Phase Separation of Aqueous Mixtures of Poly(ethylene oxide) and Dextran

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ABSTRACT: The phase behavior of aqueous mixtures of poly(ethylene oxide) (PEO) and dextran is studied as a function of the polymer concentration, the PEO molar mass, and temperature. The molar mass distributions of the two polymers in the coexisting phases are measured. From the temperature dependence we conclude that the phase separation between PEO and dextran is partially caused by sterical interactions. From the equilibrium phase volumes of the phase-separated mixture and the shape of the temperature−composition phase diagram of PEO and dextran, we conclude that also a decrease of the solvent quality of water for PEO at increasing temperatures is involved. It is suggested that the characteristics of the PEO−water interaction can affect the degree of fractionation. This suggestion is based on the observation that the degree of fractionation is not a simple exponential function of the molar mass. The phase behavior of the mixture PEO/dextran is compared to the previously studied phase behavior of the aqueous mixture of gelatin and dextran.

1. Introduction

Most foods contain mixtures of biopolymers. If the concentration of these polymers is sufficiently high, segregative phase separation might take place. This phase separation is often the basis for structuring foods. In the literature a large quantity of experimental data is available on the segregative phase separation of biopolymers.[1] However, these data are only qualitative and give no information about the effect of the temperature on the phase separation. Therefore, it is not possible to obtain any information from these data about the mechanism of the phase separation.

In a previous paper,[2] we chose an aqueous solution of gelatin and dextran as a model system for food biopolymers to study phase separation. We found that the concentration in the coexisting phases does not depend on temperature and that phase separation of this system can only be realized by increasing the total polymer concentration. This temperature independence of the phase separation suggests that phase separation between gelatin and dextran is driven by entropic interactions. Indeed, some features could be described using depletion theory.[2−4] Besides the phase diagram, also the molar mass distributions of the polymers after phase separation were measured.[5] It turned out that the phase separation results in a fractionation in molar mass and that the degree of fractionation depends exponentially on the molar mass.

To find out whether the mechanism of phase separation which we found for the system gelatin/dextran is common for mixtures of polymers or only specific for the system gelatin/dextran, we chose to study the phase behavior of the system poly(ethylene oxide) (PEO)/dextran and compare this behavior with that of the mixture gelatin/dextran. We chose PEO as a replacement for gelatin because of its experimental advantages. Apart from this, it is, just like most biopolymers, a crude material in that it is polydisperse in its molar mass.

The main reason mentioned in the literature for studying the system PEO/dextran is that it can be used for the partitioning of small biomolecules, e.g., proteins. A lot of experimental data and theoretical descriptions of the effects of several parameters (e.g., polymer concentration and molar mass) on the partitioning of biomaterials are available.[6−12] However, there are relatively few studies done on the influence of temperature and molar mass on the phase diagram. Forciniti et al., for example, performed a study on the effect of temperature and molar mass on the phase behavior of the system PEO/dextran.[13] They found that, with increasing molar mass of the polymers, the influence of temperature decreased. However, most of the PEO used in these studies had a molar mass smaller than 20 kDa.

For the study described in this paper we are interested in the phase behavior of aqueous mixtures of PEO ($M_w \geq 100$ kDa) with dextran ($M_w = 282$ kDa). The molar mass of PEO, the concentration of polymer, and the temperature at which phase separation was established, were varied. Finally, the phase behavior of the system PEO/dextran was compared to the phase behavior of the aqueous system gelatin/dextran to obtain a more general view of the phase behavior of aqueous (bio)polymer mixtures.

2. Experimental Section

2.1. Materials. Dextran with an M_w of 282 kDa was purchased from Sigma Chemicals. PEOs with M_w values of 100 and 200 kDa were purchased from Fischer Scientific. The dextran was used without further purification. PEO was purified before use. This was done by dissolving the powder in reversed osmosis (RO) water by stirring with a magnetic stirrer at room temperature. After 24 h, the solution was centrifuged (60 min, 11000*g*), and the supernatant was freeze-dried. Before using this material, the molar mass distribution

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Table 1. Overview of M_n, M_w, M_z, and Polydispersity (M_w/M_n) for PEO and Dextran before Phase Separation[a]

sample	M_n	M_w	M_z	M_w/M_n
PEO-100	39	104	253	2.6
PEO-200	57	196	758	3.4
dextran	64	299	993	4.7

[a] Molar mass averages in kilodaltons.

was measured with a size exclusion chromatograph equipped with a multiangle laser light scattering detector (SEC-MALLS) (see Table 1). Clear solutions were prepared by gravimetrically adding solvent (RO water with 0.02% sodium azide to prevent bacterial growth) to the appropriate amount of material. Dextran dissolves readily at room temperature. PEO was dissolved with stirring of the solution overnight.

2.2. Determination of the Temperature−Composition Phase Diagram. To determine the temperature−composition phase diagram, mixtures of equal mass concentrations of PEO and dextran were made. For this purpose equal masses of dextran solutions and PEO solutions with the same mass percentage of polymer were mixed. Approximately 10 g of this mixture was put into a plastic tube. To prevent evaporation of the solvent, a layer of paraffin oil was put on top of the meniscus. The tubes were held in a water bath for approximately 20 h to reach equilibrium phase separation. Equilibrium was assumed to be reached when a sharp meniscus between transparent PEO-rich and dextran-rich fluid layers was observed. Hereafter, the heights of the PEO-rich and dextran-rich phase were measured and related to the volume of the phases. The experimental error of these measurements was on the order of 1%. Samples of these phases were taken with a syringe with a hypodermic needle.

To determine the concentration of PEO and dextran in the coexisting phases, samples taken from both phases were diluted 40 times. Optical rotation was measured at 365 nm and 80 °C. Because dextran shows optical rotation and PEO does not, the concentration of dextran was measured using

$$\alpha_{meas}(c_d, \lambda = 365\ nm) = [\alpha]_{d,\lambda=365nm} c_d \quad (1)$$

where the subscripts "meas" and "d" denote measured and dextran and where [α] denotes the specific rotation (per % (w/w per dm)). The experimental error of this method is 1%.

The concentrations PEO in the coexisting phases were determined by measuring the density of the phases. By using the known concentration of dextran, the concentration of PEO (% (w/w)) could be calculated using

$$\rho_{meas} = \rho_s + \Delta\rho_P c_P + \Delta\rho_d c_d \quad (2)$$

where ρ denotes the density and $\Delta\rho$ the density increment per unit concentration. The subscripts "meas", "s", "P", and "d" denote measured, solvent, PEO, and dextran, respectively. $\Delta\rho$ was measured using a Mettler/Paar DMA 45 density meter. For this method, additivity of volumes was assumed. This method was tested by measuring the density of mixtures of PEO and dextran with various concentrations from which the concentration of PEO was calculated again. The experimental error of this method turned out to be 10%.

2.3. Determination of the Cloud Point of PEO in Water. Solutions of PEO in water were made in a concentration range between 5% and 10% (w/w). Glass tubes were almost completely filled with these solutions and sealed with a screw cap. These tubes were kept in a thermostated oil bath. The cloud point was determined by the eye (experimental error 0.5 °C).

2.4. Determination of the Phase Separation Temperatures. Mixtures of equal concentrations of PEO and dextran were made. From this mixture a series of mixtures with decreasing polymer concentration (steps of 0.02% (w/w) total polymer concentration) was made. This series was held overnight in a water bath at a fixed temperature. After 20 h the mixtures were checked on phase separation.

Table 2. Overview of the Samples Analyzed with the SEC-MALLS[a]

sample code	PEO-100	PEO-200	dextran	T_{PS}[b]
100A	3.5		3.5	60.0
100B60	3.0		3.0	60.0
100C	2.5		2.5	60.0
100B40	3.0		3.0	40.0
100B80	3.0		3.0	80.0
200A		3.5	3.5	60.0
200B60		3.0	3.0	60.0
200C		2.5	2.5	60.0
200B40		3.0	3.0	40.0
200B80		3.0	3.0	80.0

[a] Values represent the concentration of polymer (% (w/w)) in the samples before phase separation. [b] T_{PS} (°C) represents the temperature at which phase separation took place.

2.5. Determination of the Molar Mass Distributions of PEO and Dextran. An SEC-MALLS equipped with a refractive index (RI) detector was used to determine the molar mass distribution of PEO and dextran. For mixtures of PEO and dextran, an additional detector monitoring optical rotation (OR) at 365 nm was used. By combining the signals from the RI detector and the OR detector, the contribution from dextran and PEO to the two signals can be unraveled because both signals are different linear combinations of dextran and PEO contributions. This method was tested by measuring the native polymers and a mixture of these polymers. It turned out that the same molar mass distributions were found for the native polymers and the polymers in the mixture.

The conditions for determining the molar mass distributions were chosen to be the same as in ref 5. A $LiNO_3/KH_2PO_4/K_2HPO_4$ (pH 6.7) buffer was used as eluant. The flow rate was 1 mL min^{-1}. The columns that were used were a combination of TSK guard + TSK G5000PW + TSK G3000PW (TosoHaas GmbH, Stuttgart, Germany). Typically 4 mg of dry material in 200 μL was injected, resulting in a concentration of 0.2% (w/w) in the detector cells.

For the determination of the molar mass distribution of PEO and dextran in coexisting phases, samples were taken from the PEO-rich and dextran-rich phases after equilibration during about 20 h. Table 2 gives an overview of the samples from which the coexisting phases were analyzed with the SEC-MALLS. All samples from the coexisting phases were diluted 40 times in the eluant and put in vials. Besides these samples, also the starting material was analyzed. The temperatures of the SEC column and MALLS detector cell were 50 °C. The OR detector cell had a temperature of 40 °C, and the RI detector cell was not temperature controlled. For the formulas used to calculate M_n, M_w, and M_z and the polydispersity, see ref 5.

3. Results

3.1. Phase Behavior. Figure 1 presents the temperature−composition phase diagram of mixtures of 3.5% (w/w) PEO and 3.5% (w/w) dextran for the two molar masses of PEO studied. It turns out that, in the temperature range studied, no significant effect of PEO molar mass on the concentration of dextran and PEO in the coexisting phases could be detected.

Figure 2 shows the influence of the initial polymer concentration on the concentrations of the two polymers in the temperature−composition phase diagram for the mixture of PEO-100 and dextran. It turns out that the higher the initial concentration, the larger the difference is between the concentrations of polymer in the two coexisting phases. Figures 1 and 2 also show that with increasing temperature the concentration of dextran in the dextran-rich phase decreases, while the concentration PEO in the PEO-rich phase increases.

From Figure 3 it turns out that when the temperature at which phase separation is established increases, the

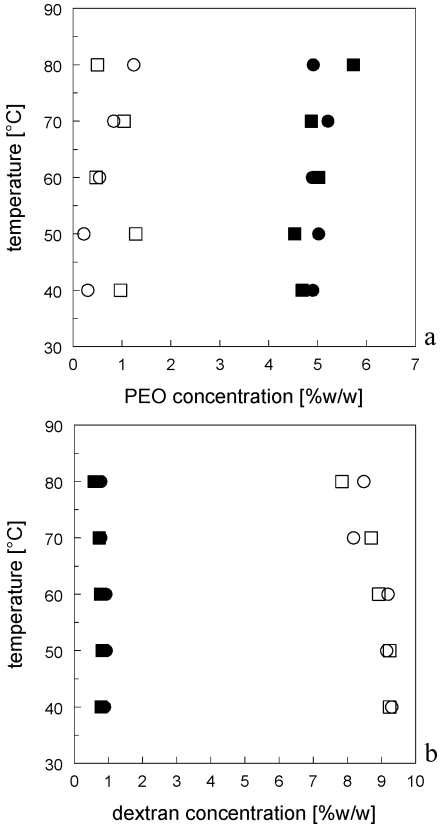

Figure 1. Coexisting phase compositions after full phase separation in 3.5% PEO/3.5% dextran/water: (a) concentrations of PEO; (b) concentrations of dextran; ●, PEO-100; ■, PEO-200; open symbols, dextran-rich phase; closed symbols, PEO-rich phase.

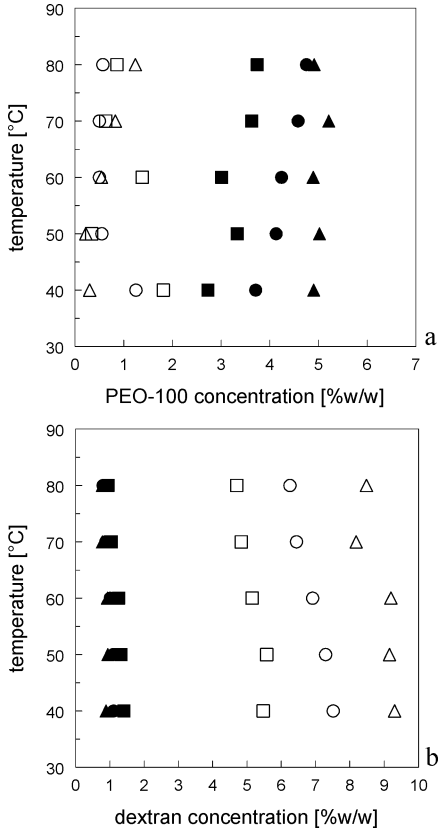

Figure 2. Coexisting phase compositions after full phase separation of the mixture PEO-100/dextran/water: (a) concentrations of PEO-100; (b) concentrations of dextran. Initial compositions of PEO-100/dextran: ▲, 3.5%/3.5%; ●, 3.0%/3.0%; ■, 2.5%/2.5%. Open symbols are for the dextran-rich phase, and closed symbols are for the PEO-rich phase.

phase volume of the PEO-rich phase decreases. It is known from the literature[14,15] that the solubility of PEO in water decreases with increasing temperature and that the cloud point for PEO in water is near 100 °C. For the PEO we used, the cloud point in a concentration range of 5−10% (w/w) PEO was determined. For all samples of PEO-100 in the concentration range measured, the cloud point was established at 106 °C, while for the samples of PEO-200, the cloud point was determined to be 103 °C.

The temperature dependence of the phase diagram was studied by determining the cloud point of the mixture PEO-100/dextran. In the temperature range between 40 and 70 °C, it appears that the position of the cloud point is located between 4.06% and 4.08% (w/w) total polymer (see Figure 4). At 80 °C, the position of the binodal shifts to a lower total polymer concentration. This is probably due to a decrease of the quality of water as a solvent for PEO.

3.2. Fractionation in Molar Mass. For dextran as well as for PEO in the coexisting phases, the molar mass distribution was determined. The molar mass of PEO, the initial polymer concentration, and the temperature at which phase separation was established were varied. An overview of the analyzed samples is given in Table 2. Only results are shown of the systems containing PEO-100.

3.2.1. Concentration Effects. Figure 5 shows the influence of the initial polymer concentration on the molar mass distributions of PEO-100 and dextran after phase separation at 60 °C. The area under the curves represents the concentration in the coexisting phases (see also ref 5).

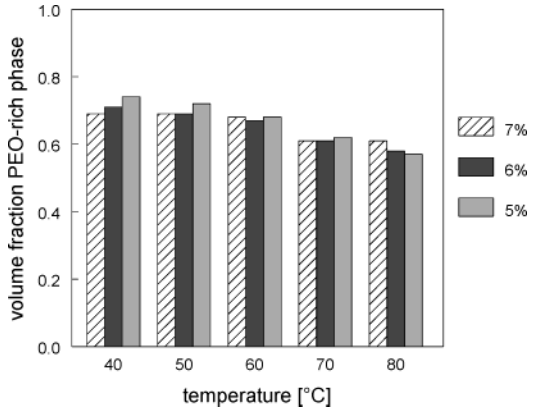

Figure 3. Volume fraction of the PEO-100-rich phase as a function of the temperature at which phase separation was established. Solute composition: 50% dextran + 50% PEO-100.

Figure 5 shows that decreasing the total polymer concentration results, for the material in the depleted phases, in broader peaks. Table 3 shows that the molar mass averages of the PEO as well as the dextran in their depleted phases increase when the initial polymer concentration is decreased. On the other hand, the molar mass averages of PEO and dextran in their enriched phase stay more or less constant with decreasing concentration. The same behavior was found for the molar mass distributions of the samples containing PEO-200. For the three different averages of the molar mass given in Table 3, the values of M_z are the most reliable. This is due to the fact that, for PEO as well as

Table 3. M_n, M_w, M_z, and the Polydispersity for PEO and Dextran for the Native Material and the Material in the Different Phases for the Different Mixtures[a]

PEO

	PEO in PEO-rich phase					PEO in dextran- rich phase				
sample	PEO concn	M_n	M_w	M_z	M_w/M_n	PEO concn	M_n	M_w	M_z	M_w/M_n
native PEO-100		39	104	253	2.6		39	104	253	2.6
100A	5.0	55	120	281	2.2	0.3	27	32	38	1.2
100B60	4.1	56	124	287	2.2	0.7	32	43	63	1.4
100C	3.2	57	123	280	2.2	1.0	37	61	107	1.6
100B40	4.1	53	103	186	1.9	0.4	30	37	47	1.2
100B80	4.8	51	102	213	2.0	0.5	30	38	50	1.3

Dextran

	dextran in PEO-rich phase					dextran in dextran- rich phase				
sample	dextran concn	M_n	M_w	M_z	M_w/M_n	dextran concn	M_n	M_w	M_z	M_w/M_n
native dextran		64	299	993	4.7		64	299	993	4.7
100A	0.9	40	86	257	2.2	9.2	104	386	1012	3.7
100B60	1.0	46	109	274	2.4	6.9	111	400	1030	3.6
100C	1.3	56	160	391	2.9	5.2	135	439	937	3.3
100B40	1.1	47	113	264	2.4	7.5	122	428	1000	3.5
100B80	1.0	41	91	250	2.2	6.3	103	368	918	3.6

[a] Concentrations in percent (w/w), molar mass averages in kilodaltons.

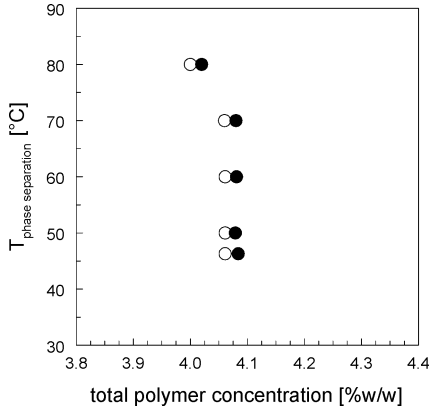

Figure 4. Phase separation temperature vs total polymer concentration. The solute consists of 50% PEO-100 + 50% dextran. Open symbols indicate no phase separation occurred, and closed symbols indicate phase separation occurred.

for dextran, the lower part of the molar mass distribution could not be measured, and this has the least effect on the value of M_z compared to the values of M_n and M_w.

3.2.2. Temperature Effects. Figure 6 shows the influence of the temperature at which phase separation was established on the molar mass distributions of the polymers. Although the results of the mixture containing PEO-200 are not shown, the same behavior as for mixtures containing PEO-100 can be observed for mixtures containing PEO-200. Figure 6 shows the opposite behavior for PEO and dextran; with increasing temperature, the peak height for PEO in its enriched phase increases, while that of dextran in its enriched phase decreases. This is in agreement with Figures 1 and 2, where it is shown that with increasing temperature the concentration of PEO in its enriched phase increases whereas that of dextran in its enriched phase decreases. From Table 3 it appears that the temperature has hardly any influence on the average molar mass values of PEO and dextran in their enriched as well as in their depleted phase.

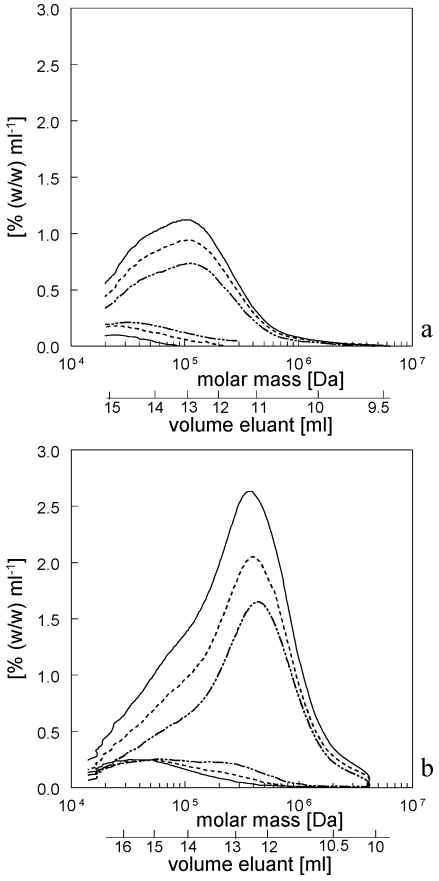

Figure 5. Molar mass distributions in coexisting phases at 60 °C, for various total polymer concentrations: (a) PEO-100; (b) dextran; upper set of lines, rich phase; lower set of lines, poor phase; —, sample 100A (3.5%/3.5%); ---, sample 100B60 (3.0%/3.0%); — •• —, sample 100C (2.5%/2.5%).

4. Discussion

4.1. Phase Behavior of PEO/Dextran. In this paper we give an overview of the phase behavior of an aqueous mixture of PEO and dextran as a function of the temperature at which phase separation is established, of the total polymer concentration, and of the molar

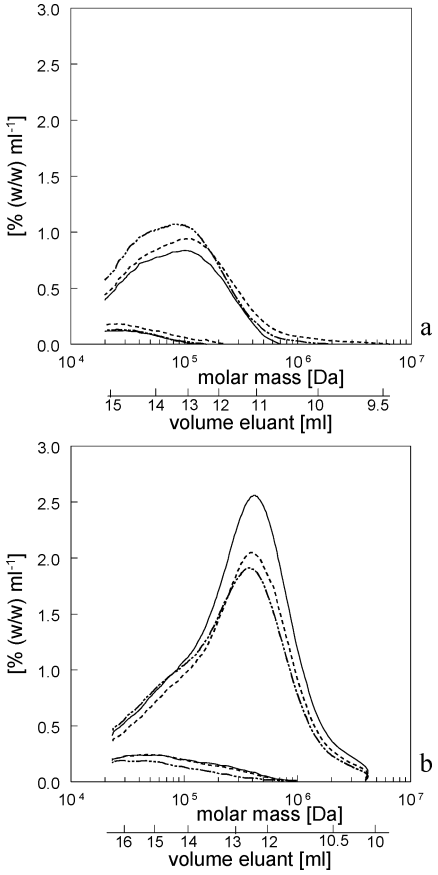

Figure 6. Molar mass distributions in coexisting phases for mixtures containing 3.0% (w/w) PEO-100 and 3.0% (w/w) dextran, at various phase separation temperatures (T_{PS}): (a) PEO-100; (b) dextran; upper set of lines, rich phase; lower set of lines, poor phase; —, sample 100B40 (T_{PS} = 40 °C); ---, sample 100B60 (T_{PS} = 60 °C); — ·· —, sample 100B80 (T_{PS} = 80 °C).

mass of PEO. It appears that the phase separation between PEO and dextran is affected by temperature and is accompanied by a fractionation in molar mass of both polymers.

It turns out that the concentration of PEO and dextran in the coexisting phases is not affected by the molar mass of PEO. This is in line with Forciniti et al.,[13] who reported that, if the molar mass of the polymers is high enough, an increase of the molar mass has no effect on the polymer concentrations in the coexisting phases.

Figure 4 shows that the position of the cloud point as a function of the total polymer concentration is hardly affected by the temperature for temperatures below 80 °C, which means that phase separation can only be established by increasing the concentration and not by decreasing the temperature. This temperature independence can be interpreted in two ways. Either the phase separation is caused by sterical interactions between the polymers, or the enthalpic and entropic contributions of the interaction parameters cancel. PEO is a relatively hydrophobic polymer. The interaction between PEO and water therefore becomes less favorable with increasing temperature, which has a negative influence on the stability of the dissolved state.[14] The solubility of dextran, on the other hand, increases with increasing temperature. In principle, the temperature dependence of phase separation behavior could vanish if solvent quality differences would cause phase separation and if these opposed tendencies in solvent qualities with temperature would cancel each other. However, since the temperature independence of the phase separation of aqueous systems of PEO and dextran is observed in systems for various molar masses of PEO and polymer concentrations, an explanation of the temperature independence in terms of solvent quality of the phase separation is not very plausible, and the phase separation being driven by sterical interactions between dissimilar polymers appears more plausible. We therefore interpret the mechanism of phase separation as phase separation induced by sterical interactions between the PEO and dextran molecules. However, at 80 °C, the total concentration at phase separation does change (it decreases with increasing temperature). Increasing the temperature also results in an increase of the PEO concentration in the PEO-rich phase, and a slight decrease of the dextran concentration in the dextran-rich phase. From the literature[14,15] and from our own observations we know that the solubility of PEO in water decreases with increasing temperature and that the cloud point of PEO lies at temperatures above 100 °C. This lower solubility of PEO in water with increasing temperature might dominate the temperature dependence observed in the temperature−composition phase diagram. Indeed, since we observed that the phase volume of the PEO-rich phase decreases at higher temperatures (due to the decreased solubility of PEO at these higher temperatures), we conclude that water is expelled from the PEO-rich phase toward the dextran-rich phase. As a consequence, the concentration of dextran in the dextran-rich phase will decrease as is shown in Figures 1 and 2.

The figures of the molar mass distributions of PEO and dextran after phase separation (Figures 5 and 6), as well as the average molar mass values in Table 3, show that for both polymers phase separation results in a fractionation in molar mass. The reason for the fractionation during phase separation of polydisperse polymers is discussed by several authors.[5,16−23] Analogous to the results of Forciniti et al.,[24] we found that the molar mass of dextran in the dextran-rich phase is independent of the molar mass of PEO.

If the degree of fractionation ($c_{x,\mathrm{poor},m}/c_{x,\mathrm{rich},m}$) is calculated and plotted versus the number of monomers, an exponential dependence on the molar mass is expected[5,16−19] when only sterical interactions (packing effects) between polymers govern the phase separation. However, Figures 7 and 8 show that this is not the case for dextran in PEO/dextran mixtures: the degree of fractionation shows a deviation from this exponential dependence. Considering the fact that the solvent quality is involved in the phase separation between PEO and dextran, it is suggested that this also plays a role in the fractionation. If this is the case, the largest effect of the solvent quality is expected in the PEO-rich phase (which is the dextran-poor phase). Presumably, the dextran molecules in this phase cannot realize the same conformation as in the dextran-rich phase. In other words, the relation between molar mass and molecular size is different for the two phases. In the case that sterical interactions between the polymers (packing effects) rule the phase separation, a nontrivial dependence of the degree of fractionation on molar mass can be expected. The involvement of solvent in the polymer conformation will be the largest at higher molar mass. This is in agreement with computer simulations that show that the dependence of the degree of fractionation

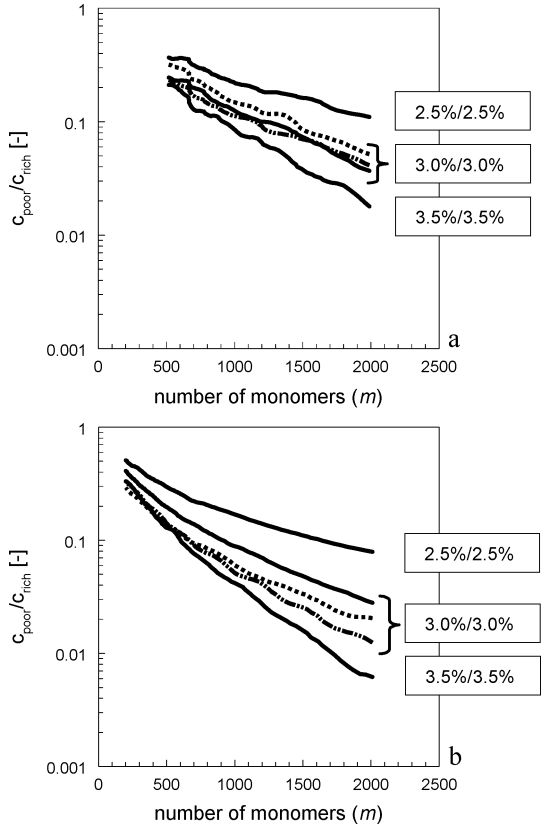

Figure 7. $c_{x,poor}/c_{x,rich}$ as a function of the number of monomers (m) in a polymer chain (a) for PEO-100 and (b) for dextran. The percentages (w/w) are those of PEO and dextran in the mixture before phase separation. For 3.0%/3.0% results for three temperatures are given: ---, $T = 40$ °C; —, $T = 60$ °C; — ·· —, $T = 80$ °C. At 2.5%/2.5% and 3.5%/3.5%, T was 60 °C.

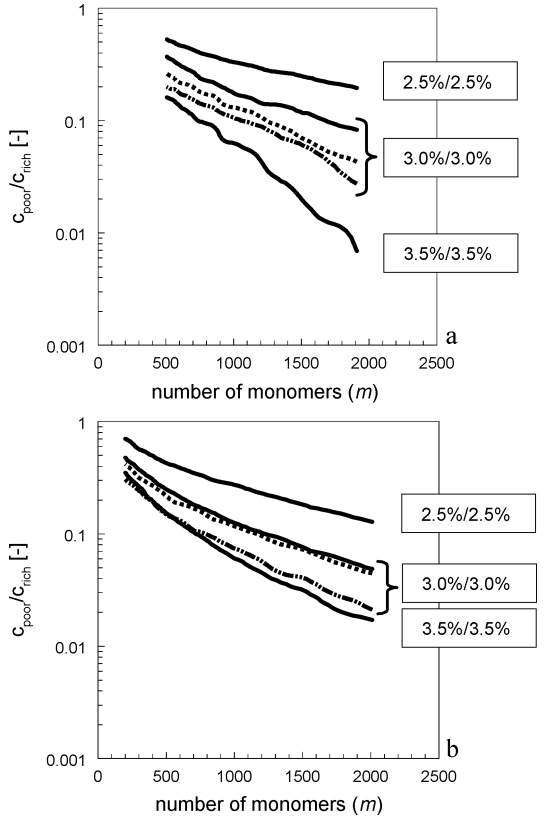

Figure 8. $c_{x,poor}/c_{x,rich}$ as a function of the number of monomers (m) in a polymer chain (a) for PEO-200 and (b) for dextran. The percentages (w/w) are those of PEO and dextran in the mixture before phase separation. For 3.0%/3.0% results for three temperatures are given: ---, $T = 40$ °C; —, $T = 60$ °C; — ·· —, $T = 80$ °C. At 2.5%/2.5% and 3.5%/3.5%, T was 60 °C.

on the degree of polymerization shows a similar concave deviation, if the radius of gyration in the poor phase is smaller than in the rich phase.[25] The result of the computer simulation would then imply that, at increasing total polymer concentration or increasing temperature, the deviation from exponential behavior would start at lower values of m. As can be seen in Figures 7 and 8, this is indeed the case. Note that the fractionation of PEO does not show a deviation of the exponential dependence. The explanation of this appearance might be found in the assumption that PEO molecules do not make a distinction between water and dextran, since the surface of dextran is very hydrophilic.

In polymer science in general, one is also interested in the phase behavior of polymers and the dependence of the degree of fractionation on the molar mass in particular. This behavior is studied experimentally[5,26] and theoretically[17−23,25,27−30] as well as with use of computer simulations.[25,30] All theoretical models use a mean field approach. However, a simple mean field approach predicts an exponential dependence of the degree of fractionation on the molar mass, where in the experimental case this dependence is not always exponential.[26] Another difference between the mean field predictions and the experimental work is that the intercept at $m = 0$ is expected to be at $c_{x,poor,m}/c_{x,rich,m} = 1$, whereas the experimental results do not show this value of the intercept when the data are extrapolated toward $m = 0$. These contradictions between the theoretical predictions and the results of experimental work show that more research is needed on this subject and that a simple mean field approach is inadequate for the prediction of the degree of fractionation.

4.2. Comparison with the System Gelatin/Dextran. As mentioned in the Introduction, the phase behavior of PEO and dextran was studied to compare it with the phase behavior of gelatin and dextran which we studied before.[2,5] We found that the phase separation between gelatin and dextran is driven by sterical interactions between dissimilar polymers and can be described with use of a depletion theory.[2−4] From the results of the research on the system PEO/dextran, it cannot be concluded that the phase separation mechanism is the same as for the system gelatin/dextran, although there are some similarities. These similarities concern the way the systems react on changing the polymer concentration and changing the molar mass of one of the polymers and the temperature dependence.[2,5]

It appears that differences between the phase behavior of gelatin/dextran and PEO/dextran are caused by the effect of solvent quality. The first difference is that for the phase separation of gelatin and dextran, the temperature has no effect on the composition of the coexisting phases,[2,5] whereas the temperature does affect the composition of the coexisting phases of phase-separated mixtures of PEO and dextran. Comparing the phase volumes of the coexisting phases, it turns out that for the mixture of gelatin and dextran the phase volumes after phase separation are the same for each temperature in the temperature range probed, whereas for the system PEO/dextran the phase volume of the PEO-rich phase decreases with increasing temperature as a result of the decreasing solvent quality of water

Table 4. Overview of the Values for the Degree of Fractionation of Dextran for $m = 1000$ for the System Gelatin/Dextran as Well as the System PEO/Dextran[a]

system	5.0%/5.0%	4.5%/4.5%	4.0%/4.0%	3.5%/3.5%	3.0%/3.0%	2.5%/2.5%
gelatin/dextran[b]	0.04	0.11	0.25			
PEO-100/dextran				0.03	0.08	0.19
PEO-200/dextran				0.02	0.05	0.14

[a] The temperature at which phase separation was established was 60 °C. [b] See ref 5.

Table 5. Overview of the Values for Free Energy ΔG (J mol^{-1}) Involved in Transferring 1 mol of Dextran Molecules of 1000 Monomers from the Enriched to the Depleted Phase for the System Gelatin/Dextran and the System PEO/Dextran[a]

system	5.0%/5.0%	4.5%/4.5%	4.0%/4.0%	3.5%/3.5%	3.0%/3.0%	2.5%/2.5%
gelatin/dextran[b]	10.05×10^3	6.73×10^3	4.85×10^3			
PEO-100/dextran				7.06×10^3	5.01×10^3	2.72×10^3
PEO-200/dextran				8.30×10^3	5.84×10^3	3.93×10^3

[a] The temperature at which phase separation was established was 60 °C. [b] Data from ref 5.

for PEO. This change in the phase volumes might also be the reason the temperature−composition phase diagram of the system PEO/dextran is slightly dependent on the temperature.

The second difference between the phase behavior of the system gelatin/dextran and PEO/dextran which might be caused by the effect of the solvent quality is the dependence of the degree of fractionation on the molar mass. If the degree of fractionation in the system PEO/dextran is considered to be exponential with the molar mass, and the slope at low m is interpreted as the slope in the (hypothetical) absence of solvent quality influence, a comparison can be made between the systems PEO/dextran and gelatin/dextran. The solvent quality dependence on molecular conformation, and therefore on fractionation, is expected to be the strongest for molecules with a higher degree of polymerization. First, consider the degree of fractionation for a dextran molecule consisting of 1000 monomers. These values are summarized in Table 4. It must be taken into account that the various systems are not the same distance from their critical point and that the data of the PEO-containing systems are the result of an extrapolation. Despite the differences between the two types of systems, the degree of fractionation is of the same order of magnitude.

Apart from this, the values for the free energy needed for the transfer of a polymer from its enriched to its depleted phase can be compared (see Table 5). These data are calculated with use of the equations mentioned in ref 5. In essence, the degree of fractionation is interpreted as a Boltzmann factor, with the exponent equal to $\Delta G(m)/RT$, where ΔG is the free energy involved in transferring from the rich phase to the poor phase 1 mol of a polymer with a certain molar mass m. This table shows that the value for the free energy for the system gelatin/dextran is of the same order of magnitude as that for the system PEO/dextran. This similarity between the two systems supports the view that when it is found that the degree of fractionation is exponential in the molar mass, entropy is governing the phase separation. An entropic ΔG of mixing of dextran with a linear polymer is not expected to depend strongly on the chemical structure of the linear polymer.

Summarizing the similarities and differences between the two different systems, it appears that the phase separation of aqueous (bio)polymer mixtures can only be established by increasing the polymer concentration and not by decreasing the temperature. This temperature independence of the phase separation suggests that this phase separation is caused by sterical interactions between the dissimilar polymers. The solvent quality difference does not appear to be the driving force of the phase separation, because if the solvent quality were to influence the mechanism of the phase separation, a stronger influence of the temperature would be expected. In this case also larger differences would be expected between the values of ΔG of the systems containing PEO or gelatin.

5. Conclusions

From the temperature dependence of the cloud points, it can be concluded that the phase separation between PEO and dextran below 80 °C is mainly caused by sterical interactions between dissimilar polymers. Due to the fact that, with increasing temperature, the solvent quality of water for PEO decreases, the phase volume of the PEO-rich phase decreases with increasing temperature. This decreasing phase volume of the PEO-rich phase affects the concentration of PEO as well as dextran in the coexisting phases.

Summarizing the similarities and differences between the phase behaviors of the system PEO/dextran and another well studied system, gelatin/dextran, it can be concluded that for both systems the main driving force is of entropic nature. It is supposed that this can be interpreted as the phase separation being the result of the sterical repulsion between the disparate polymer chains. However, the equilibrium state of the PEO/dextran mixture is also influenced by the solvent quality of water for PEO. With respect to the mass fractionation of the polymers, it appears that for both systems the effects of the molar mass and the concentration are the same. There are differences as well between the systems with respect to the degree of fractionation. For the system gelatin/dextran the degree of fractionation shows an exponential dependence on the molar mass for both gelatin and dextran, whereas this dependence in the system PEO/dextran shows a deviation from exponentiality for dextran at high molar mass. The degree of fractionation of dextran as well as the free energy of transfer of a dextran molecule from the enriched to the depleted phase is of the same order of magnitude for both systems.

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