Effect of Temperature on the Phase Equilibrium of Aqueous Two-Phase Polymer Systems

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We report experimental data showing the effect of temperature on polymer partitioning in the dextran (DEX500)-polyethylene glycol (PEG8000)-water system. Increasing temperature increases the concentration of PEG in the top phase and decreases the concentration of DEX in the bottom phase. A solution thermodynamic model based on local compositions with temperature-dependent parameters correlates the experimental data well, and an efficient Gibbs-free energy minimization algorithm for phase equilibrium calculations is described. The partial molar enthalpies and entropies of each of the solutes, calculated with the model, are negative and decrease in magnitude as temperature increases.

Introduction

Recently aqueous two-phase systems have been studied for possible use in large-scale bioseparation processes. Since their introduction by Albertsson (1958), such systems, formed by addition of the two water-soluble, but incompatible, polymers to water, have been used for the separation of cells, proteins and cell organelles (Albertsson, 1958, 1986), and have been considered for commercial-scale applications (Kula et al., 1982). Aqueous two-phase systems are suited especially for purification of biologically active materials, since each phase contains 70 to 90% water, and commonly used polymers, such as polyethylene glycol (PEG) and dextran (DEX), are nontoxic, nonimmunogenic, and nondenaturing.

Despite the fact that aqueous two-phase partitioning has been used extensively for the laboratory separation of biologically active materials, there is no accurate way to predict the partitioning of polymers and biomolecules in these systems. A fundamental understanding of biomolecule partitioning and polymer phase equilibrium in aqueous two-phase polymer systems could lead to improvements in the design and optimization of large-scale purification processes. As part of a continuing study to describe this partitioning, we consider here the effect of temperature on the relevant polymer-polymer-water-phase equilibrium. In particular, we report experimental data on the influence of temperature on liquid-liquid equilibrium for the model mixture of PEG 8000, DEX 500 and water, and develop a thermodynamically-based correlation of the results.

The thermodynamics of polymeric solutions has been studied extensively. Principal models for phase equilibrium calculations are those of Flory (1942) and Huggins (1942), who developed an expression based on lattice theory to describe the nonidealities of polymer solutions and of Edmond and Ogston (1968), who modeled nonidealities with a truncated osmotic virial expansion based on McMillan-Mayer theory (McMillan and Mayer, 1945). These models have been extended and applied to aqueous two-phase systems by others (King et al., 1988; Cabezas et al., 1990; Forciniti and Hall, 1990), and a complete description of the important thermodynamic models is given by Baskir et al. (1989). Local composition models have also been used to describe the thermodynamics of the polymer solutions. Kang and Sandler (1987) used the UNIQUAC (Abrams and Prausnitz, 1975) solution model with predetermined interaction parameters to describe polymer solution nonidealities and examined the effect of polymer molecular weight distributions. This work extends this application of the UNIQUAC model.

Experimental Methods and Results

PEG 8000 (Lot # 18F-0033, with polydispersity index = 1.1 as reported by the manufacturer) and DEX 500 (Lot # 58F-0628, polydispersity index = 2.75) were obtained from Sigma. Polymer stock solutions were prepared by the addition of weighed amounts of dry polymers to water, and concentrations

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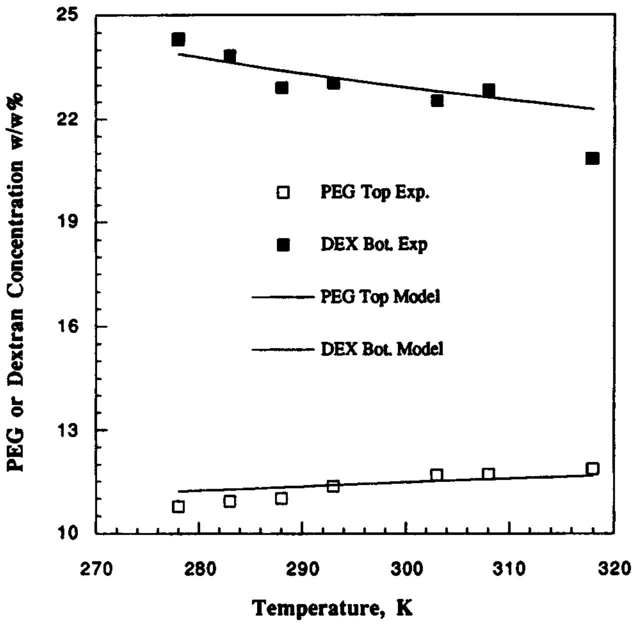

Figure 1. PEG or DEX concentration as a function of temperature for a feed of PEG 7.3% and DEX 8.4%.

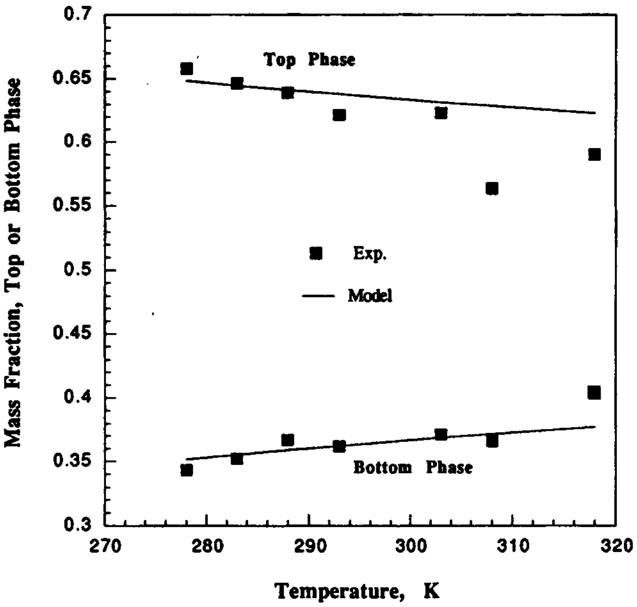

Figure 2. Mass fractions of the phases as a function of temperature for a feed of PEG 7.3% and DEX 8.4%.

were confirmed by refractive index and polarimetery measurements. Two-phase systems were then prepared gravimetrically in microcentrifuge tubes from the stock solutions and deionized water. Nine samples with various feed compositions were prepared, repeatedly mixed, and allowed to equilibrate in a water bath at temperatures between 5 and 50°C. Samples remained in the water bath for at least 24 hours after their separation into two clear phases. A sample of the top phase was withdrawn using a pipette, and a sample of the lower phase was obtained using a syringe needle inserted through the bottom of the tube. These samples were volumetrically diluted and analyzed using a high-performance liquid chromatograph with a multisolvent delivery system (Waters model 600), four gel permeation columns in series (two ultrahyhdrogel linear, one ultrahydrogel 250 and one ultrahydrogel 120), and a differential refractometer detector (Waters model 410). The mobile phase was 0.1-N sodium nitrate solution at a flow rate of 1 mL/min. Data were collected using a chromatography data station (Waters Maxima 820 software on an IBM-AT personal computer).

Increasing temperature increases the concentration of PEG in the top phase and reduces the DEX concentration in the bottom phase (Figure 1). For a fixed feed, the weights of the top and bottom phases at different temperatures were also measured; these results are presented in the Appendix and shown for one feed composition in Figure 2. When temperature increases, the mass of the bottom phase increases relative to that of the top phase. These changes in the phase compositions and amounts result from the tie lines becoming shorter and steeper with increasing temperature (Figure 3).

Model and Parameter Estimation

The equilibrium distribution of various components between the phases can be obtained either by minimizing the total Gibbs free energy of the system or by equating the partial molar Gibbs free energy of each component in each phase. The second method has two disadvantages. First, since most Gibbs free energy functions are highly nonlinear, the equations giving individual species chemical potentials are more complicated than the parent Gibbs free energy equation. Second, more computational time is needed in the second method when many components are involved (Verhegyi and Eon, 1977). For these reasons, liquid-liquid equilibrium compositions are calculated here by minimizing the total Gibbs free energy.

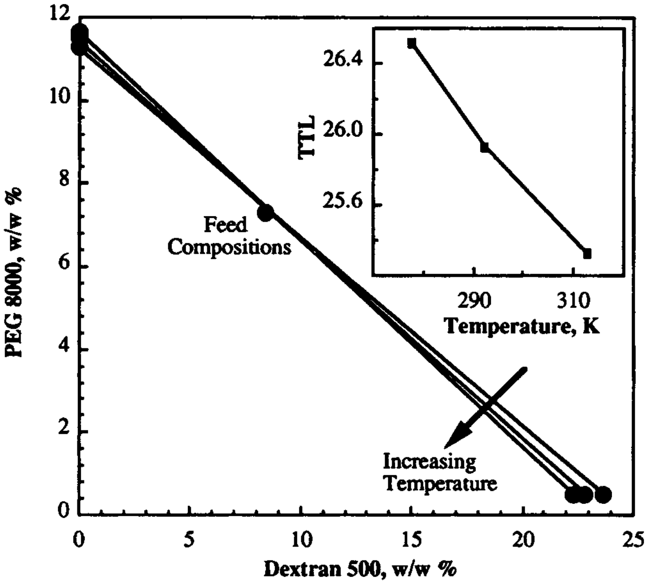

Figure 3. Tie-line slope as a function of temperature for a feed of PEG 7.3% and DEX 8.4%. Inset: Tie-line length as a function of temperature for the same feed compositions.

The UNIQUAC solution thermodynamic model (Abrams and Prausnitz, 1975) has previously been used successfully to describe the PEG-DEX-water system (Kang and Sandler, 1987). In this model, the thermodynamic excess Gibbs free energy, G^{EX}, is given by:

$$\frac{G^{\text{EX}}}{RT}=\sum_{i=1}^{n} w_i \ln\left[\frac{\Phi_i}{w_i}\right]+\frac{z}{2}\sum_{i=1}^{n} w_i M_i q_i \ln\left[\frac{\theta_i'}{\Phi_i}\right]-\sum_{i=1}^{n} w_i M_i q_i' \ln\left[\sum_{i=1}^{n}\theta_j' \tau_{ji}\right] \tag{1}$$

where

$$\Phi_i'=\frac{r_i' w_i}{\sum_j r_j' w_j} \tag{2}$$

and

$$\theta_i'=\frac{q_i' w_i}{\sum_j q_j' w_j} \tag{3}$$

Here, r_j' and q_j' are the volume and surface area parameters of the polymer j per unit mass, w_j is its weight fraction, $z=10$ is the coordination number, and τ_{ij} is given by:

$$\tau_{ij}=\exp\left(-\frac{a_{ij}}{T}\right) \tag{4}$$

where a_{ij} is the interaction parameter for components i and j, and M_j is the molecular weight of polymer j.

One of the shortcomings of local composition expressions, such as the UNIQUAC model, is that they do not simultaneously produce accurate predictions of both the excess Gibbs free energy and the excess heat of mixing (Skjold-Jorgensen et al., 1980); consequently, these models cannot accurately represent the effect of temperature on the phase equilibria. To overcome this problem, others (Skjold-Jorgensen et al., 1980; Demirel and Gecegörmez, 1989) have introduced temperature-dependent interaction parameters and achieved a significant improvement in the predictions of both the phase equilibrium compositions as a function of temperature and the excess heat of mixing. We follow the same approach here and introduce temperature-dependent interaction parameters in the UNIQUAC polymer solution model in the form:

$$a_{ij}=A_{ij}+\frac{B_{ij}}{T} \tag{5}$$

The LLE data measured at all temperatures were used simultaneously to determine the interaction parameters A_{ij} and B_{ij} by minimizing the objective function:

$$OF=\sum_{T=T_1}^{T=T_n}\sum_{p=1}^{2}\sum_{i=1}^{n}\left(w_{T,p,i}^{\text{Calc}}-w_{T,p,i}^{\text{Exp}}\right)^2 \tag{6}$$

where $w_{T,p,i}$ is the weight percent of the component i in phase p at temperature T, n_c is the number of components, and T_j is the jth temperature at which the compositions were measured. With a set of initial guesses for the values of the interaction parameters, the concentrations of the polymers and water are obtained by minimizing the total Gibbs free energy given by:

$$n\,G^{\text{Total}}=\sum_{p=1}^{2}\sum_{i=1}^{n_c} n_{ip} G_i^o+\sum_{p=1}^{2} n_p G_p^{\text{EX}}+\sum_{p=1}^{2} n_p G_p^{\text{Ideal}} \tag{7}$$

where G^{Total} is the total specific Gibbs free energy of the two-phase system, n is the total number of moles in the two-phase system, and G_i^o is the standard state Gibbs free energy of component i. For phase p, n_{ip} is the number of moles of component i in phase p, n_p is the total number of moles in phase p, and G_p^{EX} and G_p^{Ideal} are the excess and ideal Gibbs free energies of phase p, respectively.

To account for the fact that PEG and DEX are polydisperse, molecular weight distributions $F(M)$ based on the Lansig-Kramer (L-K) function:

$$F(M)=\frac{1}{\beta\sqrt{\pi}}\exp\left(-\frac{1}{\beta^2}\ln^2\frac{M}{M_0}\right) \tag{8}$$

were used for each polymer (Granath, 1958), where β and M_o are parameters. The polydispersity index is defined as:

$$b=\frac{\overline{M_w}}{\overline{M_n}}=\exp\left(\frac{2}{\beta^4}\right), \tag{9}$$

so the parameters in the distribution function (Eq. 8) are determined given either M_n and M_w, or one of the average molecular weights and b. After choosing the molecular weight distribution and given the polydispersity index for each polymer, the optimal pseudocomponents representing the continuous distribution are chosen based on the Gauss quadrature method (Shibata et al., 1987). The molecular weight M_k and weight fraction w_k of the kth pseudocomponent are calculated using the kth zero of the Hermite polynomial, z_k, and the following relations:

$$M_k=M_0\exp(\beta z_k) \tag{10}$$

$$w_k=(\text{total weight fraction of polymer in feed})\left(W_k\Big/\sum_{k=1}^{n} W_k\right) \tag{11}$$

with W_k the weight factor obtained from the Gauss-Hermite quadrature. Therefore, each polydisperse polymer is represented as a multicomponent mixture with each component described by a discrete molecular weight and concentration. In the following calculations, PEG 8000 ($b=1.1$) was assumed to be monodisperse due to its narrow molecular weight distribution, and DEX ($b=2.75$) is represented by three pseudocomponents chosen as described above. Representing a polydisperse polymer by as few as three quadrature-chosen pseudocomponents is sufficient to allow accurate predictions of phase boundaries (Kang and Sandler, 1988; Hartounian and Sandler, 1991). Using this method, the optimum pseudocom-

Table 1. UNIQUAC Temperature-Dependent Interaction Parameters Obtained from Data Reported Here

$a_{mn} = A_{mn} + (B_{mn}/T)$ T, K			
A_{mn} (K) B_{mn} (K^2)	PEG 8000	Dextran 500	Water
PEG 8000	0	−365 7,992	−584 −29,230
Dextran 500	2,498 −836	0	−532 5,724
Water	−166 47,370	−548 18,940	0

ponents for DEX 500 have molecular weights of 2.06×10^5, 8.40×10^5 and 2.08×10^6.

The Gibbs free energy minimization was done as follows. For each tie-line, through a given feed concentration, the total Gibbs free energy was computed using the experimental concentrations and a set of UNIQUAC interaction parameters as initial guesses. The unknown equilibrium concentrations were then varied using the Nelder-Mead (Nelder and Mead, 1965) optimization algorithm until a set was found that minimized the total Gibbs free energy. Then, the value of the objective function (Eq. 6) was calculated and compared with a preset tolerance (10^{-10}). If the objective function was greater than this tolerance, a new set of interaction parameters were determined using the step parameter specified in the optimization algorithm, and iterations repeated until the global minimum of the objective function was obtained.

The final interaction parameters are presented in Table 1, and the results of correlations with temperature-dependent parameters are compared with the experimental data in Figure 1 and tabulated in the Appendix. The percent errors in the model predictions with and without the temperature-dependent UNIQUAC interaction parameters are given in Table 2. As expected, the model predictions are dramatically improved by introducing temperature-dependent parameters. The model predictions follow the trend of the experimental results, namely that increasing temperature results in a decrease in the concentration of DEX in the bottom phase and an increase in PEG concentration in the top phase. This trend is not reproduced when constant parameters are used in this model.

Table 2. Model Predictions: Temperature-Dependent vs. Temperature-Independent UNIQUAC Interaction Parameters*

Feed, w/w %		Exp. w/w%		UNIQUAC Parameters: Temperature-Dependent % Error		Temperature-Independent % Error		
PEG	Dex	PEG Top	Dex Bot	PEG Top	Dex Bot	PEG Top	Dex Bot	T, K
7.3	8.4	12.22	19.16	0.09	16.1	30.62	399.0	323
7.3	8.4	11.86	21.31	1.67	50.0	27.71	165.7	318
7.3	8.4	11.71	21.31	0.85	5.96	25.44	137.8	313
7.3	8.4	12.56	22.84	7.96	0.87	27.63	76.97	308
7.3	8.4	11.69	22.53	1.54	1.82	15.06	39.68	303
7.3	8.4	11.35	23.03	0.35	1.39	1.06	0.13	293
7.3	8.4	11.00	22.90	3.00	2.88	8.18	5.28	288
7.3	8.4	10.93	23.82	3.11	0.13	11.89	12.72	283
7.3	8.4	10.77	24.32	0.08	1.11	15.78	16.45	278

*Percent error is defined as % Error = $\frac{C^{\text{Calc}} - C^{\text{Exp}}}{C^{\text{Exp}}} \times 100$.

Table 3. UNIQUAC Temperature-Dependent Interaction Parameters Obtained from Experimental Data of Forciniti and Hall (1991)

$a_{mn} = A_{mn} + (B_{mn}/T)$ T, K			
A_{mn} B_{mn}	PEG 8000	Dextran 500	Water
PEG 8000	0	−334 7,695	−535 −32,200
Dextran 500	3,011 −803	0	−531 −5,427
Water	−122 22,240	−563 15,970	0

We have also compared the UNIQUAC model predictions produced with temperature-dependent parameters with the experimental data of Forciniti and Hall (1991). Initial calculations performed using the molecular weights of the polymers given by Forciniti and Hall and the interaction parameters in Table 3 did not match the experimental data well. A new set of temperature-dependent parameters was determined by fitting the experimental data of Forciniti and Hall (1991), and these parameter values are listed as set II in Table 3. Not surprisingly, the equilibrium compositions are highly sensitive to the values of the UNIQUAC parameters. The results of the phase equilibrium calculations using set II parameters are compared with the experimental results of Forciniti and Hall (1991) in Figure 4 (for their system A of PEG 4000-DEX 10000-water). The model predictions follow the experimental data except for the prediction of low PEG concentrations in the bottom phase. Such low concentrations are difficult to measure accurately.

The model predictions using these two different set of parameters have also been compared with a third set of experimental results obtained by Connemann et al. (1991) (Figure

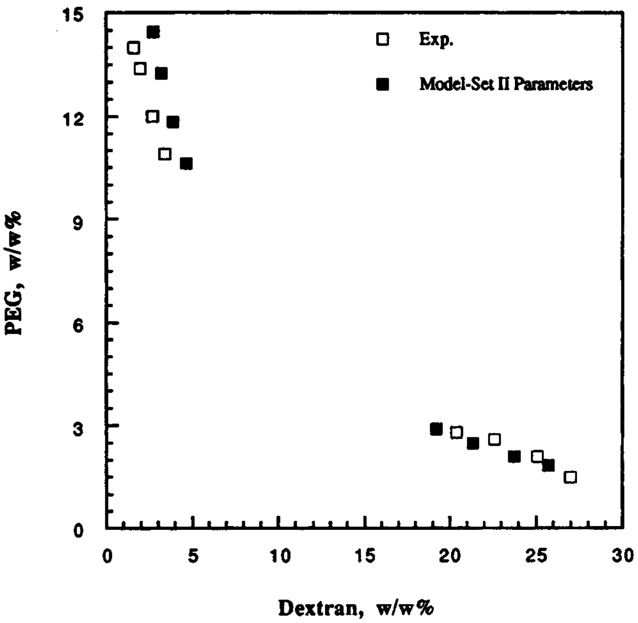

Figure 4. Binodal curve for an aqueous two-phase system of PEG4000-DEX10000-water at T = 277 K.

Data from Forciniti and Hall (1991).

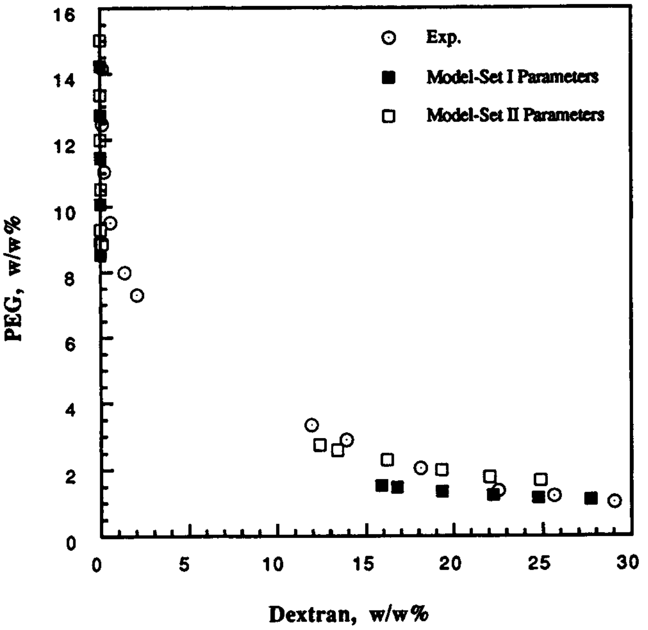

Figure 5. Comparison of the model predictions with set I and set II parameters for the binodal curve with the data of Connemann et al. (1991) at 273 K.

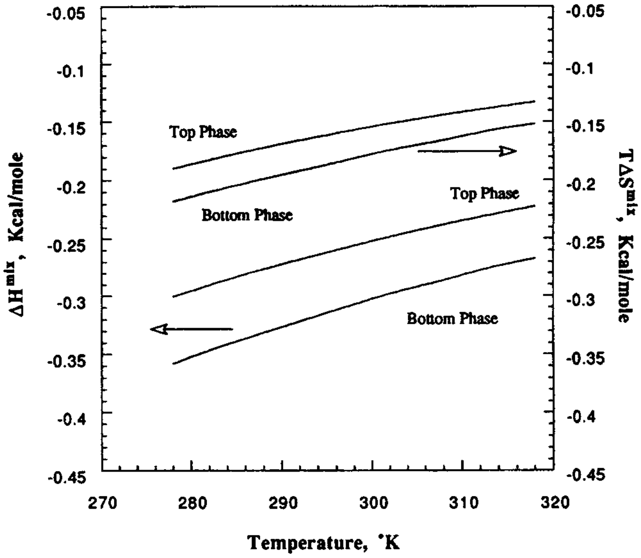

Figure 6. Enthalpy and entropy of mixing of the top and bottom phases.

5). For two-phase systems close to the plait point, the model predictions with the set II parameters are in better agreement with the experimental results than the predictions with our original set of parameters, while the opposite is true for higher feed compositions.

The heats and entropies of mixing of aqueous PEG and DEX solutions, as calculated from the Gibbs free energy model, provide additional information about the behavior of the polymers. The enthalpies of mixing for each phase are given by:

$$\Delta\underline{H}^{\text{mix}} = -RT^2 \frac{\partial}{\partial T}\left[\frac{\Delta\underline{G}^{\text{mix}}}{RT}\right] \tag{12}$$

and the entropy of mixing is calculated using

$$T\Delta\underline{S}^{\text{mix}} = \Delta\underline{G}^{\text{mix}} - \Delta\underline{H}^{\text{mix}} \tag{13}$$

where $\Delta\underline{G}^{\text{mix}} = RT\Sigma x_i \ln x_i + \underline{G}^{\text{EX}}$ with $\underline{G}^{\text{EX}}$ given by Eq. 1. The excess partial molar enthalpies and entropies of the PEG, DEX and water in both phases were calculated using the following relations:

$$\overline{G}_i = -\left[\frac{\partial N\underline{G}_i}{\partial N_i}\right]_{P,T,n_{j\neq i}} \tag{15}$$

$$\overline{S}_i = -\left[\frac{\partial \overline{G}_i}{\partial T}\right]_{P,n_j} \tag{16}$$

$$\overline{H}_i = \overline{G}_i - T\overline{S}_i \tag{17}$$

Both the enthalpies and entropies of mixing are negative (Figure 6). The calculated excess partial molar enthalpies and entropies of PEG and DEX are also negative and decrease in magnitude with increasing temperature (Figures 7 and 8).

Discussion

Increasing temperature increases the concentration of PEG in the top phase and reduces the concentration of DEX in the bottom phase (Figure 1). These trends are consistent with changes in the solubilities of these polymers with temperature and suggest that in aqueous two-phase polymer mixtures, phase separation is driven not only by polymer-polymer interactions, but importantly also by polymer-water interactions (Tjerneld et al., 1987). For aqueous two-phase systems composed of PEG 8000 and DEX 500 with feed compositions away from the critical point, the concentrations of PEG in the bottom

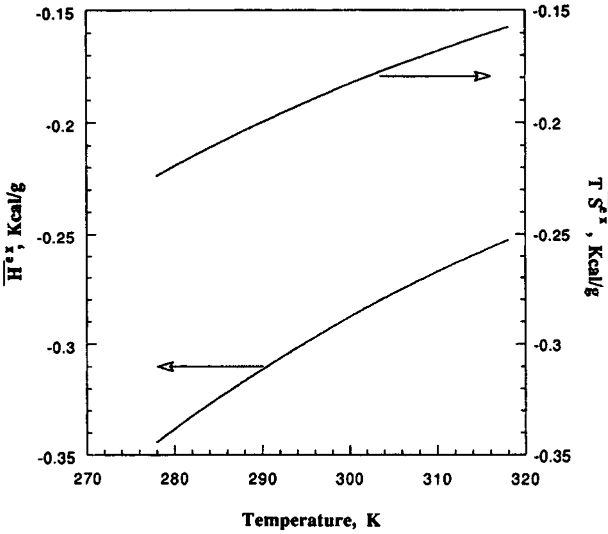

Figure 7. Excess partial molar enthalpy and entropy of PEG as a function of temperature.

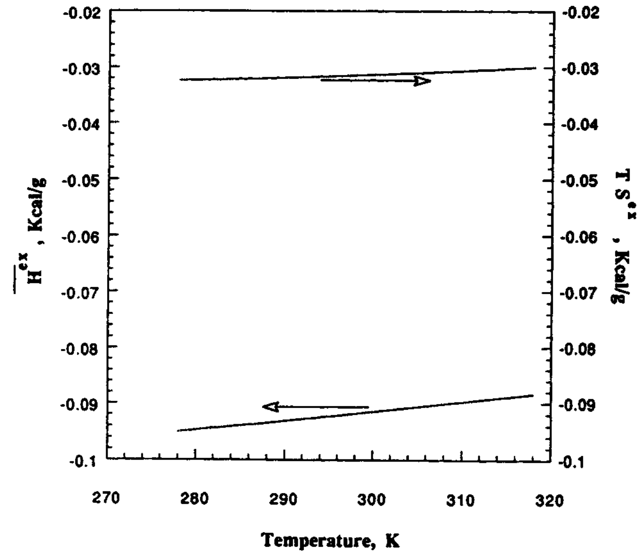

Figure 8. Excess partial molar enthalpy and entropy of dextran as a function of temperature.

phase and DEX in the top phase are very small (Appendix). Thus, for the discussion here, we can assume that the top and bottom phases are mostly aqueous solutions of PEG and DEX, respectively, and focus on the effect of temperature on the interactions between PEG and water in the top phase and DEX and water in the bottom phase.

The solubilities of both poly(ethylene oxide) and PEG in water decrease with increasing temperature (Bailey et al., 1959). The increasing hydrophobicity of these polymers with increasing temperature is related to changes of their conformations in aqueous solution. PEG chains in aqueous solution generally form a helix with approximately five monomers per turn. There are various dihedral angles around the C-O and C-C bonds of the PEG chain, so there are several conformations with different dipole moments and energies (Karlström, 1985). A mean field theory (Sjöberg and Karlström, 1989) suggests that at low temperatures low-energy polar conformations exist in PEG and lead to aqueous solubility, while higher energy and less polar conformations are favored at high temperature. Thus, the polymer becomes more hydrophobic with increasing temperature. Recent NMR measurements of PEG conformations as a function of temperature support this interpretation (Bjorling et al., 1991).

On the other hand, PEG solubility may also depend on the degree of hydration of the polymer chain in water (Kjellander and Florin, 1981; Nakayama, 1970). In this view, the ether oxygens in the PEG structure form specific orientation-dependent hydrogen bonds with water which "stiffen" the polymer (Kjellander and Florin, 1981). The hydrogen bond interactions lead to mutual stabilization of the water and polymer structure at low temperatures. The ordered structure of the PEG and the decrease in the conformational freedom of the polymer chain at low temperatures in water gives a negative contribution to the entropy of mixing. At high temperatures, hydration shells around the polymer are less developed and the entropy of mixing increases. This view is consistent with measured heats of mixing (Nakayama, 1970) and with the calculated properties in Figures 6-8.

Increasing temperature increases the slopes of the tie-lines for various polymer feed compositions (Figure 3). This happens because increasing temperature makes the PEG more hydrophobic, so water is driven from the PEG-rich phase to the DEX-rich phase, and the weight of the bottom phase increases at the expense of the top phase (Figure 2). The DEX concentration in the bottom phase then decreases with increasing temperature because of dilution. The dissolution of both PEG and DEX in water is highly exothermic at all compositions (Figure 6) (Kjellander and Florin, 1981; and Basedow et al., 1980).

The values and signs of the calculated excess partial molar enthalpies and entropies of PEG and DEX (Figures 7 and 8) give some insight into the conformational changes of the polymers. The negativity of the excess partial molar enthalpies and entropies of PEG and DEX in both the top and bottom phases are consistent with overlaps of the hydration shells of neighboring chains in solution. Increasing temperature decreases the magnitude of both the excess partial molar enthalpy and entropy of PEG in solution (Figure 7), and suggests that the structure of the hydration shell around the PEG gradually disappears as the temperature is raised. That is, the decrease in magnitude of the partial molar entropy of PEG in solution as temperature increases can be attributed to the increase in the free rotation of chain links and weakening of the polymer-solvent interactions in solution (Chew and Couper, 1976). On the other hand, increasing temperature does not significantly change either the excess partial molar enthalpy or entropy of DEX (Figure 8). Hydrolyzed DEX has a relatively compact coiled conformation in water and, once it is dissolved, its coiled conformation is not altered by dilution process (Basedow et al., 1980).

The thermodynamic properties of PEG-DEX mixtures depend significantly on specific interactions between the solvent and the solutes. Structural changes of the water due to the dissolution of the solutes play an important role in setting the properties of these nonpolar polymer solutions.

Summary

Increasing the temperature of the PEG-DEX-water two-phase system produces an increase in PEG concentration in the top phase and a decrease of DEX concentration in the lower phase. Increasing temperature makes PEG more hydrophobic, and changes in the PEG-water interactions drive water from the PEG-rich phase to the DEX-rich phase. A solution thermodynamic model based on the UNIQUAC equation with temperature-dependent parameters can represent the experimental data well, and a Gibbs free energy minimization procedure is an efficient way to calculate phase equilibrium compositions.

Notation

a_{ij} = UNIQUAC parameters defined in Eq. 4
A_{ij} = interaction parameters defined in Eq. 5
b = polymer polydispersity index
B_{ij} = interaction parameters defined in Eq. 5
$F(M)$ = molecular weight distribution function
$\underline{G}^{EX}$ = excess Gibbs free energy per mole
G_p^{EX} = excess Gibbs free energy of phase p
G^{Total} = total Gibbs free energy per mole
G_p^{Ideal} = ideal Gibbs free energy of phase p
$\underline{G}^{Mix}$ = Gibbs free energy of mixing
G_i^o = standard state Gibbs free energy

M_i = molecular weight of i
M_k = kth pseudocomponent molecular weight
M_n = number average molecular weight
M_o = parameter in the molecular weight distribution function
M_w = weight average molecular weight
n = total number of moles
n_{ip} = number of moles of i in phase p
n_p = total number of moles in phase p
OF = objective function defined in Eq. 6
q_i' = surface area parameter of i
r_i' = volume parameter of i
R = gas constant
T = temperature
w_i = weight fraction of i
w_k = kth pseudocomponent weight fraction
$w_{T,p,i}$ = weight fraction of i in phase p at temperature T
W_k = weight factor obtained from Gauss-Hermite quadrature
z = coordination number
z_k = kth zero of Hermite polynomial

Greek letters

β = parameter in the molecular weight distribution function
θ_i' = surface fraction of i component
τ_{ij} = interaction energy
Φ_i = volume fraction of i component

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Appendix: Phase Equilibrium Measurements for Aqueous Two-Phase Systems at Different Temperatures

	Feed Compositions	
System	PEG 8000 w/w %	Dextran 500 w/w %
A	5.0	7.0
B	6.5	7.0
C	5.8	8.4
D	7.3	8.4
E	5.8	6.2
F	5.0	5.0
G	6.5	5.0
H	6.0	4.0
I	7.5	4.0

System	T °C	PEG Top-Exp	PEG Top-Model	Dex Bot-Exp	Dex Bot-Model	TLL Exp	TLL Model
A	50	8.53	8.55	14.64	16.83	16.42	18.87
A	45	8.67	8.51	14.32	16.60	16.21	18.57
A	40	8.65	8.46	14.30	16.74	16.18	18.69
A	35	8.51	8.42	15.04	16.89	16.77	18.79
A	30	8.26	8.37	16.60	17.07	18.07	18.94
A	20	7.99	8.32	16.88	17.22	18.21	19.05
A	15	7.95	8.28	16.80	17.33	18.12	19.05
A	10	7.63	8.18	17.24	17.80	18.41	19.55
A	5	7.49	8.13	17.50	17.99	18.60	19.70

Appendix: Cont'd

System	T °C	PEG Top-Exp	PEG Top-Model	Dex Bot-Exp	Dex Bot-Model	TLL Exp	TLL Model
B	50	10.80	10.12	17.16	19.55	20.00	22.01
B	45	10.26	10.01	17.79	19.70	20.28	22.11
B	40	10.26	10.03	17.94	19.87	20.41	22.25
B	35	10.31	9.98	19.05	20.00	21.41	22.35
B	30	9.93	9.94	19.67	20.18	21.80	22.48
B	20	9.54	9.84	20.59	20.55	22.48	22.78
B	15	9.48	9.79	19.87	20.75	21.79	22.94
B	10	9.28	9.75	20.43	20.95	22.22	23.09
B	5	9.16	9.69	20.99	21.16	22.69	23.26
C	50	10.88	10.14	17.45	19.60	20.36	22.07
C	45	10.51	10.09	18.41	19.73	21.00	22.16
C	40	10.39	10.04	18.35	19.88	21.15	22.26
C	35	10.17	9.98	19.47	19.84	21.78	22.16
C	30	9.93	9.93	19.46	20.18	21.67	22.49
C	20	9.56	9.82	20.22	20.51	22.20	22.73
C	15	—	—	—	—	—	—
C	10	9.10	9.69	22.44	20.89	24.07	23.03
C	5	8.96	9.63	22.70	21.09	—	23.18
D	50	12.22	11.72	19.16	22.25	22.36	25.14
D	45	11.86	11.66	20.82	22.27	23.62	25.26
D	40	11.71	11.61	21.31	22.58	23.95	25.39
D	35	12.56	11.56	22.84	22.64	25.73	25.40
D	30	11.69	11.51	22.53	22.94	25.35	25.66
D	20	11.35	11.39	23.03	23.35	25.31	25.98
D	15	11.00	11.33	22.90	23.56	25.10	26.15
D	10	10.93	11.27	23.82	23.79	25.92	26.32
D	5	10.77	11.21	24.32	24.05	26.26	26.53
E	50	9.22	8.96	15.09	17.55	17.37	19.71
E	45	8.97	8.92	15.21	17.69	17.35	19.81
E	40	9.58	8.88	16.85	17.83	19.06	19.92
E	35	9.29	8.84	16.92	17.98	19.01	20.04
E	30	8.94	8.81	16.95	18.15	18.88	20.17
E	20	8.78	8.72	18.38	18.49	20.10	20.44
E	15	7.73	8.68	18.47	18.67	19.79	20.59
E	10	7.90	8.63	19.31	18.87	20.63	20.75
E	5	7.74	8.59	—	19.07	18.65	20.91
F	50	7.52	7.50	11.92	14.96	13.59	16.74
F	45	7.35	7.47	13.08	14.63	14.52	16.33
F	40	7.19	7.44	12.83	14.83	14.23	15.45
F	35	7.30	7.41	13.84	14.94	15.01	16.59
F	30	7.24	7.38	14.08	15.09	15.36	16.71
F	20	7.19	7.31	14.80	15.44	16.09	17.02
F	15	7.17	7.28	14.98	15.64	16.25	17.19
F	10	7.05	7.24	14.90	15.92	16.13	17.45
F	5	6.90	7.20	15.12	16.08	16.24	17.57
G	50	9.05	9.05	—	17.73	16.51	19.90
G	45	9.16	9.02	16.26	17.88	18.22	20.02
G	40	9.15	8.99	—	18.04	17.31	20.15
G	35	9.19	8.96	16.50	18.20	18.45	20.28
G	30	9.14	8.93	16.95	18.38	18.83	20.42
G	20	8.99	8.86	17.24	18.75	19.02	20.74
G	15	8.37	8.83	18.05	18.95	19.51	20.90
G	10	8.16	8.79	19.31	19.18	20.60	21.09
G	5	8.60	8.75	19.25	19.41	20.75	21.29
H	50	8.29	8.01	13.14	15.89	15.02	17.79
H	45	8.08	7.99	13.97	16.02	15.65	17.90
H	40	8.20	7.97	14.53	16.17	16.20	18.02
H	35	8.15	7.94	14.78	16.34	16.33	18.16
H	30	7.83	7.92	13.64	16.49	15.17	18.29
H	20	8.07	7.86	15.67	16.85	17.10	18.59
H	15	7.62	7.84	15.54	17.06	16.80	18.77
H	10	7.00	7.81	16.20	17.25	17.18	18.93
H	5	7.29	7.77	17.20	17.48	18.21	17.48
I	50	9.13	9.55	15.71	18.60	17.82	20.91
I	45	—	—	—	—	—	—
I	40	9.58	9.51	16.66	18.94	18.87	21.18
I	35	9.42	9.48	17.06	19.13	19.14	21.34
I	30	9.22	9.45	16.29	19.32	18.37	21.51
I	20	9.26	9.40	19.04	19.76	20.85	21.88
I	15	9.20	9.37	20.03	19.98	21.73	22.07
I	10	8.90	9.34	19.41	20.23	21.40	22.29
I	5	8.96	9.31	20.77	20.45	22.32	22.50

Appendix: Cont'd

System	T °C	Mass Top-Exp	Mass Top-Model	Mass Bot-Exp	Mass Bot-Model	TLL Slope Exp	TLL Slope Model
A	50	0.541	0.584	0.471	0.416	0.528	0.508
A	45	0.527	0.578	0.484	0.422	0.550	0.501
A	40	0.526	0.582	0.485	0.418	0.549	0.495
A	35	0.542	0.585	0.459	0.414	0.513	0.489
A	30	0.566	0.590	0.416	0.410	0.449	0.482
A	20	0.587	0.593	0.411	0.406	0.425	0.475
A	15	0.585	0.598	0.413	0.404	0.425	0.469
A	10	0.611	0.608	0.401	0.393	0.395	0.454
A	5	0.623	0.623	0.395	0.389	0.379	0.447
B	50	0.585	0.642	0.406	0.358	0.598	0.517
B	45	0.599	0.645	0.387	0.355	0.547	0.510
B	40	0.614	0.648	0.390	0.352	0.542	0.504
B	35	0.613	0.650	0.366	0.350	0.513	0.498
B	30	0.638	0.653	0.357	0.347	0.478	0.491
B	20	0.657	0.659	0.340	0.340	0.438	0.478
B	15	0.665	0.663	0.353	0.337	0.450	0.471
B	10	0.679	0.666	0.341	0.334	0.428	0.464
B	5	0.689	0.669	0.333	0.331	0.411	0.457
C	50	0.516	0.571	0.482	0.428	0.601	0.517
C	45	0.532	0.574	0.458	0.426	0.549	0.511
C	40	0.506	0.577	0.450	0.423	0.544	0.504
C	35	0.554	0.576	0.434	0.423	0.502	0.498
C	30	0.567	0.584	0.432	0.416	0.490	0.491
C	20	0.590	0.590	0.415	0.410	0.453	0.478
C	15	—	0.594	—	0.406	—	0.471
C	10	0.619	0.598	0.374	0.402	0.388	0.463
C	5	0.628	0.602	0.320	0.398	0.328	0.456
D	50	0.570	0.622	0.438	0.377	0.601	0.526
D	45	0.590	0.623	0.403	0.377	0.536	0.520
D	40	0.593	0.628	0.394	0.372	0.517	0.514
D	35	0.563	0.629	0.366	0.371	0.519	0.508
D	30	0.623	0.634	0.371	0.366	0.488	0.501
D	20	0.621	0.640	0.362	0.360	0.462	0.487
D	15	0.639	0.644	0.367	0.356	0.450	0.480
D	10	0.646	0.647	0.352	0.353	0.429	0.474
D	5	0.658	0.650	0.343	0.349	0.414	0.466
E	50	0.609	0.647	0.414	0.353	0.570	0.504
E	45	0.618	0.649	0.400	0.350	0.549	0.503
E	40	0.590	0.652	0.367	0.347	0.532	0.498
E	35	0.609	0.655	0.371	0.345	0.512	0.491
E	30	0.632	0.658	0.342	0.366	0.491	0.484
E	20	0.644	0.665	0.335	0.337	0.444	0.471
E	15	0.735	0.668	0.338	0.332	0.385	0.464
E	10	0.719	0.671	0.321	0.328	0.377	0.457
E	5	0.766	0.675	0.365	0.325	0.420	0.450
F	50	0.632	0.666	0.407	0.334	0.587	0.501
F	45	0.651	0.658	0.372	0.342	0.521	0.495
F	40	0.663	0.661	0.379	0.339	0.519	0.489
F	35	0.651	0.665	0.356	0.335	0.488	0.483
F	30	0.661	0.669	0.345	0.331	0.476	0.476
F	20	0.661	0.676	0.331	0.324	0.449	0.469
F	15	0.677	0.680	0.341	0.320	0.442	0.463
F	10	0.676	0.686	0.326	0.314	0.436	0.449
F	5	0.688	0.689	0.325	0.311	0.420	0.441

Appendix: Cont'd

System	T °C	Mass Top-Exp	Mass Top-Model	Mass Bot-Exp	Mass Bot-Model	TLL Slope Exp	TLL Slope Model
G	50	0.676	0.718	0.338	0.282	0.573	0.510
G	45	0.680	0.720	0.308	0.280	0.513	0.504
G	40	0.671	0.723	0.318	0.277	0.544	0.498
G	35	0.677	0.725	0.299	0.275	0.508	0.492
G	30	0.680	0.728	0.291	0.272	0.491	0.485
G	20	0.691	0.733	0.286	0.266	0.474	0.472
G	15	0.749	0.736	0.275	0.264	0.418	0.465
G	10	0.769	0.739	0.256	0.261	0.380	0.458
G	5	0.729	0.742	0.260	0.258	0.404	0.450
H	50	0.698	0.748	0.293	0.252	0.579	0.504
H	45	0.716	0.750	0.278	0.250	0.529	0.498
H	40	0.704	0.753	0.270	0.247	0.517	0.492
H	35	0.711	0.755	0.261	0.245	0.505	0.485
H	30	0.733	0.757	0.286	0.243	0.524	0.479
H	20	0.712	0.763	0.248	0.273	0.471	0.466
H	15	0.754	0.765	0.251	0.234	0.445	0.459
H	10	0.829	0.768	0.236	0.232	0.389	0.452
H	5	0.795	0.771	0.226	0.229	0.383	0.444

Appendix: Cont'd

System	T °C	Mass Top-Exp	Mass Top-Model	Mass Bot-Exp	Mass Bot-Model	TLL Slope Exp	TLL Slope Model
I	50	0.802	0.785	0.250	0.215	0.549	0.513
I	45	—	—	—	—	—	—
I	40	0.768	0.789	0.236	0.211	0.544	0.501
I	35	0.778	0.791	0.231	0.209	0.522	0.495
I	30	0.796	0.793	0.240	0.207	0.535	0.489
I	20	0.794	0.798	0.205	0.202	0.459	0.475
I	15	0.815	0.800	0.201	0.200	0.433	0.469
I	10	0.817	0.802	0.199	0.197	0.432	0.461
I	5	0.819	0.805	0.192	0.195	0.406	0.454

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