ($ARD_{solubility} = 8.47\%$). Presumably, this is caused by the experimental uncertainty (3%) as well as by the simplification used in Eq. (9). To sum up, PC-SAFT is a suitable approach for modeling thermodynamic properties over a large concentration and temperature range using solely one parameter set per solute.

6. Discussion

In general, the ectoines possess — like the amino-acid proline — a negatively charged carboxylate group attached to a cyclic ring structure that contains — in contrast to proline — a delocalized positive charge. The competition of hydrophilic (polar groups) and hydrophobic forces (nonpolar groups) influences the water–water and water–solute interactions. This influence can be revealed by activity or osmotic coefficients. In the following, especially the activity coefficients of water will be discussed for the different ectoine systems. Furthermore, they will be compared to those in aqueous solutions containing incompatible solutes (inorganic salts and urea) on the one side and to amino acids, which are known to also be compatible solutes, on the other side. Finally, also the salt influence on aqueous ectoine solutions will be investigated. In each subchapter, experimental results will also be compared to the thermodynamic modeling with PC-SAFT.

6.1. Thermodynamic properties of ectoine systems

Thermodynamic properties of aqueous ectoine solutions discussed in this work are liquid densities, osmotic and activity coefficients, and solubilities. The knowledge of density data is crucial in the engineering domain. This is due to the fact that the solution's volume (the reciprocal solution density) determines the dimension of apparatuses or reservoirs. Fig. 2 shows solution densities at 25 °C. Obviously, homoectoine and ectoine similarly influence the density behavior of the system. Only the hydroxyectoine causes higher solution densities leading to higher cellular weights at concentrated hydroxyectoine solutions. This is valid for all measured temperatures. Furthermore, the accurate description with PC-SAFT shows the quality of the equation of state modeling applying the parameters in Table 6.

Osmotic coefficients determine the osmotic pressure in a solution, i.e. solutes which cause high osmotic coefficients also cause high osmotic pressures (see Eq. (11)) making them powerful anti-stress agents in extremophile bacteria. Moreover, osmotic coefficients directly determine the water activity coefficients (WAC) (see Eq. (13)). Fig. 3 illustrates the WAC values in ectoine solutions at 30 °C. Obviously, all ectoines cause water activity coefficients lower than unity, which means that the osmotic coefficients are bigger than unity. This latter is important as it becomes obvious from Eq. (11) that the anti-stress effect would be decreased by low osmotic coefficients. However, the deviations from unity are not very pronounced which is characteristic for aqueous solutions containing biomolecules (e.g. amino acids).

Table 6

PC-SAFT parameters for ectoine (E), hydroxyectoine (HyE), homoectoine (HoE), and urea as well as deviations between model and experimental data.

Parameter	Unit	Abbr.	E	HyE	HoE	Urea
Segment number	[−]	m_{seg}	1.250	8.389	2.217	4.244
Segment diameter	[Å]	σ	5.050	2.532	4.333	2.446
Dispersion energy	[K]	u/k_B	530.00	352.56	392.98	368.23
Association sites	[−]	N	2	3	2	2
Association energy	[K]	$\varepsilon_{hb}^{AiBi}/k_B$	3500.00	2000.00	3500.00	3068.31
Association volume	[−]	κ_{hb}^{AiBi}	0.09	0.09	0.09	0.001
Melting temperature	[K]	T_0^{SL}	511.45	750.41	665.76	405.80[a]
Melting enthalpy	[K]	h_0^{SL}/R	1918.78	1462.59	1187.49	1636.91[a]
Interaction parameterat 25 °C	[−]	$k_{ij,25°C}$	$2.933 \cdot 10^{-3}$	$-5.240 \cdot 10^{-2}$	$-4.136 \cdot 10^{-4}$	$-4.380 \cdot 10^{-2}$
Interaction parameter	[−]	$k_{ij,T}$	$5.787 \cdot 10^{-4}$	–	$5.787 \cdot 10^{-4}$	–
Solution density						
T-range	[K]		288–318	288–318	288–298	298–303[b]
ARD	[%]		0.08	0.34	0.03	0.11
AAD	[kg/m³]		0.82	3.47	0.32	1.12
Solubility						
T-range	[K]		276–353	276–313	293–353	291–346[b]
ARD	[%]		2.15	17.36	5.90	3.89
AAD	[mol/kg]		0.20	1.25	0.58	0.98
Osmotic coefficients						
T-range	[K]		T^m–323	T^m–323	T^m–323	288–323[b]
ARD	[%]		1.91	0.49	2.49	0.07
AAD	[−]		0.021	0.005	0.026	<0.001

[a] Experimental melting parameters directly taken from [34] without further adjustment.

[b] Experimental data taken from literature: solution densities [35], solubilities [36], and osmotic coefficients at 25 °C [37] and between 30 and 50 °C [38].

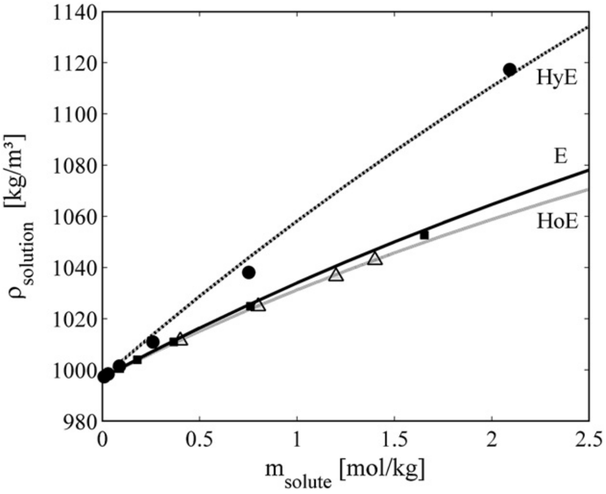

Fig. 2. Solution densities of aqueous ectoine solutions at 25 °C. Symbols represent experimental data (squares: ectoine, circles: hydroxyectoine, and triangles: homoectoine), lines are calculations with PC-SAFT.

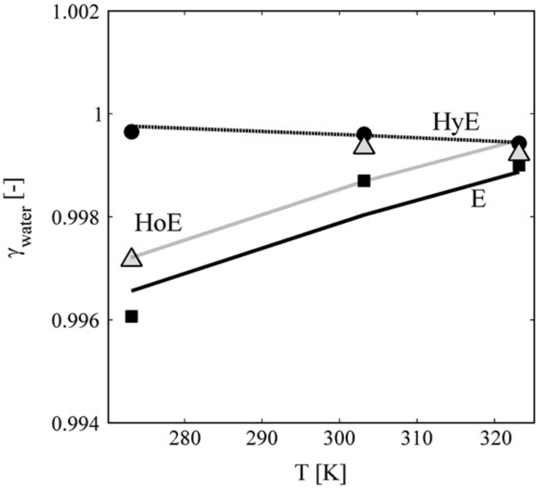

Fig. 4. Temperature dependence of water activity coefficients of one molal aqueous ectoine solutions between freezing point and 50 °C. Symbols represent experimental data (squares: ectoine, circles: hydroxyectoine, and triangles: homoectoine), lines are calculations with PC-SAFT.

Among the ectoine types experimental WAC values at 30 °C decrease in the order “hydroxyectoine > homoectoine > ectoine” as can be observed in Fig. 3, i.e. the smallest compatible solute (ectoine) causes the strongest non-ideal behavior in the solution and thus is the most effective osmolyte. This behavior is accurately captured by the PC-SAFT model (see Fig. 3).

To evaluate the temperature dependence of the anti-stress effect of ectoines, the activity coefficients of water in ectoine, hydroxyectoine, and homoectoine solutions were investigated at other temperatures than 30 °C, namely at 50 °C and at the solutions' freezing points, respectively.

Fig. 4 illustrates the influence of temperature on water activity coefficients in one molal ectoine solutions. Experimental results show a remarkably different behavior of ectoine systems with varying temperature: the WAC values of hydroxyectoine solutions slightly decrease with increasing temperature whereas for ectoine and homoectoine solutions the WAC is found to increase with elevated temperature. This means that with the increasing temperature the strength of the anti-stress effect is *weakened* for ectoine but *augmented* for hydroxyectoine. This supports the experimental observation that microorganisms like *H. elongata* to a high extent produce ectoine at ambient conditions but at elevated temperatures a remarkable amount of hydroxyectoine [7,39,40]. Another/additional explanation might be the temperature dependence of the enzymatic activity.

6.2. Comparison of ectoines and prolines

Because of the similar chemical structures it is obvious to compare the chemico-physical properties of ectoines and prolines. One osmolyte that is often produced to sustain salt stress, is the amino acid L-proline. However, proline is rather employed by halotolerant microorganisms whereas the ectoines act as osmolytes especially in halophile organisms, i.e. in surroundings with high salt concentrations. In contrast, the amino acid L-hydroxyproline is not known to be found as an anti-stress agent in microorganisms. In this chapter, the thermodynamic properties of aqueous proline solutions are compared to those of ectoines in water to detect possible differences between these similar solutes. Fig. 5 illustrates that the WAC in solutions of ectoine and proline at 30 °C shows a qualitatively similar dependence on solute molality: the WAC values always decrease with increasing solute concentration. Moreover, the two pairs ectoine/proline and

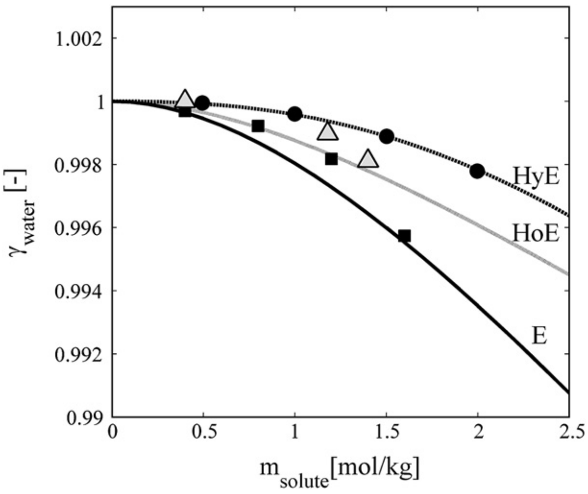

Fig. 3. Water activity coefficients of aqueous ectoine solutions at 30 °C. Symbols represent experimental data (squares: ectoine, circles: hydroxyectoine, and triangles: homoectoine), lines are calculations with PC-SAFT.

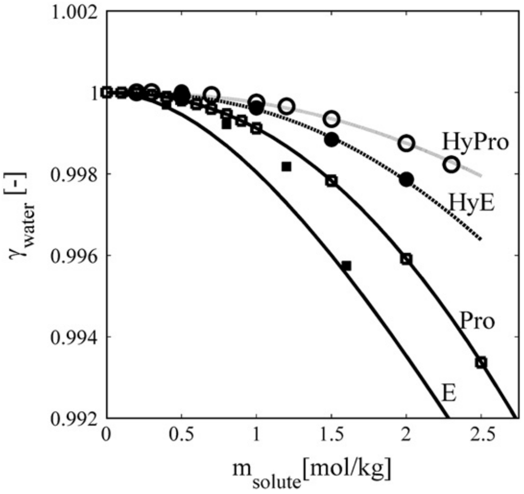

Fig. 5. Water activity coefficients of aqueous ectoine and proline solutions at 25 °C. Symbols represent experimental data (full squares: ectoine, full circles: hydroxyectoine, open squares: proline, and open circles: hydroxyproline), lines are calculations with PC-SAFT.

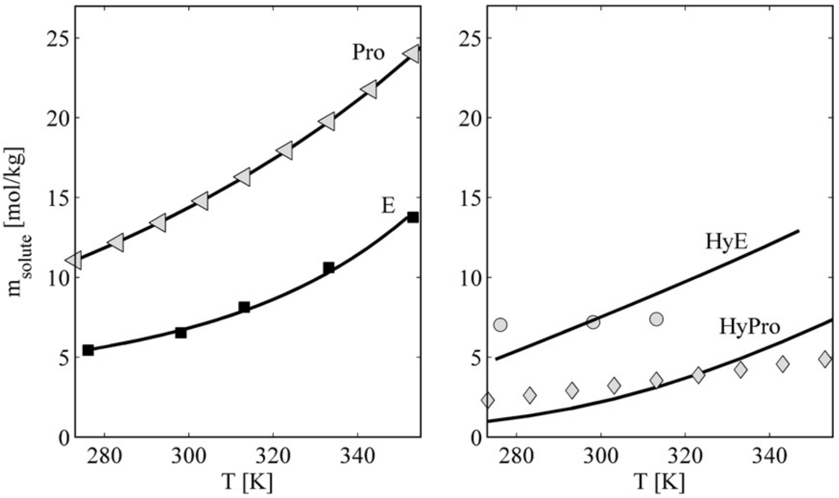

Fig. 6. Solubilities of ectoine/L-proline (a) and hydroxyectoine/L-hydroxyproline (b) in water between 0 and 80 °C. Symbols represent experimental data (squares: ectoine, circles: hydroxyectoine, triangles: proline, and rhombi: hydroxyproline), lines are calculations with PC-SAFT.

hydroxyectoine/hydroxyproline influence the WAC values in a similar way: hydroxyectoine and hydroxyproline behave almost ideally in water as the activity coefficients are very close to unity. This might be ascribed to the additional OH-group which seems to make the hydroxy solutes more water like. Therewith, the pronounced ambition of water molecules to build hydrogen bondings is satisfied and there is no need for reorientation efforts. Furthermore, we have shown earlier [12] that the addition of polar groups to amino acids (–SH, OH, peptide bonds) causes increased WACs. In contrast, ectoine as well as proline cause activity coefficients which decrease more strongly with increasing molality. These observations also thermodynamically confirm that proline — due to its similar chemical structure — is an osmolyte which behaves almost equally compared to ectoine. However, ectoine is produced by halophile organisms (moderate to high salt concentrations) while proline is rather used by halotolerant (low to moderate salt concentrations) ones [41]. This might be due to the fact that ectoine is a better anti-stress agent than proline (see Fig. 5). However, the osmotic-coefficient data cannot explain why hydroxyectoine is produced by microorganisms against stress situations whereas hydroxyproline is not. The reason for this might be another thermodynamic property: the solubility in water.

In order to be highly flexible and to survive in extremely concentrated aqueous systems with high osmotic pressures, the microorganisms have to produce a large amount of the compatible solute. Moreover, this amount of substance has to be soluble in the cell as the solute would otherwise precipitate thereby losing its anti-stress effect. This means that a good solubility in water is a prerequisite for acting as osmolyte. At ambient conditions all ectoines have a very high solubility in water (ca. 7 mol/kg, Table 2) whereas hydroxyproline possesses only a solubility of about 2 mol/kg at 25 °C (Fig. 6b). This value would certainly be further decreased in the presence of other solutes in the organism. In addition to the possibility of non-existing metabolic pathways for the formation of hydroxyproline its comparatively small solubility might be a reason that microorganisms produce hydroxyectoine instead of hydroxyproline.

As it is crucial to know the solubility of the ectoines also at other temperatures we investigated the solubility of each ectoine between 3 and 80 °C. It can be observed in Fig. 6 that the solubility of ectoine strongly increases with temperature increase whereas the solubility of hydroxyectoine only weakly depends on the system's temperature. Whereas the solubility of biomolecules like ectoine is in general accurately modeled with PC-SAFT (see Fig. 6a or Refs. [12,15]) the temperature-independent behavior of hydroxyectoine and hydroxyproline solubilities in water leads to exceptional high deviations of the model calculations (16% deviation between experiment and PC-SAFT, see Table 1). However, this is rather an exception as can be seen in Ref. [12] or in Fig. 6a, respectively.

6.3. Comparison of ectoines and salts

Almost every microorganism possesses ion pumps. However, instead of pumping in inorganic ions, they often rather produce compatible solutes against stresses although this consumes extra energy. Obviously, in contrast to compatible solutes inorganic salts negatively affect the cell milieu. In addition to that there is also a distinct difference in the thermodynamic properties. In order to survive under salt-stress conditions a certain water activity has to be adjusted [9] which — at given concentration — only depends on the activity coefficient of water.

Fig. 7 illustrates the influence of salt and ectoine on WAC values at 30 °C. Whereas ectoine has already shown to cause low water activity coefficients (and thus high osmotic pressures) the WAC values firstly *increase* by adding salts making the salt an ineffective anti-stress agent. However, at a certain (high) salt concentration (1 m for NaCl and 2 m for KCl), the WAC value starts decreasing. This behavior is characteristic for every electrolyte solution.

The following conclusions with respect to the anti-stress performance of salts compared to ectoine can be drawn:

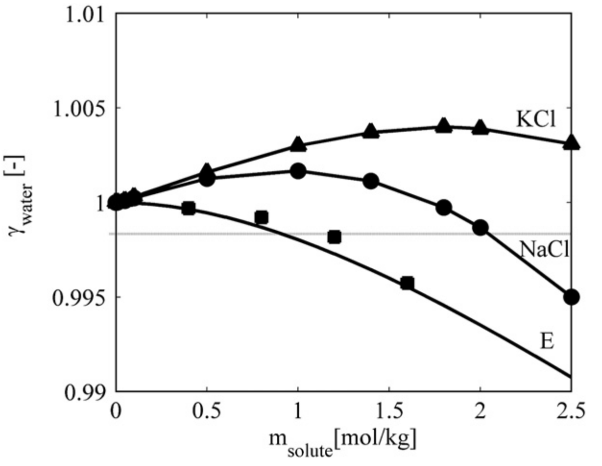

Fig. 7. Water activity coefficients at 30 °C. Circles: NaCl solution, triangles: KCl solution, and squares: ectoine solution. Symbols are experimental data from this work (ectoine) and from Lobo and Quaresma for the salts [42]. The thin grey line is the activity coefficient of water caused by 1 mol ectoine per kg water.

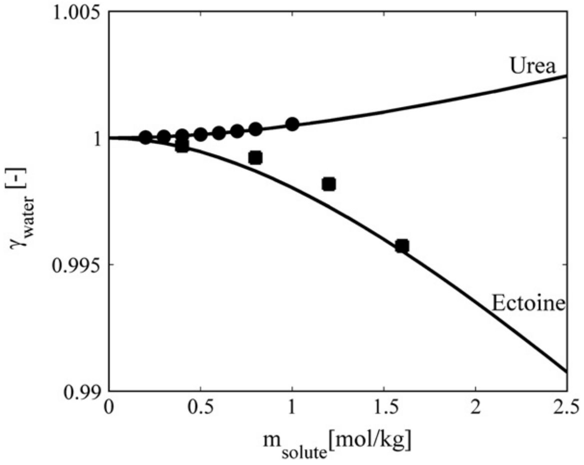

Fig. 8. Water activity coefficients of aqueous ectoine and urea solutions at 30 °C. Symbols represent experimental data (squares: ectoine, and circles: urea), lines are calculations with PC-SAFT.

- ➢ Any inorganic ion disturbs the anti-stress effect by increasing water activity coefficients and thus reducing osmotic pressures. This disadvantageous effect disappears only at high salt concentrations of 1.8 m NaCl and 3.4 m KCl, respectively.
- ➢ Compared to a one molal ectoine solution (thin grey line in Fig. 7), 2 m NaCl or 3.5 m KCl would be necessary to establish the same activity coefficients, respectively. This makes the ectoine much more effective from the thermodynamic view.
- ➢ Moreover, the accumulation of high amounts of salt (2 or 3.5 mol/kg) is well-known to be poisonous (especially Na^+) to marine organisms [43].
- ➢ Beyond thermodynamics — organic compounds might be metabolized if not needed any more which is not possible for high amounts of salts (those have to be flushed out).

All this might explain the production of compatible solutes like ectoine instead of pumping in ions from outside the organism as protection against salt stress.

6.4. Comparison of ectoine with urea

In contrast to the ectoines and proline, urea is well-known to be an incompatible solute. This means, that urea destabilizes protein structures. One reason might be the strong interaction of urea with peptide groups or aromatic side chains [44]. However, in analogy to water/salt systems, the incompatible character of urea might also be caused by its influence on the osmotic behavior of the solution. It becomes obvious from Fig. 8 that, in contrast to the ectoine solutions, water activity coefficients directly *increase* by adding urea. This means that urea is a very ineffective anti-stress agent which seems to be characteristic for incompatible solutes. The same osmotic behavior was observed for glycine solutions [12] in which water activity coefficients are higher than unity. To give an example, a 2 molal urea solution causes an osmotic coefficient of 0.93 which is more than 25% lower compared to an equimolal ectoine solution (see Table 4), i.e. the osmotic pressure within a cell containing urea will be remarkably reduced compared to cells containing ectoine.

6.5. Salt influence on aqueous ectoine solutions

The importance of understanding the phase behavior of "simple" binary ectoine/water systems was illustrated in the previous chapters. However, biological solutions never contain one solute only.

To study the influence of electrolytes on the thermodynamic behavior of ectoine solutions, also osmotic coefficients of aqueous solutions containing both, ectoines as well as salt, were investigated. Here, we applied the vapor-pressure osmometer for the experimental investigations at 30 °C. Table 7 summarizes the experimental results of these ternary systems at potassium chloride concentrations of 0.5 and 1 mol/kg.

Fig. 9 shows the influence of hydroxyectoine (Fig. 9a) and of potassium chloride (Fig. 9b) on water activity coefficients at 30 °C. Just as in the binary ectoine/water systems where WACs are always decreased by the ectoines (Fig. 3), the addition of hydroxyectoine to aqueous KCl solutions (Fig. 9a) causes also decreased WAC values. This means that increased osmotic pressures can be realized in cells containing salts (KCl) also by adding ectoines (independent of salt concentration). Moreover, this also gives a first hint to the effect of osmolytes in more complex systems: obviously, hydroxyectoine influences solutions by increasing their osmotic coefficients *independent of the presence of other solutes*. This supports the statement of other authors ([9,10]) that the ability of a molecule to act as biological osmolyte is mostly determined by the properties of the osmolyte/water binary system.

Fig. 9b shows the salt influence to binary osmolyte/water systems. It can be observed that the addition of salt to aqueous hydroxyectoine solutions strongly increases the WACs in such ternary systems. As the addition of salt to pure water also causes increased WACs this is an expected experimental result. This means that increasing salt concentrations defeat the benefit of ectoines as anti-stress agents. Thus, from thermodynamic point of view, the cell should avoid accumulating salts. This might explain why microorganisms do not produce ectoines and simultaneously pump in inorganic compounds. However, it is possible that other salts do not cause such unfavorable effects which still have to be investigated.

In order to evaluate the applicability of ePC-SAFT to these complex multi-solute solutions, the activity coefficients and osmotic coefficients were predicted for these systems (no parameter fitting). Fig. 9 exemplarily illustrates the model predictions in hydroxyectoine/KCl/water solutions at 30 °C. With the hydroxyectoine parameters given in Table 6 and the KCl parameters as determined earlier [26] the influence of KCl on WAC of hydroxyectoine/water solutions can be predicted with ePC-SAFT at several temperatures and concentrations. Fig. 9a shows that the WACs even at both salt concentrations (0.5 and 1 mol/kg) are *predicted* by the model quantitatively, i.e. the results are based on the binary salt/water and ectoine/water systems only and no adjustable parameters k_{ij} between ions and hydroxyectoine had to be applied. This shows that ePC-SAFT is a suitable model for describing

Table 7
Experimental water and osmotic coefficients of aqueous KCl/hydroxyectoine solutions at 30 °C. Solute activity coefficients γ^* were obtained by the ePC-SAFT model.

30 °C, 0.5 m KCl					30 °C, 1 m KCl				
m_{HyE} [mol/kg]	Φ [−]	γ_{water} [−]	γ^*_{KCl} [−]	γ^*_{HyE} [−]	m_{HyE} [mol/kg]	Φ [−]	γ_{water} [−]	γ^*_{KCl} [−]	γ^*_{HyE} [−]
0.401	0.929	1.0014	0.630	0.986	0.204	0.928	1.0021	0.594	0.967
0.799	0.962	1.0008	0.626	0.996	0.400	0.931	1.0020	0.591	0.971
1.200	0.990	0.9996	0.623	1.009	0.600	0.941	1.0016	0.588	0.976
1.600	1.007	0.9986	0.619	1.026	0.791	0.957	1.0009	0.586	0.981

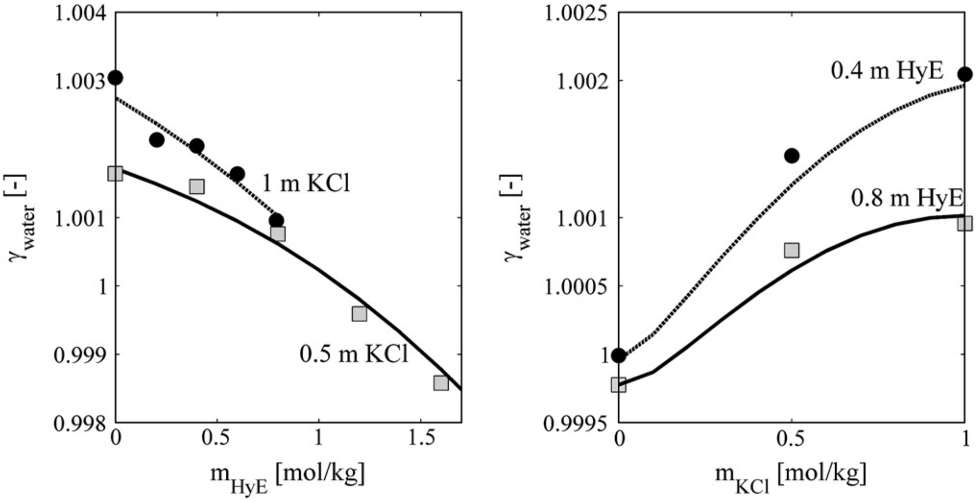

Fig. 9. (a) Water activity coefficients of aqueous hydroxyectoine solutions containing different concentrations of potassium chloride at 30 °C. Symbols represent experimental data (squares: 0.5 m KCl and circles: 1 m KCl), lines are predictions with ePC-SAFT. (b) Water activity coefficients of aqueous potassium chloride solutions containing different concentrations of hydroxyectoine at 30 °C. Symbols represent experimental data (circles: 0.4 m HyE and squares: 0.8 m HyE), lines are predictions with ePC-SAFT.

the liquid-phase properties of solutions containing biomolecules or salt and even of multi-solute solutions.

7. Summary

In this work we investigated the interactions in aqueous solutions containing the compatible solutes ectoine, hydroxyectoine, and homoectoine. Whereas the latter is a synthetic compound, ectoine and hydroxyectoine are produced by halophilic organisms against salt stress. In the first part of the work we presented for the first time solution densities between 15 and 45 °C, solubilities between 3 and 80 °C, and activity coefficients between freezing point and 50 °C. Furthermore, the influence of KCl on activity coefficients in aqueous hydroxyectoine solutions at 30 °C was measured.

Based on these data, comparisons between the different kinds of ectoines were carried out showing that the smallest osmolyte (ectoine) causes the lowest water activity coefficients and thus has the best anti-stress effect. It could be observed that all thermodynamic properties between ectoine and proline as well as between hydroxyectoine and hydroxyproline are very similar except the comparably low solubility of the latter solute. This might explain the fact that organisms do not produce hydroxyproline to protect themselves against salt/pressure stresses.

Comparing the influence of urea (an incompatible solute) and salts to the one of ectoines and prolines (compatible solutes) on the thermodynamic properties of aqueous solutions revealed pronounced differences. Whereas salts and urea increase the WAC, all ectoines and prolines were shown to reduce these WAC values even at small concentrations. Thus, osmolytes cause much higher osmotic pressures than incompatible solutes at the same concentrations. That means that, e.g. less ectoine is necessary to establish a certain osmotic pressure (i.e. low water activity) compared to urea. This makes the osmolytes unique compared to salts or urea and explains why organisms avoid pumping in ions but rather produce organic compounds like the ectoines.

In the last part of the work the ternary system water/KCl/hydroxyectoine was investigated. It could be shown that the anti-stress effect of hydroxyectoine in the binary system without salt is qualitatively the same as for the ternary systems with added salt. That supports earlier findings, that the ability of a molecule to act as biological osmolyte is for the most part already determined by the properties of the binary osmolyte/water systems.

Finally, the thermodynamic model PC-SAFT has been applied for the modeling of solution densities ($ARD_{density} = 0.15\%$) as well as for vapor-liquid ($ARD_{osmotic\ coefficient} = 1.63\ \%$) and solid–liquid ($ARD_{solubility} = 8.47\%$) phase behaviors of aqueous ectoine solutions. PC-SAFT was shown to accurately describe all the thermodynamic properties with only one single parameter set per solute. One exception is the modeling of hydroxysolute solubilities which show an almost temperature-independent solubility behavior; this cannot be captured well with PC-SAFT (up to 16% deviation between model and experiment). Moreover, it is possible to predict the salt influence on aqueous ectoine solutions with ePC-SAFT, i.e. the calculations are based on binary water/salt and water/ectoine data only and do not require any additional adjustable parameters.

8. Symbols

Roman symbols

a	[J] Helmholtz free energy per number of particles
a	[−] activity
A	[J] Helmholtz free energy
Δh^{SL}	[kJ/kg] melting enthalpy
k_B	[J/K] Boltzmann constant, $1.38065 \cdot 10^{-23}$ J/K
k_{ij}	[1/K] binary interaction parameter
$k_{ij,T}$	[1/K] temperature-dependent binary interaction parameter
$k_{ij,25°C}$	[1/K] binary interaction parameter at 25 °C
K_{cryo}	[kgK/mol] cryoscopic constant of water, 1.86 kgK/mol
K_{eb}	[kgK/mol] ebullioscopic constant of water, 0.52 kgK/mol
m	[mol/kg] molality (moles solute i per kg solvent)
M	[g/mol] molecular weight
m_{seg}	[−] number of segments
n	[−] number of moles
N	[−] total number of particles
N	[−] number of association sites
p	[kPa, bar] pressure
R	[J/mol/K] ideal gas constant
T	[K] temperature
T^{SL}	[K] melting temperature
X	[−] mole fraction
V	[m³] volume
Z	[−] real gas factor

Greek symbols

γ_i	[−] symmetrical activity coefficient of component i (related to pure component)
γ_i^*	[−] asymmetrical activity coefficient of component i (related to infinite dilution)
φ_i	[−] fugacity coefficient of component i
u/k_B	[K] dispersion-energy parameter
$\varepsilon_{hb}^{AiBi}/k_B$	[K] association-energy parameter
κ_{hb}^{AiBi}/k_B	[−] association-volume parameter
μ_i	[−] chemical potential of component i
Φ	[−] osmotic coefficient
ρ	[kg/m³] density
π	[bar] osmotic pressure
ν	[−] stoichiometric factor
σ_i	[Å] temperature-independent segment diameter of molecule i

Subscripts

i, j	component indexes
T	function of temperature
seg	segment
W	water
0	pure substance

Superscripts

assoc	association
b	boiling
disp	dispersion
f	freezing
hc	hard chain
m	based on molality
res	residual
∞	infinitely diluted
*	related to infinite dilution

Abbreviations

AAD	absolute average deviation
ARD	absolute average relative deviation
E	ectoine
EOS	equation of state
ePC-SAFT	electrolyte Perturbed-Chain Statistical Association Theory
HoE	homoectoine
HyE	hydroxyectoine
HyPro	hydroxyproline
P	proline
WAC	water activity coefficient

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