Aqueous Two-Phase Systems of Poly(ethylene glycol) and Sodium Citrate: Experimental Results and Modeling

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Aqueous two-phase systems of poly(ethylene) glycol and a salt are considered as extraction media for large-scale downstream processing of industrially important enzymes. The present paper deals with one of such two-phase systems. New experimental results are presented for the composition of the coexisting liquid phases of the system (water + poly(ethylene glycol) + sodium citrate) at 298.15 K for three poly(ethylene) glycols of different molar masses of about (600, 1500, and 3000) g·mol^{-1}, respectively. The experimental results are described within experimental uncertainty by a semiempirical expression for the excess Gibbs energy.

Introduction

The liquid−liquid equilibrium of aqueous two-phase systems that are observed when two hydrophilic, but nevertheless incompatible solutes (for example, polymer/salt or polymer/polymer), are simultaneously dissolved in water has attracted a lot of interest in the past decades. One of the reasons for that interest comes from their suitability as extraction media for the downstream processing in biotechnology, for example, for the separation of cells, cell organelles, and proteins (see Albertsson[1] and Kula et al.[2]). For the extraction of proteins, polymer/salt systems are preferred over polymer/polymer systems for economical reasons, and they often reveal a higher selectivity (Vernau and Kula[3]). Besides some advantages, there might also be some problems in the industrial use of such systems. For example, when aqueous two-phase systems are formed with the most commonly studied salts potassium phosphate or sodium sulfate in a large scale process, the aqueous effluents from such plants carry a lot of these salts. This can result in some environmentally undesired wastes. As an alternative approach, Vernau and Kula[3] reported a study with aqueous two-phase systems where one of the phase-forming hydrophilic components is sodium citrate which is a biodegradable salt.

More recently, Zafarani-Moattar et al.[4] presented experimental results (at 298.15, 308.15, and 318.15 K) for the liquid−liquid equilibrium that is observed when sodium citrate and a high-molecular poly(ethylene glycol) with molar mass about 6000 g·mol^{-1} (PEG 6000) are simultaneously dissolved in water. They correlated these data with two models for the Gibbs excess energy. One model was based on the osmotic virial equation, and the other one was an extension of the UNIQUAC equation. The extension consisted of an additional Debye−Hückel term to account for interactions between ionic species.

Murugesan and Perumalsamy[5] and Perumalsamy et al.[6] reported two sets of experimental results for the liquid−liquid equilibrium in aqueous systems of PEG and sodium citrate at temperatures between (298.15 and 318.15) K. In both sets of experiments, different PEGs were used (in the first part,[5] PEG 2000; in the second part,[6] PEG 6000).

Recently, Porto et al.[7] published experimental results for the cloud-point curve of various (PEG + sodium citrate) aqueous two-phase systems which were used (at adjusted pH) to partition some proteases.

Here, we present new experimental results for the liquid−liquid equilibrium at 298.1 K in aqueous solutions of sodium citrate that contain three poly(ethylene glycols). All PEGs are nearly monodisperse, but they differ in the average molar mass (PEG 600, PEG 1500, and PEG 3000, respectively). The new experimental results are correlated with the VERS model (virial equation with relative surface fractions) for the excess Gibbs energy of aqueous solutions of low molecular electrolytes and neutral polymers.[8,9] That model was also used to predict the influence of temperature and molecular mass of PEG on the liquid−liquid equilibrium and for a comparison with literature data for such liquid−liquid equilibria.

Experimental Section

Materials. Three samples of poly(ethylene glycol), commercially designated as PEG 600, PEG 1500, and PEG 3000, of different molar mass (the commercial names comprehend the approximated molar masses in g·mol^{-1}) were used. PEG 600 was from Hoechst/Germany (lot 663095), PEG 1500 from Clariant/Germany (lot 106329400), and PEG 3000 from Fluka/Switzerland (lot 405674/1). Dehydrated sodium citrate (lot A118748903) and water (for chromatography) were both from Merck/Germany. All chemicals were used without further purification. Previous investigations of similar PEG samples by gel chromatography (GPC)[10] showed that such polymer samples are nearly monodisperse, the polydispersity (i.e., ratio of the weight averaged molar mass to the number averaged molar mass) being close to one. Furthermore, the suppliers' characterizations of the molecular mass proved to be reliable.

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Table 1. Experimental Results for the Liquid−Liquid Equilibrium of Systems (PEG (1) + Sodium Citrate (2) + Water (3)) at 298 K (Mass Fraction w_i of Component i with Experimental Uncertainty)

feed solution		top phase		bottom phase	
w_1	w_2	w'_1	w'_2	w''_1	w''_2
Mixtures with PEG 600					
0.3005	0.2000	0.552 ± 0.018	0.016 ± 0.003	0.032 ± 0.007	0.381 ± 0.029
0.2800	0.1798	0.522 ± 0.008	0.0194 ± 0.0003	0.016 ± 0.001	0.367 ± 0.002
0.2610	0.1589	0.457 ± 0.002	0.029 ± 0.001	0.019 ± 0.005	0.324 ± 0.003
0.2400	0.1510	0.417 ± 0.006	0.0384 ± 0.0002	0.056 ± 0.002	0.269 ± 0.001
Mixtures with PEG 1500					
0.2606	0.0894	0.521 ± 0.001	0.0146 ± 0.0001	0.0003 ± 0.0001	0.321 ± 0.001
0.2398	0.1033	0.489 ± 0.007	0.0190 ± 0.0001	0.0001 ± 0,0001	0.293 ± 0.001
0.2207	0.1204	0.446 ± 0.001	0.026 ± 0.001	0.0052 ± 0.0007	0.269 ± 0.005
0.2000	0.1197	0.39 ± 0.01	0.034 ± 0.002	0.008 ± 0.001	0.239 ± 0.001
0.1800	0.1463	0.334 ± 0.005	0.044 ± 0.002	0.016 ± 0.004	0.210 ± 0.004
0.1597	0.1098	0.247 ± 0.004	0.071 ± 0.001	0.046 ± 0.001	0.172 ± 0.001
Mixtures with PEG 3000					
0.2197	0.1400	0.469 ± 0.001	0.0191 ± 0.0006	0.0013 ± 0.0005	0.2620 ± 0.0002
0.1999	0.1310	0.434 ± 0.002	0.02343 ± 0.0001	0.0016 ± 0.0001	0.239 ± 0.002
0.1797	0.1210	0.384 ± 0.024	0.0296 ± 0.0008	0.0039 ± 0.0015	0.213 ± 0.001
0.1590	0.1130	0.333 ± 0.016	0.038 ± 0.002	0.0067 ± 0.0009	0.190 ± 0.004
0.1550	0.1046	0.284 ± 0.011	0.0442 ± 0.0002	0.010 ± 0.001	0.174 ± 0.001
0.1251	0.1001	0.210 ± 0.020	0.0687 ± 0.0004	0.0372 ± 0.0003	0.141 ± 0.001

Table 2. Surface Parameters of Species k According to Bondi[16]

species	number of groups	q_k
H_2O	1	1.40
sodium ion	1	1.40
citrate ion	1	1.40
PEG middle group	−	1.32
PEG end group	−	1.74
PEG 400[a]	2 end groups + 7 middle groups	
PEG 600	2 end groups + 12 middle groups	
PEG 1500	2 end groups + 33 middle groups	
PEG 2000	2 end groups + 44 middle groups	
PEG 3000	2 end groups + 67 middle groups	
PEG 6000[a]	2 end groups + 135 middle groups	
PEG 8000[a]	2 end groups + 179 middle groups	

[a] For the prediction and comparison with literature data.

Table 3. Interaction Parameters of the VERS Model

sodium citrate + water[a]	
$a^{(0)}_{Na^+,Cit^{3-}}$	$2.744\cdot10^{-1}$
$a^{(1)}_{\mathrm{Na^+,Cit^{3-}}}$	5.3772
$b_{\mathrm{Na^+,Na^+Cit^{3-}}}$	$-5.25\cdot10^{-3}$
PEG + water	
$a^{(00)}_{\mathrm{EG,EG}}$	$1.05\cdot10^{-3}$
$a^{(00)}_{\mathrm{MG,EG}}$	$1.56\cdot10^{-3}$
$a^{(00)}_{\mathrm{MG,MG}}$	$7.78\cdot10^{-3}$
$a^{(01)}_{\mathrm{MG,EG}}$	0.0453
$a^{(01)}_{\mathrm{MG,MG}}$	0.1334
$a^{(02)}_{\mathrm{MG,MG}}$	-0.1685
PEG + sodium citrate + water	
$a^{(0)}_{\mathrm{EG,Na^+}}$	$1.39\cdot10^{-3}$
$a^{(0)}_{\mathrm{MG,Na^+}}$	$5.568\cdot10^{-2}$

[a] From Schunk and Maurer.[17]

Experimental Procedures. Feed solutions of about 20 g were prepared in 25 cm^3 glass centrifuge tubes by mixing known masses of stock solutions of the single components. The concentrations of the solutes (polymer/salt) in these stock solutions were known from gravimetric preparations. The feed mixtures were at first stirred for about 1 min using a vortex mixer and afterward placed into a water bath for about 1 h. The temperature of that bath was kept at (298.1 ± 0.1) K. Then the mixture was again agitated for about 1 min and kept in the water bath for about 1 day. Afterward, the tubes were centrifuged at 3500 rpm for 15 min in a thermostatted centrifuge (model Rotina 48R, Hettich, Tuttlingen, Germany) at 298.1 K and placed again in the thermostatted water bath for one more hour. Samples of the coexisting phases were then carefully removed and analyzed for PEG and sodium citrate. All analyses were repeated at least once, in most cases twice.

Analysis. The concentration of sodium citrate in a sample was determined by HPLC with a SUPELCOGEL C-610H column (SUPELCO, USA) and a UV detector (model SPD-6AV, Shimadzu, Japan). All samples analyzed by HPLC were diluted with an exactly known amount of the corresponding eluent and injected by syringes through filters (pore size: 0.45 μm). The eluent was a 0.1 % by volume aqueous solution of phosphoric acid. The flow rate was 0.5 cm^3·min^{-1} resulting in a retention time of about 11.2 min. The detector's signal was recorded and integrated by an integrator (model C-R3A. Shimadzu, Japan). Tests were performed to verify that the presence of PEG has no influence on the analytical results for sodium citrate. These tests revealed that there was no detectable influence as long as the PEG mass fraction (of the sample that was injected into the HPLC) was below 2 %. At higher PEG mass fractions, there is a small influence that can result in a

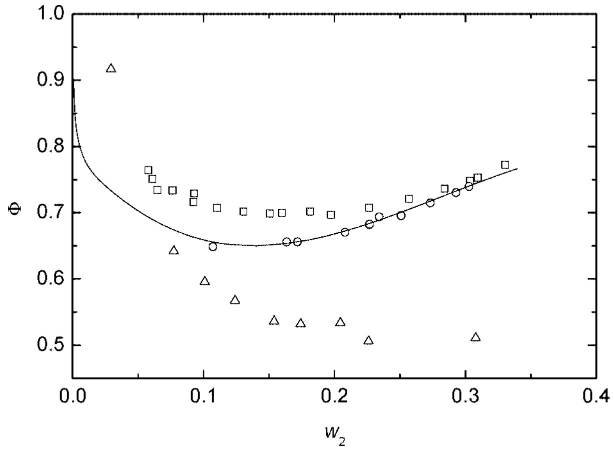

Figure 1. Aqueous solutions of sodium citrate (2): osmotic coefficients Φ at 298.15 K as a function of w_2. Experimental results from: □, Sadeghi;[18] ○, Schunk and Maurer;[17] Δ, Salabat et al.;[19] −, calculation results (from the VERS model).

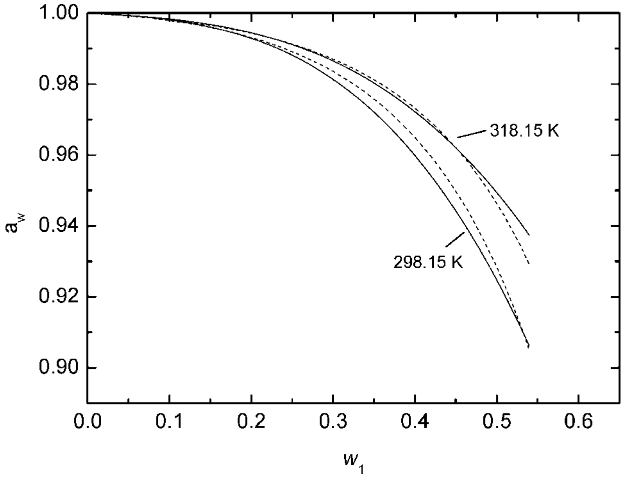

Figure 2. Activity of water in aqueous solutions of PEG 1500 (1) at (298.15 and 318.15) K as a function of w_1: −, pseudo-experimental data; - - -, correlation by the VERS model.

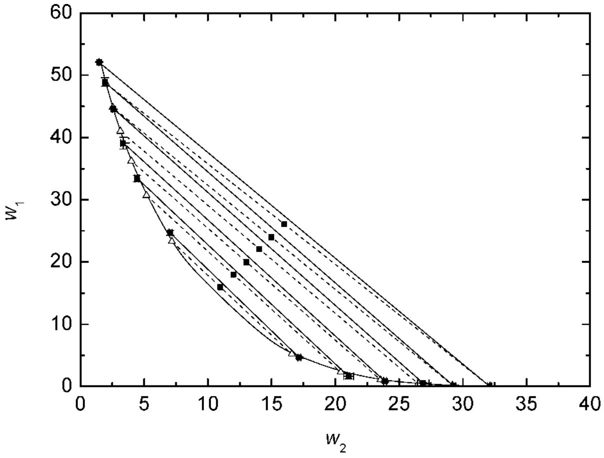

Figure 3. Liquid−liquid equilibrium of the system (PEG 1500 (1) + sodium citrate (2) + water (3)) at 298.15 K: ■, new experimental results; Δ, correlation with the VERS model for the compositions of the coexisting phases; −, prediction with the VERS model for the binodal line.

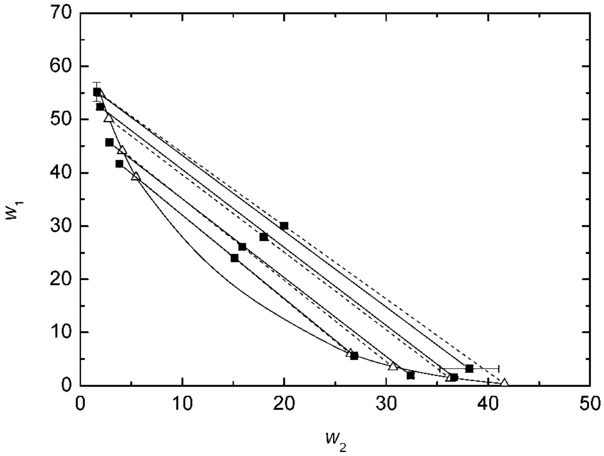

Figure 4. Liquid−liquid equilibrium of the system (PEG 600 (1) + sodium citrate (2) + water (3)) at 298.15 K: ■, new experimental results; Δ, predictions with the VERS model for the compositions of the coexisting phases; −, prediction with the VERS model for the binodal line.

relative deviation of at maximum 3.8 % in the peak area of sodium citrate. However, an influence of the molar mass of PEG on the peak area of sodium citrate was not found. Therefore, two calibration curves were determined, one for aqueous solutions without PEG and the other for aqueous solutions that contained the maximum expected PEG concentration. For samples with PEG mass fractions below 5 %, the calibration curve for the PEG-free solution was used, whereas for samples with higher PEG mass fractions, the other calibration curve was

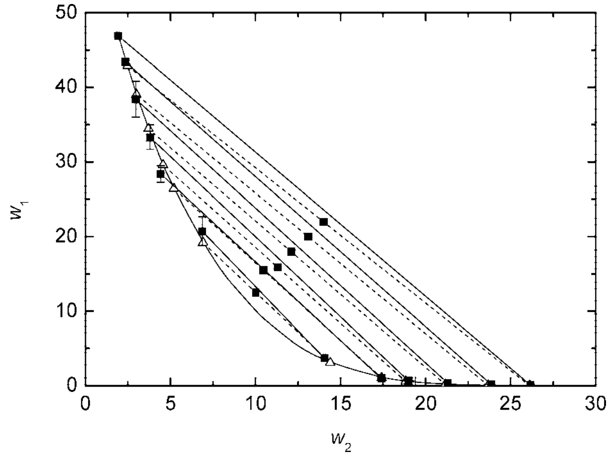

Figure 5. Liquid−liquid equilibrium of the system (PEG 3000 (1) + sodium citrate (2) + water (3)) at 298.15 K: ■, new experimental results; Δ, predictions with the VERS model for the compositions of the coexisting phases; −, prediction with the VERS model for the binodal line.

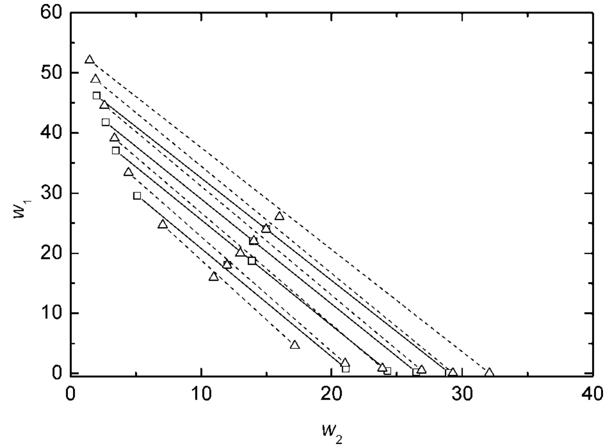

Figure 6. Liquid−liquid equilibrium for the system (PEG 1500 (1) + sodium citrate (2) + water (3)) at 298.15 K: □, experimental results from Vernau and Kula;[3] Δ, experimental results from the present work.

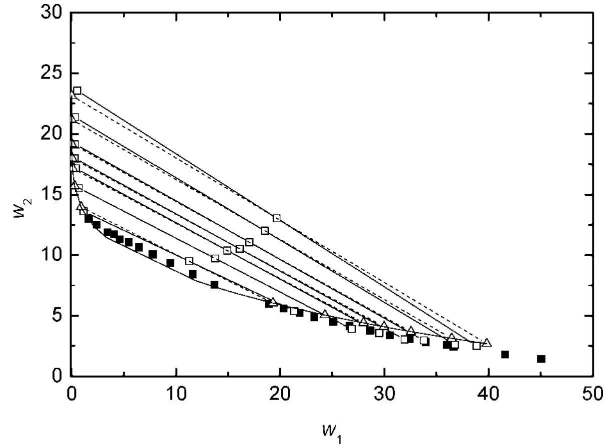

Figure 7. Liquid−liquid equilibrium of the system (PEG 6000 (1) + sodium citrate (2) + water (3)) at 298.15 K: ■,□ experimental results by Zafarani−Moattar et al.[4] for the cloud-point line and for the tie-lines, respectively; −, predictions by the VERS model for the binodal curve; Δ, predictions by the VERS model for the tie-lines.

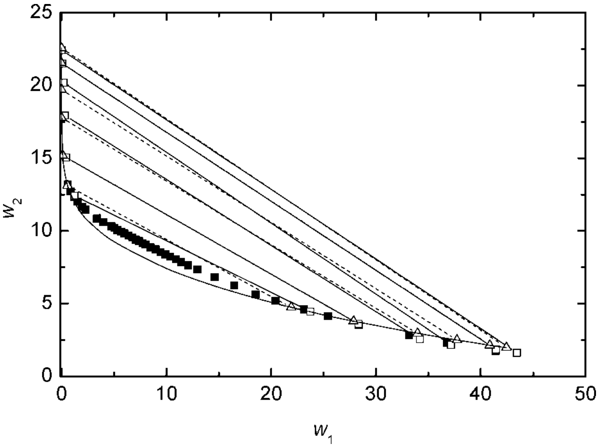

Figure 8. Liquid−liquid equilibrium of the system (PEG 6000 (1) + sodium citrate (2) + water (3)) at 308.15 K: ■,□ experimental results by Zafarani-Moattar et al.[4] for the cloud-point line and for the tie-lines, respectively; −, predictions by the VERS model for the binodal curve; Δ, predictions by the VERS model for the tie-lines.

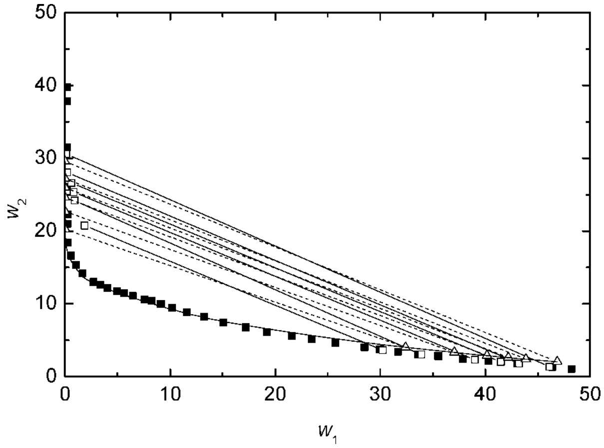

Figure 10. Liquid−liquid equilibrium of the system (PEG 6000 (1) + sodium citrate (2) + water (3)) at 293.15 K: ■,□, experimental results by Perumalsamy et al.[6] for the cloud-point line and for the tie-lines, respectively; −, predictions by the VERS model for the binodal curve; Δ, predictions by the VERS model for the tie-lines.

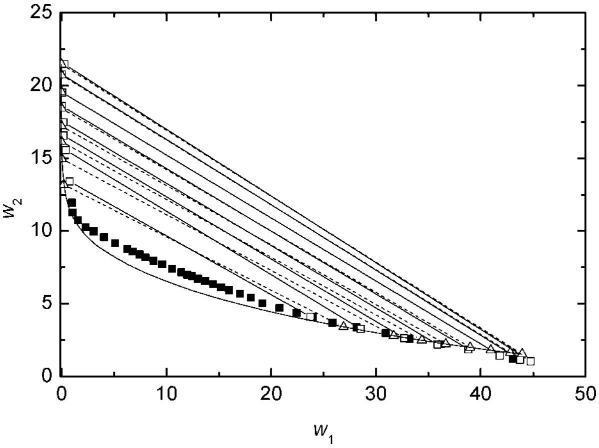

Figure 9. Liquid−liquid equilibrium of the system (PEG 6000 (1) + sodium citrate (2) + water (3)) at 318.15 K: ■,□, experimental results by Zafarani-Moattar et al.[4] for the cloud-point line and for the tie-lines, respectively; −, predictions by the VERS model for the binodal curve; Δ, predictions by the VERS model for the tie-lines.

used. It is estimated that the relative uncertainty in the experimental results for the mass fraction of sodium citrate in each of the coexisting phases does not surmount 2 %. The absolute experimental uncertainty for the salt mass fraction in the salt-rich (i.e., lower) phase is about 0.13 % and in the PEG-rich (i.e., upper) phase is about 0.08 %.

The mass fraction of PEG 1500 (and PEG 3000) in a liquid phase was also determined by HPLC using two GPC columns (model TSK-GEL G3000 PWXL, Tosoh Haas, Germany) in series and a refractive index detector (model RID-6A, Shimadzu, Japan). The eluent was, as proposed by Rämsch et al.,[11] a 10 mM aqueous solution of citric acid (pH $\approx$ 2.0). The flow rate was 0.6 $cm^3 \cdot min^{-1}$, resulting in a retention time of about 30 min. The peak areas were recorded and integrated by the aforementioned integrator. A different procedure was used to determine the mass fraction of PEG 600. For that, rather low-molecular PEG, it was not possible to separate sodium citrate from PEG in the GPC column. Therefore, the amount of PEG 600 in a sample was determined from a mass balance from the analytical results for the amount of sodium citrate and the amount of water that was determined by freeze-drying. In such analysis, a known amount of a sample (about 2 g) was poured into a small aluminum pot (diameter, 95 mm; height, 25 mm) and further diluted with about 40 g of water. The dilution was to ensure that the freeze-drying process results in a fine dry powder. The aluminum pots were cooled to about −190 °C with liquid nitrogen. The frozen sample was allowed to dry at 0.05 kPa (corresponding to a sublimation temperature of about −30 °C) for 3 days in a freeze-dryer (model Lyovac GT 2, Amsco/Finn-Aqua, Hürth, Germany). Afterward, the resulting powder was transferred to a vacuum oven (model VT 5050 EKP, Heraeus, Germany) and kept there for further drying at 60 °C for 2 days. Finally, the dried samples were weighted with a precision balance. The water content was obtained via a mass balance, and the amount of PEG 600 in a sample was also calculated via a mass balance. All freeze-drying analyses were done at least in duplicate. The uncertainty of the experimental results for the mass fraction of PEG 600 in typical samples from the coexisting phases is estimated from the uncertainties of the single steps by (0.7 and 0.2) % for the PEG-rich (i.e., upper) and for the salt-rich (i.e., lower) phases, respectively. These estimated uncertainties were confirmed by tests with typical aqueous solutions of PEG 600 and sodium citrate where the composition was known from preparation.

Experimental Results. Experiments were carried out with four feed solutions containing PEG 600, six feed solutions containing PEG 1500, and six feed solutions of PEG 3000. The experimental results for the feed solutions as well as for the resulting coexisting phases are given in Table 1 together with the estimated experimental uncertainties. The experiments were carried out sufficiently far away from the critical points of the liquid−liquid equilibrium. In the upper (PEG-rich) phase, the mass fraction of PEG and of sodium citrate are about (20 to 60) % and rarely more than 2 %, respectively. In the lower (salt-rich) phase, the mass fractions are about (15 to 40) % for sodium citrate and up to about 6 % for PEG. The experimental results are discussed and compared with literature data in the following chapters.

Thermodynamic Modeling. The aqueous two-phase liquid−liquid equilibrium is described here by the VERS (virial equation with relative surface fractions) model.[8,9] This model is a modification of Pitzer's equation for the excess Gibbs energy of aqueous electrolyte solutions.[12] To enable an extension to aqueous polymer solutions, it uses a group-contribution approach and replaces the molality scale by a surface-fraction scale. The activity coefficients resulting from the VERS model are normal-

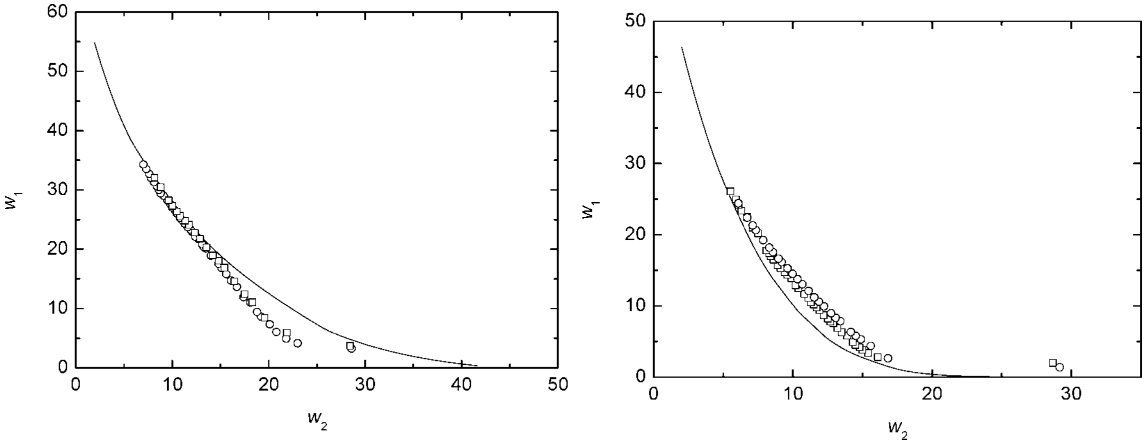

Figure 11. Comparison between experimental cloud-point lines from Porto et al.:[7] □, at pH = 6; ○, at pH = 8; −, predicted binodal curves by the VERS model at 298.15 K.

ized according to the asymmetric convention. The reference state for the chemical potential of the solvent (water) is the pure liquid at the temperature of interest. The influence of pressure on the chemical potential is neglected. The reference state for a solute component is a hypothetical one-molal solution of the solute in the pure solvent also at the temperature of the solution. Again, the influence of pressure on the chemical potential is neglected. The solute concentration in the equation for its activity coefficient is expressed by its "normalized surface fraction", that is, its surface fraction Θ_i per 1000 g of water divided by the surface fraction of water Θ_w.

The surface fraction of a component i is given by

$$\Theta_i = \frac{m_i Q_i}{\sum\limits_{\text{all comp}, j} m_j Q_j} \tag{1}$$

where m_i and Q_i are the molality and surface parameter of component i,respectively.

When $\nu_k^{(i)}$ represents the number of groups of type k in component i and q_k is the surface parameter of group k, the surface parameter of component i, Q_i, becomes

$$Q_i = \sum_{\text{all groups } k} \nu_k^{(i)} q_k \tag{2}$$

The equation for the activity of the solvent (i.e., water), a_w, from the VERS model is

$$\ln a_\mathrm{w} = -\frac{M_{\mathrm{H_2O}}}{1000}\left[\sum_{j\neq w} m_j - 2A_\varphi \frac{I_m^{1.5}}{1+b\sqrt{I_m}}\right] - \left(\frac{1000}{M_{\mathrm{H_2O}}}\right)\sum_{i\neq \mathrm{w}}\sum_{j\neq \mathrm{w}} \frac{\Theta_i}{\Theta_\mathrm{w}}\frac{\Theta_j}{\Theta_\mathrm{w}}[A_{i,j}^{(0)} + A_{i,j}^{(1)} \exp\{-\alpha\sqrt{I_m}\}] - 2\left(\frac{1000}{M_{\mathrm{H_2O}}}\right)^2 \sum_{i\neq \mathrm{w}}\sum_{j\neq \mathrm{w}}\sum_{k\neq \mathrm{w}} \frac{\Theta_i}{\Theta_\mathrm{w}}\frac{\Theta_j}{\Theta_\mathrm{w}}\frac{\Theta_k}{\Theta_\mathrm{w}} B_{i,j,k} \tag{3}$$

where $M_{\mathrm{H_2O}} = 18.02\ \mathrm{g\cdot mol^{-1}}$ is the molar mass of water and I_m is the ionic strength (on molality scale) of the solution

$$I_m = \frac{1}{2}\sum_{i=1}^{S} m_i z_i^2 \tag{4}$$

z_i is the charge number of species i; A_φ is the Debye−Hückel constant; and subscript w stands for water. $A_{i,j}^{(0)}$, $A_{i,j}^{(1)}$, and $B_{i,j,k}$ are parameters for interactions between two solutes (i and j) and three solutes (i, j, and k), respectively. The parameters b and α were adopted from Pitzer:[13] $b = 1.2\ \mathrm{kg^{1/2}\cdot mol^{-1/2}}$; $\alpha = 2.0\ \mathrm{kg^{1/2}\cdot mol^{-1/2}}$.

The binary and ternary interaction parameters between solute species are expressed using a group contribution approach

$$A_{i,j}^{(0)} = \sum_{\text{all groups } k}\ \sum_{\text{all groups } l} \vartheta_k^{(i)}\vartheta_l^{(j)} a_{k,l}^{(0)} \tag{5}$$

$$A_{i,j}^{(1)} = \sum_{\text{all groups } k}\ \sum_{\text{all groups } l} \vartheta_k^{(i)}\vartheta_l^{(j)} a_{k,l}^{(1)} \tag{6}$$

$$B_{i,j,k} = \sum_{\text{all groups } d}\ \sum_{\text{all groups } l}\ \sum_{\text{all groups } m} \vartheta_d^{(i)}\vartheta_l^{(j)}\vartheta_m^{(k)} b_{d,l,m} \tag{7}$$

$$\vartheta_d^{(i)} = \frac{\nu_d^{(i)} q_d}{Q_i} \tag{8}$$

where $\vartheta_k^{(i)}$ is the relative contribution of group k to the surface parameter of species i, while $a_{k,l}^{(0)}$, $a_{k,l}^{(1)}$, and $b_{d,l,m}$ are binary and ternary interaction parameters between groups, respectively. These parameters are symmetric; for example, $b_{d,l,m} = b_{l,m,d} = b_{m,d,l}$.

The activity of a solute species i, $a_{i,m}{}^*$, from the VERS model is

$$\ln a_{i,m}^* = \ln m_i - A_\varphi z_i^2\left[\frac{\sqrt{I_m}}{1+b\sqrt{I_m}} + \frac{2}{b}\ln(1+b\sqrt{I_m})\right] + 2\left(\frac{1000}{M_{\mathrm{H_2O}}}\right)\frac{q_i}{q_\mathrm{w}}\sum_{j\neq \mathrm{w}}\frac{\Theta_j}{\Theta_\mathrm{w}}[A_{i,j}^{(0)} + A_{i,j}^{(1)} f_2(I_m)] - z_i^2 f_3(I_m)\left(\frac{1000}{M_{\mathrm{H_2O}}}\right)^2 \sum_{j\neq \mathrm{w}}\sum_{k\neq \mathrm{w}}\frac{\Theta_j}{\Theta_\mathrm{w}}\frac{\Theta_k}{\Theta_\mathrm{w}} A_{j,k}^{(1)} + 3\left(\frac{1000}{M_{\mathrm{H_2O}}}\right)^2 \frac{q_i}{q_\mathrm{w}}\sum_{j\neq \mathrm{w}}\sum_{k\neq \mathrm{w}}\frac{\Theta_j}{\Theta_\mathrm{w}}\frac{\Theta_k}{\Theta_\mathrm{w}} B_{i,j,k} \tag{9}$$

with

$$f_2(I_m) = \frac{2}{\alpha^2 I_m}[1 - (1+\alpha\sqrt{I_m})\exp\{-\alpha\sqrt{I_m}\}] \tag{10}$$

and

$$f_3(I_m) = \frac{1}{\alpha^2 I_m^2}\left[1 - \left(1 + \alpha\sqrt{I_m} + \frac{\alpha^2 I_m}{2}\right)\exp\{-\alpha\sqrt{I_m}\}\right] \tag{11}$$

All summations in eqs 3 and 9 are over the solute species only (i.e., there is no term from the solvent). Some preliminary calculations revealed that in the liquid mixtures of (PEG + water + sodium citrate) under the conditions of the present work sodium citrate is completely dissociated, and the protonation of citrate ions as well as the autoprotolysis of water can be neglected. Therefore, an aqueous solution of sodium citrate and PEG is considered as an electroneutral mixture of water, PEG-molecules, sodium ions, and citrate ions. Instead of considering sodium ions and citrate ions as different solute species, one might consider sodium citrate as a single solute. The activity of sodium citrate then is

$$a^*_{\mathrm{Na_3Citrate},m} = (a^*_{\mathrm{Na^+},m})^3 a^*_{\mathrm{Citrate},m} \qquad (12)$$

The calculation of activities requires the Debye–Hückel parameter (A_φ) for water (at 298.15 K: A_φ = 0.39147 $\mathrm{kg^{1/2}\cdot mol^{-1/2}}$),[14] surface parameter of group k (q_k), and binary ($A^{(0)}_{i,j}$, $A^{(1)}_{i,j}$) as well as, if necessary, ternary ($B_{i,j,k}$) interaction parameters. Water is treated as a single group with surface parameter q_w = 1.4. The surface parameter of water was also used for sodium ions as well as for citrate ions. Following Groβmann et al.[8] and Tintinger et al.,[15] a poly(ethylene glycol) molecule is split into (N_P−1) PEG middle groups and 2 PEG end groups. The middle group is (−$\mathrm{CH_2}$−O−$\mathrm{CH_2}$−), and the end group is (−$\mathrm{CH_2OH}$). The total number of groups N_P is estimated from the number averaged molar mass M_n of a PEG sample as follows

$$N_\mathrm{P} = (M_\mathrm{n} - 18.015)/44.053 \qquad (13)$$

The nomenclatures of the supplier for the PEG samples were used to estimate the number-averaged molecular masses of the poly(ethylene glycol)s. The surface parameters of the middle and the end groups of PEG were calculated as proposed by Bondi.[16] Table 2 gives the number of groups of each species and the surface parameter of each group.

Binary and ternary parameters for interactions between ions carrying electrical charges of the same sign are (as usual) set to zero. The remaining binary and ternary parameters for interactions between sodium ions on one side and citrate ions on the other side were taken from Schunk and Maurer.[17] These authors reported experimental results for the activity of water in aqueous solutions of sodium citrate at 298.15 K and used that data to adjust two binary and one ternary parameters for interactions (cf. Table 3). These binary and ternary parameters were also used here.

Recently, also Sadeghi[18] and Salabat et al.[19] published experimental results for the activity of water in aqueous solutions of sodium citrate. These data show comparatively large deviations from the data of Schunk and Maurer. This is demonstrated in Figure 1. The solid curve represents calculation results for the osmotic coefficients by the VERS model with parameters from Schunk and Maurer.[17] Those data agree with the results by Sadeghi[18] only at salt mass fractions above 25 %. At lower salt mass fractions, the experiments by Sadeghi resulted in larger numbers for the osmotic coefficient. On the other side, the results reported by Salabat et al.[19] systematically lie below the results of Schunk and Maurer at salt mass fractions above about 10 %. The binary and ternary parameters for interactions between sodium and citrate ions from Schunk and Maurer were also used here.

Only binary parameters were taken into account for interactions between groups of PEG. As PEG consists of middle groups (MG) and end groups (EG), there are three of such parameters: $a^{(0)}_{\mathrm{EG,EG}}$, $a^{(0)}_{\mathrm{MG,EG}}$, and $a^{(0)}_{\mathrm{MG,MG}}$. These parameters were fitted to the activity of water in aqueous solutions of all three PEGs (PEG 600, PEG 1500, and PEG 3000) at 298.15 K at polymer mass fractions up to about 55 %. Experimental results for the activity of water in such solutions were reported by Hasse et al.[20] for temperatures from (278 to 333) K and PEGs with relative molar masses from approximately 200 to 40 000. These authors described their experimental results by an osmotic virial equation taking into account the second and the third osmotic virial coefficient. The interaction parameters of the VERS model were determined from that osmotic virial equation, i.e, that osmotic virial equation was used to calculate "pseudo-experimental" results for the activity of water up to the high polymer concentrations covered in the present work at temperatures between (293 and 318) K. That pseudo-experimental data was used to fit the parameters for interactions between PEGgroups in the VERS model. A good agreement between that pseudo-experimental data and the correlation results by the VERS model was also found for PEGs with other molar masses. As one of the goals of the present work was to calculate the liquid–liquid phase equilibrium in an extended temperature range, the influence of temperature on the interaction parameters was considered through

$$a^{(0)}_{k,l} = a^{(00)}_{k,l} + a^{(01)}_{k,l}\cdot\left(\frac{T}{298.15\ \mathrm{K}} - 1\right) + a^{(02)}_{k,l}\cdot\ln\left(\frac{T}{298.15\ \mathrm{K}}\right) \qquad (14)$$

The resulting binary parameters are given in Table 3. Figure 2 shows a comparison between calculation results from the osmotic virial equation and the VERS model for the activity of water in aqueous solutions of PEG 1500 at (298.15 and 318.15) K. Although the agreement is not perfect, the absolute differences in the numbers for the activity of water calculated by both methods amount to 0.002 for the whole range of solute concentrations, whereas the experimental uncertainty of the water activity data reported by Hasse et al.[20] is 0.001.

Only binary interaction parameters between solute species that originate from different solutes (PEG and sodium citrate) were considered in the extension of the VERS model from the single solute systems to the mixed solute systems. However, as there is always a constant ratio of sodium to citrate ions, some parameters cannot be determined independently. For example, only the following combination of parameters between a PEG group and sodium citrate ($A^{(0)}_{\mathrm{PEG,Na^+}}$ and $A^{(0)}_{\mathrm{PEG,Citrate}}$) appears in all equations for the activities

$$A^{(0)}_{\mathrm{PEG,Na_3Citrate}} = 3\cdot A^{(0)}_{\mathrm{PEG,Na^+}} + A^{(0)}_{\mathrm{PEG,Citrate}} \qquad (15)$$

Therefore, we arbitrarily set

$$A^{(0)}_{\mathrm{PEG,Citrate}} = 0 \qquad (16)$$

The remaining interaction parameter $A^{(0)}_{\mathrm{PEG,Na^+}}$ follows from eq 5

$$A^{(0)}_{\mathrm{PEG,Na^+}} = \vartheta^{(\mathrm{PEG})}_{\mathrm{EG}}\vartheta^{(\mathrm{Na_3Citrate})}_{\mathrm{Na^+}} a_{\mathrm{EG,Na^+}} + \vartheta^{(\mathrm{PEG})}_{\mathrm{MG}}\vartheta^{(\mathrm{Na_3Citrate})}_{\mathrm{Na^+}} a_{\mathrm{MG,Na^+}} \qquad (17)$$

All ternary parameters for interactions between groups from different solutes were neglected (i.e., set to zero). The two nonzero parameters ($a_{\mathrm{EG,Na^+}}$ and $a_{\mathrm{MG,Na^+}}$) were fitted to the new experimental results for the liquid–liquid equilibrium of the system (PEG 1500 + sodium citrate + water). For this parameter estimation process, the average deviation Δw was minimized

$$\Delta w = \left[\frac{\sum_{i=1}^{3}\sum_{n=1}^{C}[(w_i^{\mathrm{I,exptl}} - w_i^{\mathrm{I,calcd}})^2 + (w_i^{\mathrm{II,exptl}} - w_i^{\mathrm{II,calcd}})^2]_n}{6C}\right]^{1/2} \tag{18}$$

where w_i^{I} and w_i^{II} are the mass fractions of component i in the coexisting liquid phases (I and II). C is the number of experimentally investigated tie-lines. Superscripts exptl and calcd designate the experimental data and the calculated results, respectively. Table 3 shows the entire set of group interaction parameters used to calculate the liquid−liquid equilibrium presented in this work. All other parameters of the VERS model for interactions between groups were neglected (i.e., set to zero).

Figure 3 gives a comparison between the experimental data and the correlation results for the compositions of the coexisting phases of the system (PEG 1500 + sodium citrate + water) at 298.15 K. Figures 4 and 5 show comparisons between the experimental data and prediction results from the VERS model for the liquid−liquid equilibrium of the systems (PEG 600 + sodium citrate + water) and (PEG 3000 + sodium citrate + water), respectively. All calculation results shown in Figures 3 to 5 (as well as the calculations required in the parameter estimation procedure) were performed in a flash-type procedure; i.e., the experimental results for the composition of the feed were used to calculate the compositions of the coexisting phases. Generally, the correlation as well as the prediction results agree with the new experimental data within the experimental uncertainties for the compositions of the coexisting phases. Only for the system with PEG 600, the concentration of sodium citrate in the polymer-rich liquid phase is somewhat underestimated (cf. Figure 4). This is no surprise as the group contribution concept will fail for low molecular polymers.

Comparison with Literature Data. Vernau and Kula[3] reported experimental results for the liquid−liquid equilibrium of the system (PEG 1500 + sodium citrate + water) at 298.15 K. These experimental results are compared with the results of the present work in Figure 6. There are some small deviations between the experimental results from both investigations in particular at the polymer-rich phase (that is, the top phase). However, we believe that the differences are smaller than the accumulated experimental uncertainties of both investigations. Furthermore, from our experience, we assume that the UV-photometric method which was used by Vernau and Kula for measuring the composition of PEG is less accurate than the chromatographic method used in the present work. The differences in the experimental results from both investigations for the composition of the salt-rich phase (i.e., the bottom phase) are smaller than the experimental uncertainty of the present work.

Figures to 9 show the comparisons between the experimental results from Zafarani−Moattar et al.[4] for PEG 6000 + sodium citrate + water and results obtained with the VERS model for the binodal curve as well as for the compositions of the coexisting phases (from flash calculation with a feed that was composed of equal amounts of the coexisting liquid phases) at (298.15, 308.15, and 318.15) K. There is a very good agreement, with some small deviation for the extension of the two-phase region near the critical point at (308.15 and 318.15) K.

Figure 10 shows a comparison of the experimental results of Perumalsamy et al.[6] for the system (PEG 6000 + sodium citrate + water) at 293.15 K and the prediction results from the VERS model. The predictions nicely agree with the experimental results for the binodal curve. However, the experimental results for the composition of the salt-rich phase (determined in phase equilibrium experiments) deviate systematically from the results for the binodal curve (determined by cloud-point measurements). The results for the polymer concentration in the salt-rich phase (as determined in phase equilibrium experiments) are always somewhat larger than the cloud-point data.

Murugesan and Perumalsamy[5] reported experimental results for the liquid−liquid equilibrium of the system (PEG 2000 + sodium citrate + water) at temperatures from (25 to 45) °C. These data reveal somewhat larger deviations from the predictions by the VERS model. In particular, the extent of the two-phase region reported by those authors is always somewhat smaller than predicted.

Porto et al.[7] published experimental results for the cloud-point line of the system (PEG + sodium citrate + water) for four PEG samples (PEG 400, PEG 600, PEG 1500, PEG 3000, and PEG 8000). The experiments were performed at constant pH (in the range from 6 to 8). Figure 11 shows two comparisons (for systems with PEG 600 and with PEG 1500, respectively) between these experimental results and model predictions. For the low-molecular PEGs, there is a good agreement between these experimental data for the polymer-rich branch of the cloud-point curve, whereas the model predicts a smaller two-phase region in the salt-rich area. For the high-molecular PEGs, the experimental results (that cover mainly the region around the critical point) reveal a smaller than predicted two-phase region. The reason for these differences is not obvious. Neglecting the presence of citric acid (that was added in the experiments in unknown amounts to adjust the pH), experimental problems with the observation of the cloud-point, but also model deficiencies, might be responsible for these differences.

Conclusions

New experimental results for the compositions of coexisting phases of aqueous two-phase systems of three poly(ethylene glycol)s (PEG) and sodium citrate at 298.15 K are presented. The three PEG samples differed in the molar mass (from about 600 to about 3000). The new experimental data are described using the VERS model for the excess Gibbs energy of aqueous solutions of neutral polymers and strong electrolytes. The model enables a good correlation of the underlying experimental data. It is also able to predict quantitatively the influence of the polymer molecular mass and the temperature on the liquid−liquid equilibrium.

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