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Thermodynamic properties of aqueous osmolyte solutions at high-pressure conditions

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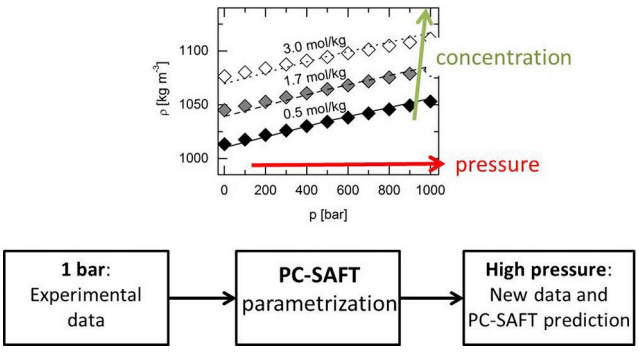
HIGHLIGHTS

- New osmotic coefficients were measured for aqueous osmolyte systems
- New high pressure density data were measured for aqueous osmolyte systems
- PC-SAFT was used to model density and osmotic coefficients at 1 bar
- High pressure effects on density of osmolyte solutions were predicted using PC-SAFT
- PC-SAFT accurately predicts combined influence of pressure and concentration

GRAPHICAL ABSTRACT



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ABSTRACT

Living organisms can be encountered in nature under extreme conditions. At the seabed, pressure may reach 1000 bar. Yet microorganisms can be found that still function under these conditions. On the one hand, it is known that high pressure even has a positive effect on piezophile enzymes increasing their activity. On the other hand, such microorganisms might contain up to very high concentrations of osmolytes that counteract osmotic stress. To better understand high-pressure influences on biochemical systems, fundamental knowledge about pressure effects on thermodynamic properties of such osmolytes is important. However, literature data is scarce and experiments at high-pressure conditions are challenging. Hence, new high-pressure density data of aqueous osmolyte solutions were measured in this work at temperatures between 298.15 K and 318.15 K and at osmolyte concentrations up to 3 mol/kg water. Further, the thermodynamic model PC-SAFT has been applied recently to successfully model vapor pressures of water and density of water up to 10 kbar [M. Knierbein et al., Density variations of TMAO solutions in the kilobar range: experiments, PC-SAFT predictions, and molecular dynamics simulations, *Biophysical chemistry*, (2019)]. This allowed accurately predicting effects of temperature and osmolyte concentration on thermodynamic properties (especially mixture densities) up to very high pressures. Common osmolytes (trimethylamine-N-oxide, urea, ectoine, glycerol, glycine) as well as the dipeptides acetyl-*N*-methylglycine amide, acetyl-*N*-methylalanine amide, and acetyl-*N*-methylleucine amide were under investigation.

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1. Introduction

Biochemical systems have been studied intensively at atmospheric conditions. The influence of temperature and concentration effects on many systems has been investigated and are already quite well understood. This knowledge can be applied for the optimization of biochemical production processes as well as for better understanding the metabolism in microorganisms. Another influence factor that has recently gained attraction is high pressure [1–8]. Multiple microorganisms can be encountered in nature under extreme conditions, e.g. under high-pressure conditions at the bottom of the sea where pressures of up to 1000 bar can be encountered [9,10]. Studies have shown that microorganisms do not only survive under high-pressure conditions but also that high pressure has a positive influence on piezophile enzymes [5,11–14] that have adopted high-pressure conditions. The mechanisms that are influenced by high pressure conditions, e.g. enzyme folding and enzymatic kinetics, can be complex and are subject of recent studies [3,4,15–17]. These works have shown that enzyme functionalities depend strongly on the presence of osmolytes, which are usually present in microorganism to counteract stress conditions such as stress by osmotic pressure.

Osmolytes are molecules of low molecular weight, and even the simplest ones have a strong influence on osmotic pressure and therefore on the stability of enzymes and the functionality of microorganisms [18–21]. Molecules that counteract unfavorable concentration effects and increase enzyme stability are called compatible solutes, e.g. trimethylamine-N-oxide (TMAO), glycerol, ectoine [18,19,22–28]. In contrast to that molecules that increase osmotic stress and decrease enzyme stability are called incompatible solutes, e.g. urea [19,29–32]. From microorganisms living under extreme conditions it is known that they have the ability to synthesize compatible solutes that do not interfere with the microorganism's metabolism in order to protect themselves against osmotic stress [9,10,18,19,33]. Furthermore, it has been shown that marine organisms contain TMAO and urea to counteract osmotic stress as well as to counteract high hydrostatic pressure effects and that the concentration of these two solutes increases with increasing depth [34,35]. In this work, all biological molecules that influence the osmotic pressure are further denoted as osmolytes.

In order to get further insight into high-pressure effects on biochemical systems it is important to investigate and quantify the influence of high pressure on fundamental thermodynamic properties, e.g. density, in a first step. At ambient conditions concentration and temperature effects on biochemical systems have already been studied [18,19]. However, the effect of high pressure on biochemical systems is still much less investigated. For example, high-pressure density data is known for aqueous solutions of some osmolytes [36–38]; besides, high-pressure data of biochemical systems is scarce in literature. Furthermore, commercial devices only exist to perform measurements and experiments under high pressure up to a few hundred bar. However, special equipment and the fulfillment of many regulations is required for measurements within the kilobar range. Therefore, it is meaningful to apply models and simulations additional to experiments. Recent molecular dynamics (MD) simulations were used to explore the effects of high pressure on osmolyte solutions [39,40]. The results are very detailed, yet the computational effort is high. In contrast, thermodynamic equations of state allow for investigating high pressure effects on a different molecular scale with much less computational effort than MD simulations. In literature high-pressure effects on density data is often described by empirical correlations [41,42]. However, these empirical approaches do not explicitly account for thermodynamics. Consequently, thermodynamic models are used in literature to model pressure effects on liquid density data [43,44]. The thermodynamic model PC-SAFT (Perturbed-Chain Statistical Associating Fluid Theory) [45] has already been successfully applied to predict concentration and temperature effects on osmolyte systems at ambient pressure [18,19,46–48]. Since PC-SAFT explicitly accounts for pressure disturbances it is hence a promising model to investigate high-pressure effects on thermodynamic properties of osmolyte solutions.

In this work, an approach to predict temperature, concentration and especially high-pressure effects on thermodynamic properties of osmolyte solutions is presented. For this the thermodynamic model PC-SAFT is used to predict osmotic coefficients of osmolyte solutions and high-pressure solution densities of osmolyte solutions. Prediction results are evaluated using literature data and own experimental data where required. Biologically meaningful ranges of pressure, temperature, and concentration were studied. In conventional biological systems typically temperatures between 20 °C and 37 °C are most interesting. However, at deep-sea conditions temperatures may decrease to −2 °C while pressure may reach 1000 bar. Under these conditions osmolyte concentrations of up to 1 mol/kg may be encountered in organisms. Monomeric osmolytes under investigation were TMAO, ectoine and glycerol, urea, and glycine. In order to analyze the behavior of more complex molecules the acetylated dipeptides acetyl-*N*-methylglycine amide (Ac-Gly-NHMe), acetyl-*N*-methylalanine amide (Ac-Ala-NHMe) and acetyl-*N*-methylleucine amide (Ac-Leu-NHMe) were studied as well.

2. Experimental methods

2.1. Vibrating-tube density meter

All density measurements shown in this work were performed using an Anton Paar vibrating-tube density meter DMA 4200 M. This apparatus uses the pulsed excitation method that allows highly accurate density measurements in the pressure range of 1 bar to 500 bar. The manufacturer's specified reproducibility is 0.1 kg/m^3 while the temperature is kept constant at a controlled temperature with an accuracy of ± 0.03 K. Water and *n*-heptane were used to calibrate the density meter and the method was validated by measuring the density of an aqueous 1 mol/kg sodium chloride solution at 298.15 K and pressures from 1 bar to 500 bar. The experimental results of this validation measurement were evaluated using literature data [49] and the average absolute deviation (AAD) for this validation was 0.29 kg/m^3.

All samples were prepared using water from Millipore water purification system. The supplier of the substances under investigation and their purity is given in Table 1. The substances were weighed using a Sartorius laboratory balance with an accuracy of 0.0001 g in order to obtain solutions of desired and defined composition. All density measurements were performed at least twice.

2.2. Freezing-point depression osmometer

The freezing-point depression osmometer Osmomat O10 from Gonotec was used to determine osmotic coefficients in this work. Osmotic coefficient (ϕ) and rational molality-based activity coefficient ($\gamma_{osmolyte}^{*}$) of the osmolyte can be converted into each other according to (1) where $m_{osmolyte}$ is the molality of the osmolyte as moles per kg pure water.

Table 1
Supplier and purity of all substances studied in this work.

Substance	Supplier	Purity
Urea	Merck	> 99.5%
Trimethylamine-N-oxide	TCI	> 98%
Glycerol	VWR	> 99.5%
Ectoine	Bitop	> 95%
Acetyl-*N*-methylglycine amide	Bachem	> 99%
Acetyl-*N*-methylalanine amide	Bachem	> 99%
Acetyl-*N*-methylleucine amide	Bachem	> 99%
Glycine	Sigma Aldrich	> 99%

$$ln(\gamma^{*}_{osmolyte}) = (\phi - 1) + \int_{0}^{m_{osmolyte}} \frac{(\phi - 1)}{m_{osmolyte}} dm_{osmolyte} \quad (1)$$

This device allows measuring the freezing point depression of an aqueous solution which depends on the molality of the osmolyte and molecular interactions. A sample of the solution is supercooled to 264.15 K and a stainless-steel needle is then used to induce crystallization. At the end of the crystallization process the solution reaches its freezing temperature which is lower than the freezing temperature of the pure solvent, i.e. water in this work. This freezing-point depression depends on the activity of water a_{water} according to (2).

$$\Delta T^{SL} = \frac{R \bullet (T^{SL}_{0water})^2}{\Delta h^{SL}_{0water}} \bullet \ln(a_{water}) \quad (2)$$

Finally, the osmotic coefficient is then calculated according to (3), where 1.86 is the cryoscopic constant of water.

$$\phi = \frac{\Delta T^{SL}}{-1.86\ [K\ kg\ mol^{-1}] \bullet m_{osmolyte}} \quad (3)$$

All freezing-point depression measurements were performed at least three times. The manufacturer specifies the accuracy of the device's temperature measurement with 0.00186 K leading to an uncertainty of the osmotic coefficients of 0.6%.

3. PC-SAFT modeling

The thermodynamic model PC-SAFT was used to model osmotic coefficients of osmolyte solutions and to predict the density of aqueous osmolyte solutions of pressures up to 1000 bar. Within PC-SAFT each molecule is described as a chain of spherical segments that are defined by the segment diameter (σ_i) and the number of segments (m_i^{seg}). The attractive interactions between the molecules are characterized by dispersion (u_i/k_B) and association forces. Based on these model contributions PC-SAFT calculates the residual Helmholtz free energy A^{res} of a fluid. A^{res} is calculated as the sum of Helmholtz energy contributions from the hard chain A^{HC}, dispersion A^{disp} and association A^{assoc} to the residual Helmholtz free energy. The number of association sites as well as the corresponding association-energy parameter (ε^{AiBi}/k_B) and the association-volume parameter (κ^{AiBi}) are required for A^{assoc}. PC-SAFT is suited to predict pressure effects since it explicitly accounts for pressure disturbances [45]. Furthermore, in scientific literature PC-SAFT has proven to predict fluid densities very accurately [18,19,50,51].

PC-SAFT predictions were performed for aqueous solutions at defined conditions (pressure p, temperature T, and molality of the solute m_i). The PC-SAFT pure-component parameters for all components used in this work are given in Table 2 and the binary interaction parameters used are given in Table 3. Induced association was accounted for between water and TMAO according to Kleiner et al. [52]. Binary interaction parameters were determined in this work and are valid only with the parameters for water according to Table 2. The binary interaction parameters k_{ij} between solutes and water were fitted to osmotic-coefficient data. The pure-component parameters of the peptides were fitted in this work to experimental density data (see Table 6) and osmotic-coefficient data of the peptide solutions at atmospheric pressure (see Table 4).

4. Results and discussion

4.1. Osmotic coefficients and activity coefficients at atmospheric pressure conditions

In this work thermodynamic properties of osmolyte solutions were measured and modeled with PC-SAFT. Osmolytes counteract osmotic stress and are thus actively used by living organisms to counteract concentration effects as well as to counteract pressure effects at extreme conditions [20,21,35]. The strength of this osmoprotectant effect can be classified by their influence on osmotic coefficients or by their rational activity coefficients. This is shown illustratively using two examples in Figs. 1 and 2.

In Fig. 1, the rational activity coefficients of two osmolytes, ectoine and glycine, are presented. Aqueous mixtures of ectoine were analyzed at different temperature and concentration conditions. From the literature it is well known that ectoine has a strong influence on the osmotic pressure [18]. The rational activity coefficient can be used to quantify the effect of ectoine on osmotic pressure. Fig. 1 illustrates that an increased concentration of ectoine in aqueous solution leads to higher values of $\gamma_{ectoine}^{*}$. This is equal to higher values of the osmotic coefficient (according to Eq. 1). This means that rising concentrations of ectoine effectively counteract the effects of higher (osmotic) pressure. This effect is highest at low temperatures where low ectoine concentrations already have a strong effect on $\gamma_{ectoine}^{*}$ and consequently on the osmotic pressure of a solution. At higher temperature, this effect decreases. The observed effects of concentration and temperature are thus very important for living organisms under high osmotic stress conditions. It is known that living organisms take advantage of the effect of ectoine on osmotic pressure to counteract the effects of high salt concentrations [55].

The thermodynamic model PC-SAFT was used to model the molecular interactions in the ectoine + water solutions (see Table S.1). The results given in Fig. 1 show that PC-SAFT describes the combined concentration and temperature effects on $\gamma_{ectoine}^{*}$ very well with a maximum deviation of < 0.25%.

The amino acid glycine has an opposite effect on aqueous solutions (see Fig. 1). Glycine dissolved in water has rational activity coefficients $\gamma_{glycine}^{*}$ lower than one, which means that osmotic coefficients are also lower than one and glycine is a less effective osmolyte than ectoine. In nature, glycine can be found in marine organisms where it is supposed to counteract pressure effects [10]. Thus, the finding of rational activity coefficients lower than one does not necessarily mean that glycine is a protein destabilizer. Compared to ectoine (which is strongly hydrated), glycine might form dimers. This explains the observed effects that lead to different efficiencies of counteracting osmotic stress. Summing up,

Table 2
PC-SAFT pure-component parameters and binary interaction parameters with water used in this work.

	m_i^{seg} [−]	σ_i [Å]	u_i/k_B [K]	ε^{AiBi}/k_B [K]	κ^{AiBi} [−]	Assoc. Scheme	Ref.
Water*	1.442	2.612	113.47	2171.8	0.0700	2:2	[53]
Urea	4.300	2.625	400.05	2100.1	0.0010	2:2	[54]
TMAO	8.928	2.248	245.44	0#	0.0700	1:1	[19]
Glycerol	2.007	3.815	430.82	4633.5	0.0019	1:1	[19]
Ectoine	1.250	5.050	530.00	3500.0	0.0900	1:1	[18]
Ac-Gly-NHMe	5.445	3.403	560.71	1652.4	0.0781	2:2	this work
Ac-Ala-NHMe	5.337	3.608	631.24	1488.6	0.0769	2:2	this work
Ac-Leu-NHMe	7.293	3.542	579.15	1538.6	0.0614	2:2	this work
Glycine	4.851	2.327	216.96	2598.1	0.0393	1:1	[46]

* Water parameters especially designed for high-pressure conditions, # induced association as introduced by Kleiner et al. [52].

Table 3
Binary interaction parameters k_{ij} between components used in this work. Only valid with the pure-component parameters from Table 2.

	Ac-Gly-NHMe	Ac-Ala-NHMe	Ac-Leu-NHMe	glycine	glycerol	ectoine	TMAO	water
Urea	0.0123	0.03	0.027	0	–	–	0.26	−0.221
TMAO	0.135	0.16	0.14	–	–	–	–	*
Water	−0.136	−0.120	−0.102	−0.03	−0.01	0.096	*	–

* k_{ij} between water and TMAO $k_{ij} = -0.15–0.005417 \cdot T[K]$, k_{ij} values with water were fitted in this work to osmotic-coefficient data.

Table 4
Experimentally determined osmotic coefficients of aqueous dipeptide solutions at 273.15 K and 1 bar.

m [mol/kg]	0.3	1.0	1.2	1.5
Ac-Gly-NHMe	0.9771	1.0263	1.0453	1.0706
Ac-Ala-NHMe	0.9590	1.0643	1.0805	1.1443
Ac-Leu-NHMe	0.9250	1.0122	1.0235	1.0387

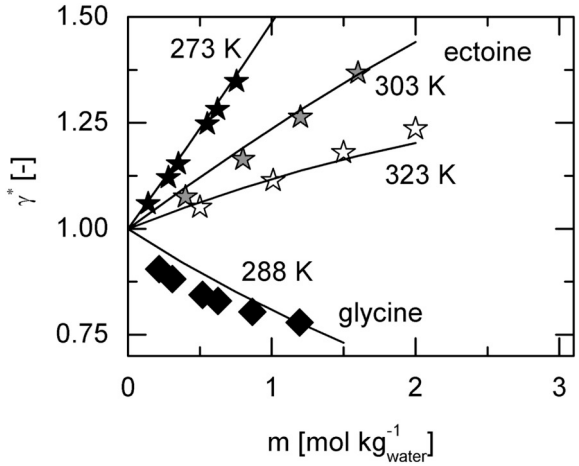

Fig. 1. Rational activity coefficient of osmolytes vs. molality of the osmolyte. Symbols: literature data (diamonds: glycine at 288.15 K [56], stars: ectoine at 273.15 K, 303.15 K and 323.15 K [18]), lines: PC-SAFT modeling results using parameters from Table 2.

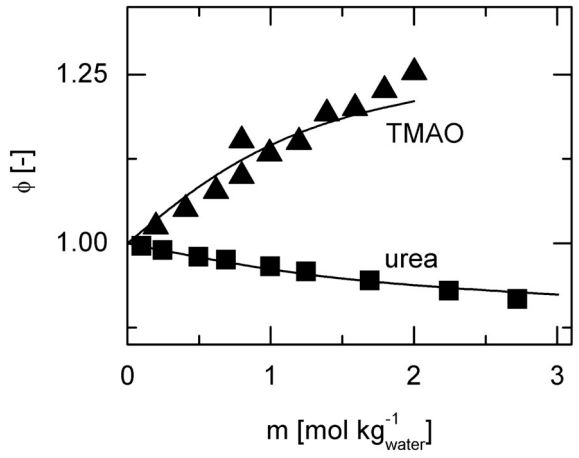

Fig. 2. Osmotic coefficient of aqueous osmolyte solutions vs. molality of the osmolyte. Symbols: literature data (squares: urea at 298.15 K [57], triangles: TMAO at 310.15 K [19]), lines: PC-SAFT modeling results using parameters from Table 2.

PC-SAFT correctly describes the opposing effects of ectoine and glycine in aqueous solution on the rational activity coefficient at different concentration and temperature conditions.

Similar to the different behavior of ectoine and glycine, also the osmolytes urea and TMAO show contradictive effects in water. Both, urea and TMAO, are known to influence the osmotic pressure of aqueous solutions. Literature results show that urea decreases the osmotic coefficient in aqueous solution [57], while TMAO strongly increases the osmotic coefficient [19]. From deep-sea organisms it is known that especially a 2:1 mixture of urea and TMAO is beneficial to counteract the high-pressure effects at deep sea conditions [4]. PC-SAFT was used to predict concentration effects of urea and TMAO on the osmotic coefficient of the corresponding aqueous solutions at 298.15 K (see Table S.2). PC-SAFT correctly describes that TMAO increases the osmotic coefficient strongly. Further, the opposing effect of urea which decreases the osmotic coefficient of the solution is also described with high accuracy. This means that PC-SAFT is able to distinguish the effects of the common osmolytes urea and TMAO on aqueous solutions correctly.

Glycerol is also considered to be a compatible solute with a strong effect on osmotic stress. Gylcerol can be found in marine organisms [9]. Since glycerol and water are completely miscible the osmotic coefficient is known for a broad concentration range (see Fig. 3). It should be noted that a molality of 50 mol/kg is equal to a mole fraction of > 0.5 and a mass fraction of > 0.8. Even though these highly concentrated conditions will not be encountered in natural organisms they are of high interest to evaluate the accuracy of PC-SAFT. Literature data shows that low concentrations of glycerol (< 6 mol/kg) increase the osmotic coefficient at 298.15 K while higher concentrations strongly decrease the osmotic coefficient [37]. It is thus reasonable that glycerol naturally occurs only at rather low concentrations where glycerol is an effective protectant against osmotic stress. PC-SAFT describes these effects correctly in almost quantitative agreement for this vast concentration range (see Fig. 3 and Table S.3).

Besides analyzing different compatible and incompatible solutes that can be found in nature, three different amino-acid-based dipeptides were also analyzed in this work as model compounds for more complex osmolytes. The dipeptides Ac-Gly-NHMe, Ac-Ala-NHMe and Ac-Leu-NHMe have a functional acetyl group, further they are methylated to

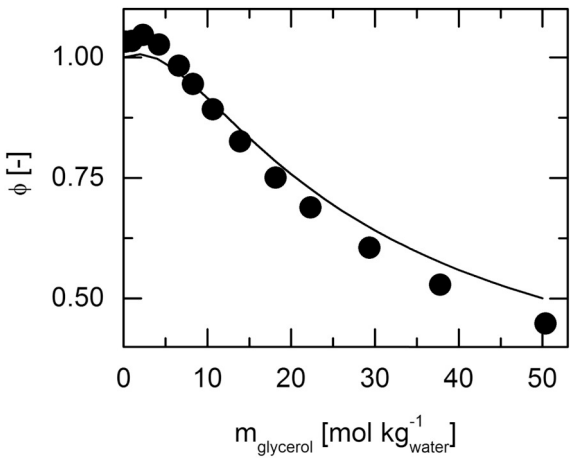

Fig. 3. Osmotic coefficient of an aqueous glycerol solution vs. molality of glycerol at 298.15 K. Symbols: literature data [58], line: PC-SAFT modeling results using parameters from Table 2.

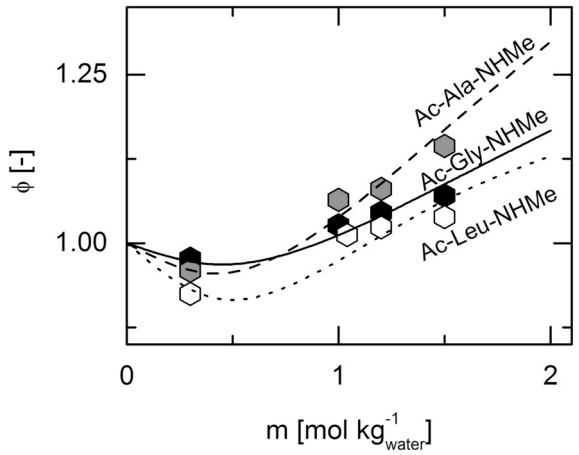

Fig. 4. Osmotic coefficients of aqueous dipeptide solutions vs. molality at 273.15 K. Symbols: experimental data (this work), lines: PC-SAFT modeling using parameters from Table 2 and Table 3. Black symbols and full line: acetyl-*N*-methylglycine amide, gray symbols and dashed line: acetyl-*N*-methylalanine amide, white symbols and dotted line: acetyl-*N*-methylleucine amide.

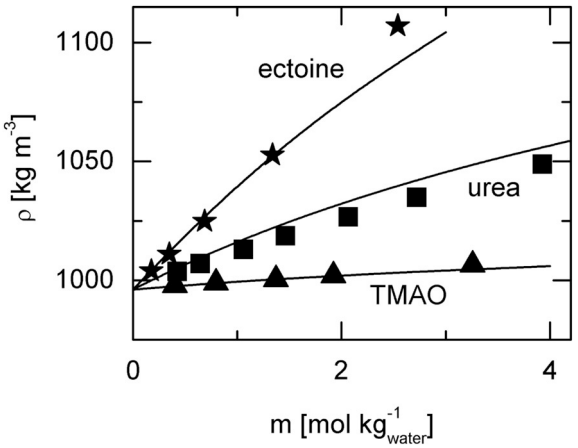

Fig. 5. Density of aqueous osmolyte solutions vs. molality of the osmolyte at 298.15 K. Symbols: literature data (triangles: TMAO [36], squares: urea [59], stars: ectoine [18]), lines: PC-SAFT prediction results using parameters from Table 2.

decrease reactivity and ensure stability for experimental investigations.

The osmotic coefficient was determined in this work for the three dipeptides at 273.15 K and molalities up to 2 mol/kg (see Fig. 4 and Table 4). The results show that all three dipeptides cause osmotic coefficients greater than unity at concentrations higher than 1 mol/kg. This means that the dipeptides can also be characterized as compatible solutes. This effect is weakest for Ac-Leu-NHMe and strongest for Ac-Ala-NHMe. The PC-SAFT modeling results are in good agreement with experimental data. That is, PC-SAFT might also be used to model thermodynamic properties of oligopeptides or even small proteins or protein fragments (see Table S.4).

4.2. Mixture densities at atmospheric-pressure conditions

In order to be able to use the thermodynamic model PC-SAFT to predict the effects of different osmolytes in aqueous solution it is not only important to account for energetic properties, e.g. activity coefficient and osmotic coefficient, but also to account for volumetric properties, e.g. density.

Even though the density of low concentrated aqueous osmolyte solutions does not deviate strongly from the density of pure water, occurring volume effects are well measurable. The observed effects of different osmolytes on the density of their aqueous solution do not only depend on the osmolyte's concentration and molecular size but also on molecular interactions, e.g. hydrogen bonding, hydration / solvation. The thermodynamic model PC-SAFT does not explicitly account for the exact arrangement of a hydration shell. However, it explicitly accounts for molecular interactions in the form of association and dispersion. Consequently, it is expected that PC-SAFT is an appropriate model to quantify the osmolyte effect on the density of the aqueous solutions. The PC-SAFT results that are presented in this work are predictions in a sense that parameters have not been adjusted to mixture density data.

In Fig. 5 the density of aqueous solutions of ectoine, urea and TMAO is shown for different molalities at 298.15 K. It can be seen that ectoine strongly increases the solution density while TMAO barely has an effect. Urea, in contrast, has a medium effect on the solution density. The results show that this behavior does not correlate with a simple quantity like the osmolyte's molar mass. Hence, molecular interactions between osmolyte/osmolyte and osmolyte/water also make substantial contributions to the density data.

The PC-SAFT prediction results correctly describe the effects of the osmolytes under investigation on the solution's density in very good agreement to literature data (see Table S.5). This shows that molecular interactions and their contributions to effects on volumetric properties are correctly accounted for in this thermodynamic model.

A wider concentration range has been investigated for quantifying the effect of glycerol on the density of its aqueous solutions. At low molalities glycerol strongly increases the density of the aqueous solution while the concentration effect on density does not increase much at higher glycerol molalities. This effect is also predicted correctly by PC-SAFT (see Table S.6) (Fig. 6).

Summing up, the results show that PC-SAFT is a useful tool to predict both energetic and volumetric properties of aqueous solutions of common osmolytes under different concentration and temperature conditions at atmospheric pressure using a water parameter set that was intentionally designed to be valid also at high pressure.

4.3. Mixture densities at high-pressure conditions

In the sections above the thermodynamic model PC-SAFT was used to predict thermodynamic properties of aqueous osmolyte solutions at atmospheric pressure. The results show that PC-SAFT is well suited to perform predictions at different concentration and temperature conditions. The following sections investigate the effect of pressure on aqueous osmolyte solutions and evaluate the performance of PC-SAFT to predict these pressure effects on aqueous solution densities.

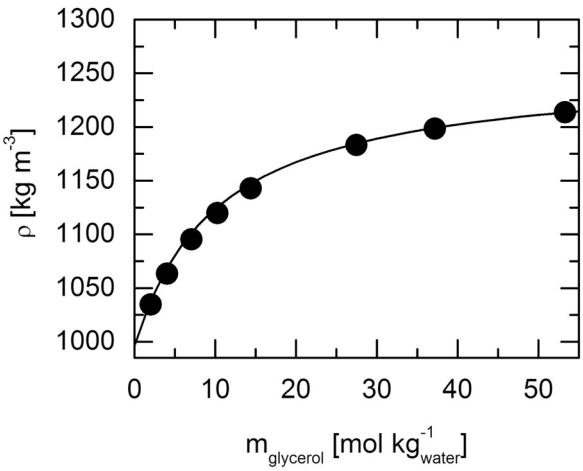

Fig. 6. Density of an aqueous glycerol solution vs. molality of glycerol at 298.15 K. Symbols: literature data [37], line: PC-SAFT prediction results using parameters from Table 2.

Table 5
Experimentally determined densities of aqueous osmolyte solutions in kg/m^3 vs. pressure.

	T[K]	1 bar	100 bar	200 bar	300 bar	400 bar	500 bar
0.5 mol/kg ectoine	298.15	1015.51	1021.22	1026.84	1032.37	1037.79	1043.16
0.5 mol/kg urea	300.15	1004.31	1008.72	1013.02	1017.24	1021.35	1025.35
0.586 mol/kg (0.5 mol/L) TMAO	300.15	998.37	1002.515	1006.61	1010.68	1014.67	1018.51
0.5 mol/kg urea + 0.5 mol/kg TMAO	300.15	1007.47	1011.29	1015.19	1019.05	1023.09	1027.11

First, the effect of pressure on the density of aqueous osmolyte solutions was investigated experimentally (see Table 5). In Fig. 7 the density of urea, TMAO and ectoine solutions with a molality of 0.5 mol/kg at 298.15 K is presented for pressures between 1 bar and 500 bar. Within this pressure range the density of each solution rises by 20–28 kg/m^3 upon pressurization. This effect is correctly predicted by PC-SAFT in good agreement to experimental data (see Table S.7).

Second, the pressure influence on the density of the ternary system containing 0.5 mol/kg urea and 0.5 mol/kg TMAO aqueous solution was investigated experimentally. From 1 bar to 500 bar the density increases by 20 kg/m^3. Even for this ternary mixture the pressure effect on the density is predicted correctly by PC-SAFT, which can be also seen from Fig. 7.

Furthermore, the pressure effects on 0.5 mol/kg solutions of dipeptide solutions were experimentally investigated at different temperatures (see Table 6). The results for the experimentally determined densities of Ac-Ala-NHMe solutions are illustrated in Fig. 8. It can be seen that both temperature and pressure have a strong effect on solution density. Further, under same temperature and pressure conditions solution density is lowest for Ac-Leu-NHMe solutions while it is highest for Ac-Gly-NHMe solutions. For each dipeptide under investigation PC-SAFT correctly predicts the effects of species, temperature and pressure (see Table S.8).

For the investigation of pressure effects on aqueous glycerol solutions density data was taken from the literature at 298.15 K and different concentration and pressure conditions [37] up to 1000 bar (see Fig. 9). The data shows that compressibility is lower compared to the low concentrated osmolyte solutions discussed above. Furthermore, compressibility is lower for higher glycerol concentrations. Even though the concentration range and pressure range of this density data are wide, the PC-SAFT predictions for pressure and concentration effects on density data of the aqueous glycerol solutions are in almost quantitative agreement with literature data (see Table S.9).

For studies of pressure and concentration effects on the density of aqueous glycine solutions data was also taken from the literature [38]. For the aqueous glycine solutions compressibility decreases with increasing glycine concentrations (see Fig. 10). The PC-SAFT prediction results show that PC-SAFT correctly predicts concentration and pressure effects on the density of aqueous glycine solutions for pressures up to 1000 bar (see Table S.10).

The modeling results are based on pure-component parameters for water that have been adjusted to vapor pressures and densities up to very high pressures. In contrast, the pure-component parameters of the osmolytes were fitted to densities and osmotic coefficients at ambient pressure. This shows that a high accuracy for modeling water density is the basis for modeling properties of aqueous solutions. To conclude, these results show that the thermodynamic model PC-SAFT allows predicting concentration, temperature and pressure effects on the density of binary and ternary aqueous solutions containing important osmolytes.

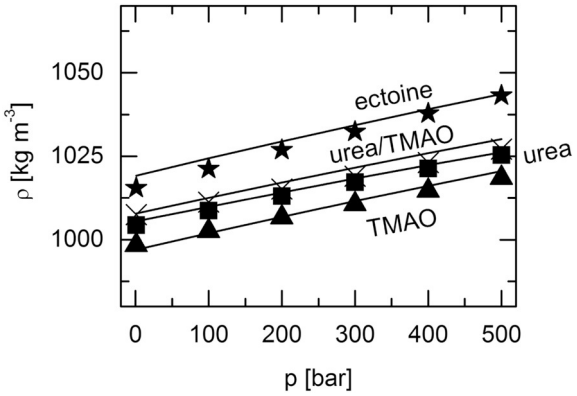

Fig. 7. Density of aqueous osmolyte solutions vs. pressure. Symbols: experimental data (triangles: 0.586 mol/kg (0.5 mol/L) TMAO at 300.15 K, squares: 0.5 mol/kg urea at 300.15 K, stars: 0.5 mol/kg ectoine at 298.15 K, crosses: mixture of 0.5 mol/kg TMAO and 0.5 mol/kg urea at 298.15 K), lines: PC-SAFT prediction results using parameters from Table 2.

4.4. Osmotic coefficients of ternary systems containing two osmolytes and water

In the section above focus was laid on temperature, concentration and pressure effects on volumetric data, i.e. density, of aqueous osmolyte solutions. In this section energetic data, i.e. osmotic coefficient, is analyzed for ternary aqueous osmolyte solutions. In Fig. 11 the osmotic coefficient of aqueous solutions containing Ac-Gly-NHMe and urea as well as Ac-Gly-NHMe and TMAO is plotted different molalities of the dipeptide (see Table S.11 and Table S.12). The results show that higher dipeptide molality leads to a higher osmotic coefficient. The addition of TMAO strongly increases the osmotic coefficient while urea decreases the osmotic coefficient. This behavior was quantitatively described by PC-SAFT by using one binary parameter between two osmolytes. The results for the other dipeptides are given in Table S.13

Table 6
Experimentally determined densities of 0.5 mol/kg aqueous dipeptide solutions vs. pressure at 298.15 K, 308.15 K and 318.15 K.

	p [bar]	1	100	200	300	400	500
Ac-Gly-NHMe	298.15 K	1008.59	1012.49	1016.46	1020.38	1024.56	1028.68
	308.15 K	1004.20	–	–	–	–	–
	318.15 K	999.13	–	–	–	–	–
Ac-Ala-NHMe	298.15 K	1007.15	1011.00	1014.91	1018.99	1023.05	1027.06
	308.15 K	1002.71	1006.86	1010.82	1014.93	1018.93	1022.98
	318.15 K	998.55	1002.79	1006.93	1010.85	1014.76	1018.46
Ac-Leu-NHMe	298.15 K	1004.75	1008.57	1012.38	1016.33	1020.21	1024.09
	308.15 K	1000.09	1004.19	1008.27	1012.20	1016.12	1020.11
	318.15 K	995.65	999.90	1003.96	1007.93	1011.82	1015.57

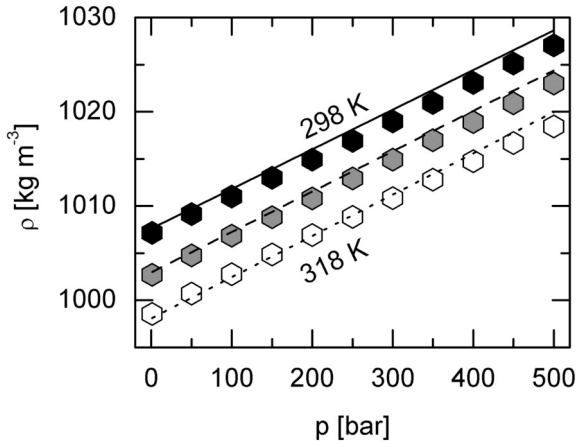

Fig. 8. Density of aqueous 0.5 mol/kg acetyl-*N*-methylalanine amide solutions vs. pressure. Symbols: experimental data (black: 298.15 K, gray: 308.15 K, white: 318.15 K), lines: PC-SAFT prediction results using parameters from Table 2.

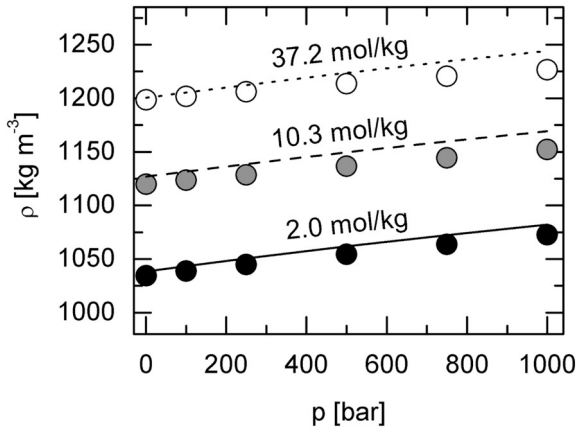

Fig. 9. Density of aqueous glycerol solutions vs. pressure at 298.15 K. Symbols: literature data [37], lines: PC-SAFT prediction results using parameters from Table 2.

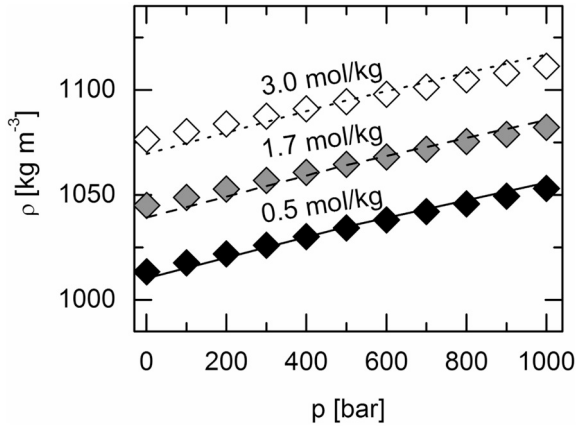

Fig. 10. Density of aqueous glycine solutions vs. pressure at 298.15 K. Symbols: literature data [38], lines: PC-SAFT prediction results using parameters from Table 2.

to Table S.16.

Also literature data was used to determine concentration effects on the osmotic coefficient of ternary aqueous solutions containing urea and glycine [60] (Fig. 12). The results show that low (< 0.5 mol/kg) urea concentrations lead to a higher osmotic coefficient, while higher

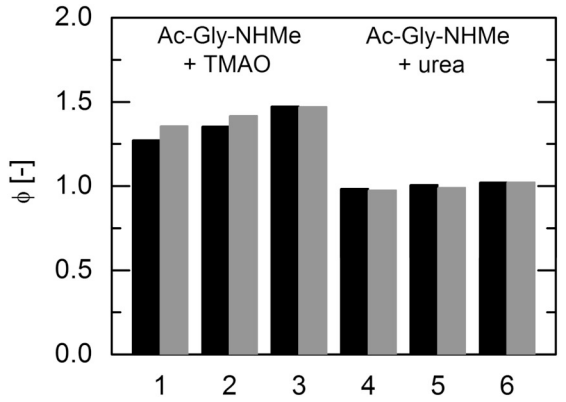

Fig. 11. Osmotic coefficient of ternary aqueous solutions containing two osmolytes (Ac-Gly-NHMe and TMAO) and (Ac-Gly-NHMe and urea) at different molalities (N° 1–6: molalities of all osmolytes see Table S.11 and Table S.12) at 273.15 K. Black: experimental data, gray: PC-SAFT modeling results using parameters from Tables 2 and 3.

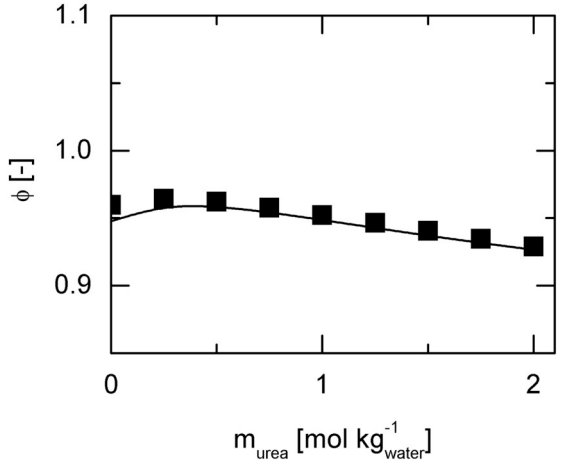

Fig. 12. Osmotic coefficient of ternary aqueous solutions containing glycine and urea vs. molality of urea ($m_{glycine}$ = 0.5 mol/kg) at 298.15 K. Symbols: literature data [60], line: PC-SAFT modeling results using parameters from Tables 2 and 3.

urea concentrations decrease the osmotic coefficient. These concentration effects on the osmotic coefficient of ternary aqueous urea + glycine solutions are described by PC-SAFT in quantitative agreement to the experimental data (see Table S.17).

5. Conclusions

High-pressure effects on different biochemical systems were investigated in this work where a special focus was laid on pressure effects on osmolyte solutions. Osmolytes under investigation were TMAO, urea, ectoine, glycerol, glycine as well as the dipeptides acetyl-*N*-methylglycine amide, acetyl-*N*-methylalanine amide, and acetyl-*N*-methylleucine amide. In order to get a complete picture, temperature (273.15 K – 323.15 K) and concentration effects (typically up to 3 mol/kg) were also analyzed. New experimental high-pressure density data was determined for aqueous osmolyte solutions at pressures up to 500 bar. The thermodynamic model PC-SAFT was used to predict the influence of pressure, temperature and concentration on solution density of aqueous osmolyte systems. The prediction results agreed very well with experimental data as well as with literature data. Further, the influence of osmolyte concentration and of temperature on osmotic coefficients at ambient pressure was modeled accurately with the same PC-SAFT parameters. Finally, PC-SAFT was validated by successfully

modeling osmotic coefficients of ternary solutions containing two osmolytes. This shows that PC-SAFT is very well suited for modeling biochemical solutions even at high pressure. Since the fundamental thermodynamic influence factors pressure, temperature and concentration are correctly accounted for by PC-SAFT this work provides the basis for further investigations of more complex systems. This knowledge can be used in future work to approach more challenging problems, e.g. pressure effects on enzymatic reactions.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bpc.2019.106211.

References

[1] R. Winter, D. Lopes, S. Grudzielanek, K. Vogtt, Towards an understanding of the temperature/pressure configurational and free-energy landscape of biomolecules, Journal of Non-Equilibrium Thermodynamics 32 (2007) 41–97.
[2] L. Smeller, Pressure–temperature phase diagrams of biomolecules, Biochimica et Biophysica Acta (BBA)-Protein Structure and Molecular Enzymology 1595 (2002) 11–29.
[3] M.W. Jaworek, V. Schuabb, R. Winter, The effects of glycine, TMAO and osmolyte mixtures on the pressure dependent enzymatic activity of α-chymotrypsin, Phys. Chem. Chem. Phys. 20 (2018) 1347–1354.
[4] T.Q. Luong, R. Winter, Combined pressure and cosolvent effects on enzyme activity–a high-pressure stopped-flow kinetic study on α-chymotrypsin, Phys. Chem. Chem. Phys. 17 (2015) 23273–23278.
[5] M.J. Eisenmenger, J.I. Reyes-De-Corcuera, High pressure enhancement of enzymes: A review, Enzym. Microb. Technol. 45 (2009) 331–347.
[6] K. Akasaka, H. Matsuki, High pressure bioscience, basic concepts, Applications and Frontiers (Springer, Heidelberg, 2015).
[7] S. Imoto, P. Kibies, C. Rosin, R. Winter, S.M. Kast, D. Marx, Toward extreme biophysics: deciphering the infrared response of biomolecular solutions at high pressures, Angew. Chem. Int. Ed. Eng. 55 (2016) 9534–9538.
[8] J.L. Silva, A.C. Oliveira, T.C. Vieira, G.A. de Oliveira, M.C. Suarez, D. Foguel, High-pressure chemical biology and biotechnology, Chem. Rev. 114 (2014) 7239–7267.
[9] P.H. Yancey, M.E. Clark, S.C. Hand, R.D. Bowlus, G.N. Somero, Living with water stress: evolution of osmolyte systems, Science 217 (1982) 1214–1222.
[10] P.H. Yancey, M.E. Gerringer, J.C. Drazen, A.A. Rowden, A. Jamieson, Marine fish may be biochemically constrained from inhabiting the deepest ocean depths, Proc. Natl. Acad. Sci. U. S. A. 111 (2014) 4461–4465.
[11] S.A. Hawley, Reversible pressure-temperature denaturation of chymotrypsinogen, Biochemistry 10 (1971) 2436–2442.
[12] F. Abe, K. Horikoshi, The biotechnological potential of piezophiles, Trends Biotechnol. 19 (2001) 102–108.
[13] E. Ohmae, Y. Miyashita, C. Kato, Thermodynamic and functional characteristics of deep-sea enzymes revealed by pressure effects, Extremophiles 17 (2013) 701–709.
[14] Q. Huang, J.M. Rodgers, R.J. Hemley, T. Ichiye, Extreme biophysics: enzymes under pressure, J. Comput. Chem. 38 (2017) 1174–1182.
[15] C.A. Royer, Revisiting volume changes in pressure-induced protein unfolding, Biochim. Biophys. Acta Protein Struct. Mol. Enzymol. 1595 (2002) 201–209.
[16] J.B. Rouget, T. Aksel, J. Roche, J.L. Saldana, A.E. Garcia, D. Barrick, C.A. Royer, Size and sequence and the volume change of protein folding, J. Am. Chem. Soc. 133 (2011) 6020–6027.
[17] F. Meersman, L. Smeller, K. Heremans, Protein stability and dynamics in the pressure-temperature plane, Biochim. Biophys. Acta 1764 (2006) 346–354.
[18] C. Held, T. Neuhaus, G. Sadowski, Compatible solutes: thermodynamic properties and biological impact of ectoines and prolines, Biophys. Chem. 152 (2010) 28–39.
[19] C. Held, G. Sadowski, Compatible solutes: thermodynamic properties relevant for effective protection against osmotic stress, Fluid Phase Equilib. 407 (2016) 224–235.
[20] M. Gao, C. Held, S. Patra, L. Arns, G. Sadowski, R. Winter, Crowders and Cosolvents-major contributors to the cellular milieu and efficient means to counteract environmental stresses, Chemphyschem. 18 (2017) 2951–2972.
[21] E.A. Galinski, Compatible solutes of halophilic eubacteria: molecular principles, water-solute interaction, stress protection, Experientia 49 (1993) 487–496.
[22] S.S. Cho, G. Reddy, J.E. Straub, D. Thirumalai, Entropic stabilization of proteins by TMAO, J. Phys. Chem. B 115 (2011) 13401–13407.
[23] C.Y. Hu, G.C. Lynch, H. Kokubo, B.M. Pettitt, Trimethylamine N-oxide influence on the backbone of proteins: an oligoglycine model, Proteins 78 (2010) 695–704.
[24] R. Kumar, J.M. Serrette, E.B. Thompson, Osmolyte-induced folding enhances tryptic enzyme activity, Arch. Biochem. Biophys. 436 (2005) 78–82.
[25] J. Ma, I.M. Pazos, F. Gai, Microscopic insights into the protein-stabilizing effect of trimethylamine N-oxide (TMAO), Proc. Natl. Acad. Sci. U. S. A. 111 (2014) 8476–8481.
[26] D.L. Pincus, C. Hyeon, D. Thirumalai, Effects of trimethylamine N-oxide (TMAO) and crowding agents on the stability of RNA hairpins, J. Am. Chem. Soc. 130 (2008) 7364–7372.
[27] Q. Zou, B.J. Bennion, V. Daggett, K.P. Murphy, The molecular mechanism of stabilization of proteins by TMAO and its ability to counteract the effects of urea, J. Am. Chem. Soc. 124 (2002) 1192–1202.
[28] H. Sun, B. Glasmacher, N. Hofmann, Compatible solutes improve cryopreservation of human endothelial cells, CryoLetters 33 (2012) 485–493.
[29] I. Baskakov, A. Wang, D.W. Bolen, Trimethylamine-N-oxide counteracts urea effects on rabbit muscle lactate dehydrogenase function: A test of the counteraction hypothesis, Biophys. J. 74 (1998) 2666–2673.
[30] K. Göller, E. A. Galinski, Protection of a model enzyme (lactate dehydrogenase) against heat, urea and freeze-thaw treatment by compatible solute additives, J. Mol. Catal. B Enzym. 7 (1999) 37–45.
[31] T.-Y. Lin, S.N. Timasheff, Why do some organisms use a urea-methylamine mixture as osmolyte? Thermodynamic compensation of urea and trimethylamine N-oxide interactions with protein, Biochemistry 33 (1994) 12695–12701.
[32] P.V. Sundaram, R. Venkatesh, Retardation of thermal and urea induced inactivation of alpha- chymotrypsin by modification with carbohydrate polymers, Protein Eng. Des. Sel. 11 (1998) 699–705.
[33] A. Oren, Thermodynamic limits to microbial life at high salt concentrations, Environ. Microbiol. 13 (2011) 1908–1923.
[34] P.H. Yancey, J.F. Siebenaller, Co-evolution of proteins and solutions: protein adaptation versus cytoprotective micromolecules and their roles in marine organisms, J. Exp. Biol. 218 (2015) 1880–1896.
[35] R.H. Kelly, P.H. Yancey, High contents of trimethylamine oxide correlating with depth in deep-sea teleost fishes, skates, and decapod crustaceans, Biol. Bull. 196 (1999) 18–25.
[36] D.M. Makarov, G.I. Egorov, A.M. Kolker, Density and volumetric properties of aqueous solutions of trimethylamine N-oxide in the temperature range from (278.15 to 323.15) K and at pressures up to 100 MPa, J. Chem. Eng. Data 60 (2015) 1291–1299.
[37] G.I. Egorov, D.M. Makarov, Volumetric properties of binary liquid-phase mixture of (water + glycerol) at temperatures of (278.15 to 323.15)K and pressures of (0.1 to 100)MPa, J. Chem. Thermodyn. 79 (2014) 135–158.
[38] C. Yokoyama, S. Takahashi, PVT and derived thermodynamic properties for the glycine-water system at temperatures from 298 to 323 K and pressures up to 300 MPa, Int. J. Thermophys. 10 (1989) 35–45.
[39] C. Holzl, P. Kibies, S. Imoto, R. Frach, S. Suladze, R. Winter, D. Marx, D. Horinek, S.M. Kast, Design principles for high-pressure force fields: aqueous TMAO solutions from ambient to kilobar pressures, J. Chem. Phys. 144 (2016) 144104.
[40] S. Imoto, H. Forbert, D. Marx, Water structure and solvation of osmolytes at high hydrostatic pressure: pure water and TMAO solutions at 10 kbar versus 1 bar, Physical Chemistry Chemical Physics 17 (2015) 24224–24237.
[41] H. Teng, A. Yamasaki, M.K. Chun, H. Lee, Solubility of liquid CO2in water at temperatures from 278 K to 293 K and pressures from 6.44 MPa to 29.49 MPa and densities of the corresponding aqueous solutions, J. Chem. Thermodyn. 29 (1997) 1301–1310.
[42] Z. Li, M. Dong, S. Li, L. Dai, Densities and Solubilities for binary Systems of Carbon Dioxide + water and carbon dioxide + brine at 59 °C and pressures to 29 MPa, J. Chem. Eng. Data 49 (2004) 1026–1031.
[43] L.M.C. Pereira, A. Chapoy, R. Burgass, B. Tohidi, Measurement and modelling of high pressure density and interfacial tension of (gas + n -alkane) binary mixtures, J. Chem. Thermodyn. 97 (2016) 55–69.
[44] K. Liu, Y. Wu, M.A. McHugh, H. Baled, R.M. Enick, B.D. Morreale, Equation of state modeling of high-pressure, high-temperature hydrocarbon density data, J. Supercrit. Fluids 55 (2010) 701–711.
[45] J. Gross, G. Sadowski, Perturbed-chain SAFT: an equation of state based on a perturbation theory for chain molecules, Ind. Eng. Chem. Res. 40 (2001) 1244–1260.
[46] C. Held, L.F. Cameretti, G. Sadowski, Measuring and modeling activity coefficients in aqueous amino-acid solutions, Ind. Eng. Chem. Res. 50 (2011) 131–141.
[47] J.-B. Grosse Daldrup, C. Held, F. Ruether, G. Schembecker, G. Sadowski, Measurement and modeling solubility of aqueous multisolute amino-acid solutions, Ind. Eng. Chem. Res. 49 (2010) 1395–1401.
[48] J.-B. Grosse Daldrup, C. Held, G. Sadowski, G. Schembecker, Modeling pH and Solubilities in aqueous multisolute amino acid solutions, Ind. Eng. Chem. Res. 50 (2011) 3503–3509.
[49] P.S.Z. Rogers, K.S. Pitzer, Volumetric properties of aqueous sodium chloride solutions, J. Phys. Chem. Ref. Data 11 (1982) 15–81.
[50] L.F. Cameretti, G. Sadowski, Modeling of aqueous amino acid and polypeptide solutions with PC-SAFT, Chem. Eng. Process. Process Intensif. 47 (2008) 1018–1025.
[51] A.P. Carneiro, O. Rodríguez, C. Held, G. Sadowski, E.N.A. Macedo, Density of mixtures containing sugars and ionic liquids: experimental data and PC-SAFT modeling, J. Chem. Eng. Data 59 (2014) 2942–2954.
[52] M. Kleiner, G. Sadowski, Modeling of polar systems using PCP-SAFT: an approach to account for induced-association interactions, J. Phys. Chem. C 111 (2007) 15544–15553.
[53] M. Knierbein, C. Held, M. Paulus, G. Sadowski, C. Sternemann, J. Nase, Density measurements and simulations of pressurized TMAO solutions, Biophys. Chem. (2019) (Submitted).
[54] C. Hölzl, D. Marx, S. Kast, D. Horinek, Prediction of the density and apparent molar volume of aqueous urea solutions at 10 kbar, Biophys. Chem. (2019) (Submitted).

[55] E.A. Galinski, H.G. Trüper, Microbial behaviour in salt-stressed ecosystems, FEMS Microbiol. Rev. 15 (1994) 95–108.
[56] C.M. Romero, M.E. González, Osmotic and activity coefficients of glycine, dl-α-alanine and dl-α-aminobutyric acid in aqueous solutions at temperatures between 288.15 and 303.15K, Fluid Phase Equilib. 250 (2006) 99–104.
[57] D.N. Kurhe, D.H. Dagade, J.P. Jadhav, S.P. Govindwar, K.J. Patil, Thermodynamic studies of amino acid–denaturant interactions in aqueous solutions at 298.15 K, J. Solut. Chem. 40 (2011) 1596–1617.
[58] A.M. Rudakov, V.V. Sergievskii, Activities of the components of glycerol-water binary solutions at 298.15 K, Russ. J. Phys. Chem. 80 (2006) 1804–1808.
[59] F.T. Gucker Jr., F.W. Gage, C.E. Moser, The densities of aqueous solutions of urea at 25 and 30 and the apparent Molal volume of Urea1, J. Am. Chem. Soc. 60 (1938) 2582–2588.
[60] L. Rafflenbeul, W.-M. Pang, H. Schönert, K. Haberle, Zur Thermodynamik der hydrophoben Wechselwirkung; die Systeme Wasser + Glycin + Harnstoff und Wasser + Alanin + Harnstoff bei 25° C/thermodynamics of Hydrophobie interaction in the systems water + Glycin + urea and water + Alanin + urea at 25° C, Z. Naturforsch. C 28 (1973) 533–554.