Phase Behavior in Aqueous Two-Phase Systems Containing Micelle-Forming Block Copolymers

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ABSTRACT: The phase behavior of the three-component PEO–PPO–PEO/dextran/water system has been studied. Pluronic L64, P105, and F68 were used, and they are members of a family of triblock copolymers with the structure PEO–PPO–PEO, where PEO is poly(ethylene oxide) and PPO poly(propylene oxide). Moreover, these triblock copolymers tend to self-aggregate in aqueous solution at increasing temperature. The phase behavior of the three-component system was studied both experimentally and theoretically at different temperatures corresponding to the unimer (monomolecular) and the micellar region of the PEO–PPO–PEO polymers. The determination of the unimer and micellar regions was performed with a dye solubilization technique using 1,6-diphenyl-1,3,5-hexatriene (DPH) as a spectroscopic probe. A strong temperature effect on the phase behavior in PEO–PPO–PEO/dextran/water systems was observed in the case of Pluronic L64 and P105. At elevated temperature (40 °C) the micellization of the PEO–PPO–PEO polymers leads to a drastic change of the tie-line slopes as well as the shape of the binodals. Qualitative agreements were found between experimental findings and theoretical calculations using a Flory–Huggins lattice theory extended with internal degrees of freedom by modeling the PEO–PPO–PEO polymers as a poly(ethylene oxide) chain. This supports the prevailing picture that the more hydrophobic PPO block is shielded from water contacts by forming hydrophobic domains surrounded by EO segments.

Introduction

Partitioning in aqueous polymer two-phase systems is an important separation technique in biochemistry and biotechnology. The system that has been most often used in laboratory-scale separations is composed of the polymers dextran and poly(ethylene oxide) (PEO) in water (PEO will here be considered as equivalent to poly(ethylene glycol) (PEG)). The PEO/dextran/water system has been used for separation of macromolecules, cell particles, membrane fractions, and whole cells.[1,2] The phase separation between the two polymers is segregative, with PEO enriched in the upper phase and dextran in the lower. The partitioning of macromolecules and particles between the two polymer phases can be influenced in several ways, e.g., by changing polymer molecular weight or concentration, adding salts, or changing the pH.[2,3] By using hydrophobic groups bound to one of the polymers, the partitioning of hydrophobic molecules can be influenced.[4] Biospecific ligands can be used for affinity partition.[5]

Recently, the use of ethylene oxide (EO)–propylene oxide (PO) random copolymers in aqueous two-phase systems was introduced.[6] The EO–PO random copolymers were used in two-phase systems together with dextran or hydroxypropyl starch for protein[6–8] and steroid[9] purification. The decreased solubility of these polymers on temperature increase was utilized for temperature-induced phase separation. With EO–PO random copolymers in the upper phase instead of PEG, it was possible after phase separation to remove the upper phase and by a temperature increase above the copolymer cloud point to obtain a new phase system with a water upper phase and a polymer lower phase. With this temperature-induced phase separation it was

possible to obtain the target protein in the water phase essentially free from polymer.[6,8]

In this work we have investigated aqueous two-phase systems containing triblock copolymers of poly(ethylene oxide) and poly(propylene oxide) (PPO) with the structure PEO–PPO–PEO, also referred to as Pluronic (trademark of BASF Corp.). The triblock copolymers have been studied in phase systems together with dextran; i.e., the block copolymers have replaced PEG in the traditional two-phase system. Just as for the random EO–PO copolymers, it is the effect of temperature on the solution behavior of the polymers that is of primary interest. PEO–PPO–PEO block copolymers are known to self-associate in a water solution at increased temperature.[10–12] In particular, Pluronic polymers with different PEO and PPO block lengths have been shown to self-aggregate in the form of micelles with a core dominated by PPO blocks and a corona of hydrated swollen PEO chains.[10–20] The micellization is temperature dependent, and critical micellization temperatures have been determined for a range of Pluronic polymers.[21]

The use of micelle-forming polymers in aqueous polymer two-phase systems has great potential. The partitioning could be improved by regulating the environment of the target object by controlling the micellar size and hydrophobic character. Drastic changes in the partitioning of added substances in two-phase systems containing Pluronic polymers can thus be expected by changing the temperature. Another potential advantage is that the separation of the target object from the phase polymers may be facilitated by dissolving the Pluronic micelle by a temperature reduction. Solubilization of aromatic compounds by micelle-forming block copolymers has been studied.[22,23]

We have determined phase diagrams for Pluronic/dextran/water systems at different temperatures covering the unimeric and micellar state of the Pluronic polymers and found large differences in the phase diagrams whether the Pluronic polymers are in their

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Table 1. Composition of Pluronic Block Copolymers Used

polymer	mol wt[a]	PPO segment wt[a]	no. of EO units	no. of PO units
L64	2900	1740	2 × 13	30
F68	8400	1680	2 × 76	29
P105	6500	3250	2 × 37	56

 a Data from the manufacturer.[27]

unimeric or micellar state. By use of a spectroscopic probe the micellization of the Pluronic polymers was be proved. Model calculations have been made using a theoretical model based on the Flory–Huggins lattice theory[24] extended with internal degrees of freedom.[25] In order to realistically model EO-containing polymers, it is necessary to have temperature-dependent segment–segment interaction parameters.[26] The model calculations gave a qualitative description of the mechanisms behind the strong effects observed in the phase diagrams upon temperature change.

Experimental Section

Materials. Pluronic L64, P105, and F68 were obtained from BASF Corp., Parsippany, NJ, and used without further purification. Table 1 gives their composition according to the manufacturer.[27] No polydispersity or impurity check was performed on these block copolymers. Dextran T500 with molecular weight 500 000 was purchased from Pharmacia Bio Process Technology, AB, Uppsala, Sweden, and used as received. The spectroscopic probe used to follow the micellization process was 1,6-diphenyl-1,3,5-hexatriene (DPH) obtained from Sigma Chemical Co. and was used as received. DPH gives rise to an absorbance peak at 356 nm when in hydrophobic surroundings. Millipore water was used in all experimental preparations.

Determination of Phase Diagrams. Ternary phase diagrams of the Pluronic/dextran/water system were determined in two temperature regimes: below and above the critical micelle temperature (cmt). The lower temperature (5 °C) was achieved in an medical refrigerator with an accuracy of ±0.5 °C, while the higher temperature (40–50 °C) was obtained by using a Julabo UC water bath with an accuracy of ±0.1 °C. The prepared samples were left to phase separate overnight, but approximately 1 h was often enough to get two macroscopic well-defined phases.

The polymer concentrations in the separated phases were determined in two steps. First, after separating the two phases from each other and after suitable dilution, the dextran concentration in both phases was determined by polarimetry (optical rotation). The polarimetric measurements were performed by a digital polarimeter (Model DIP-360) from Jasco International Co., Ltd., Japan, equipped with water-thermostated sample cells connected to a Haake F3 water circulator. All polarimetric measurements were performed at 25 ± 0.1 °C. Second, the concentration of the Pluronic polymer in the phases was after suitable dilution then determined by measuring the total refractive index of the solutions and then subtracting the refractive index of dextran. To do this, standard curves were established for aqueous solutions of Pluronic L64, P105, and F68 and dextran T500. The validity of refractive index additivity was also confirmed. A Model 2142 differential refractometer from LKB, Bromma, Sweden, was used for the refractive index measurements.

Spectroscopic Measurements. To ensure that the phase diagrams were established in the unimeric and the micellar regions, respectively, a dye solubilization technique was applied. As the spectroscopic probe, the well-studied 1,6-diphenyl-1,3,5-hexatriene (DPH) was chosen. DPH has been used in fluorescence as well as in UV–vis studies of micelle processes.[21,28] The DPH molecule possesses nonpolar properties, can easily be solubilized in the hydrophobic interior of a Pluronic micelle, and shows a characteristic absorbance spectrum with maximum absorbance at 356 nm. Spectra are
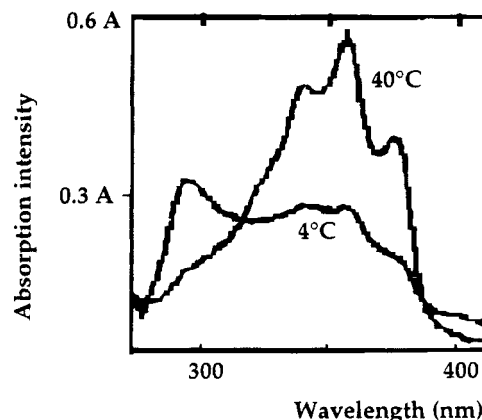

Figure 1. Absorbance spectra in the UV–vis region of a 10 wt % P105 Pluronic block copolymer solution containing DPH as a spectroscopic probe. The spectra are obtained at two different temperatures, 4 and 40 °C, which represent two extremes, the unimer and micellar regions, respectively. When increasing the temperature, the DPH probe gives rise to a maximum peak at 356 nm caused by the micellization of the Pluronic block copolymers.

shown in Figure 1. To dissolve a detectable amount of DPH into the solution (DPH is almost insoluble in pure water), a few DPH crystals were shaken overnight in a 10 wt % aqueous solution of Pluronic P105 block copolymer. The absorbance measurements of DPH dissolved in Pluronic solution at different temperatures (4–41 °C) were performed with a thermostated spectrophotometer from Shimadzu, Japan, Model UV-160A. The temperature was measured directly in the cell, with an accuracy of 0.1 °C, because of the temperature difference (ca. 5 °C) between the water bath and the cell. The sample cell was connected to a Haake DC5 water bath, and by using an ethanol/water mixture as the circulating liquid, low temperature was achieved in the sample cell.

Theoretical Modeling

The Flory–Huggins lattice theory,[24] extended with internal degrees of freedom of the ethylene oxide (EO) groups,[25] was used to model the phase behavior. This extension provides a possibility to model effective segment–segment interaction parameters which are temperature as well as concentration dependent. The physical arguments behind this theory originate from the distribution of conformations of the −OCCO− segments. Since the conformers have different dipole moments, their population will depend on the polarity of neighboring molecules. In the model, this is captured by introducing two conformational classes of the −OCCO− segment, one class being polar and having a lower energy and one being less polar, or nonpolar, and having higher statistical weight. At low temperatures, the former class (or state) dominates and the interaction between the polymer and solvent is favorable, whereas at higher temperatures, the latter state dominates, resulting in an unfavorable polymer−solvent interaction.[25]

The basis of the phase diagram calculations is the expression for the mixing free energy. For a multicomponent system consisting of solvent(s) and polymer(s) with internal states, the Helmholtz free energy of mixing becomes[29,30]

$$\beta(A - A^*) = \beta(A_{\mathrm{int}} - A_{\mathrm{int}}^*) - \ln\frac{\Omega}{\Omega^*} + \beta(U - U^*) \quad (1)$$

where A_{int} and U represent the internal free energy and configurational energy in the mixed system, respec-

Table 2. Internal State Parameters U_{AB} (Energy) and g_{AB} (Statistical Weight) as Well as Flory–Huggins Interaction Parameters ($\chi_{BB'}$) (Energy in kJ mol⁻¹)

species	state	U_{AB}	g_{AB}
water		0	1
EO	polar	0	1
EO	nonpolar	5.086[a]	8[a]
dextran		0	1

		$RT\chi_{BB'}$	
state	EO, polar	EO, nonpolar	dextran
water	0.6508[a]	5.568[a]	1.096[b]
EO, polar		1.266[a]	0.360[b]
EO, nonpolar			1.542[b]

[a] From the fit to the experimental PEO/water phase diagram.[25]
[b] From the fit to the experimental PEO/dextran/water phase diagram.[40]

tively, while $\ln(\Omega/\Omega^*)$ is the mixing conformational entropy divided by the Boltzmann constant, and $\beta = 1/kT$, with k being the Boltzmann constant and T the absolute temperature. Starred quantities refer to the reference system where the components are in pure amorphous states. The internal free energy, the mixing configurational entropy, and the interaction energy are given by[29,30]

$$\beta A_{int} = \sum_x \sum_A n_x r_{Ax} \sum_B P_{AB} \left[\beta U_{AB} + \ln \frac{P_{AB}}{g_{AB}} \right]$$

$$\ln \frac{\Omega}{\Omega^*} = -\sum_x n_x \ln \frac{n_x r_x}{L} \tag{2}$$

$$\beta U = \frac{1}{2} \sum_x \sum_A \sum_{A'} \sum_B \sum_{B'} n_x r_{Ax} P_{AB} \chi_{BB'} P_{A'B'} \phi_{A'}$$

Here r_{Ax} denotes the number of segments of type A in component x, r_x the total number of segments in component x, n_x the total number of molecules of type x, L the total number of lattice sites, ϕ_A the volume fraction of species A, and $\chi_{BB'}$ the Flory–Huggins interaction parameter between species A in state B and species A' in state B'. Moreover, P_{AB} is the fraction of species A in state B, U_{AB} the zero-energy level of species A in state B, and g_{AB} the degeneration of species A in state B. At equilibrium P_{AB} is given by[29,30]

$$P_{AB} = \frac{X_{AB}}{\sum_B X_{AB}}$$

$$X_{AB} = g_{AB} \exp[-\beta U_{AB} - \sum_{A'} \sum_{B'} \chi_{BB'} P_{A'B'} \phi_{A'}] \tag{3}$$

We can now determine the equilibrium distribution $\{n_x{}^\alpha\}$ of given total amounts of the components in the system $\{n_x\}$ over the coexisting phases $\{\alpha\}$ as the one that minimizes the total free energy, $A^{tot} = \sum_\alpha A^\alpha(\{n_x{}^\alpha\})$, under the restriction $\sum_B (P_{AB})^\alpha = 1$ for all species A in each phase α, where A^α is given by eqs 1 and 2. By selecting different $\{n_x\}$, the binodal as well as the tie lines in a phase diagram can be calculated. The values of the parameters U_{AB}, g_{AB}, and $\chi_{BB'}$ were taken from
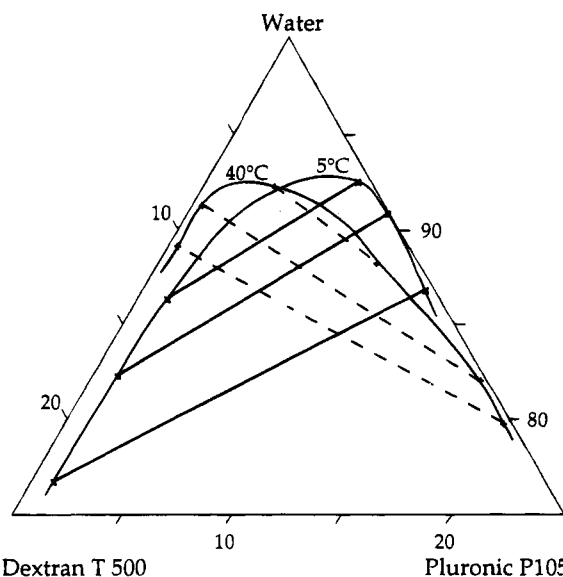
Figure 2. Experimental phase diagram for the Pluronic P105/dextran T500/water system at 5 and 40 °C with tie lines. Concentration is in weight percent.
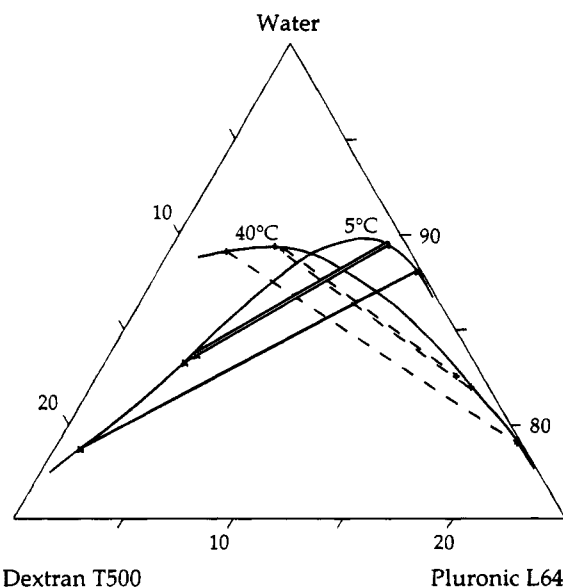
Figure 3. Experimental phase diagram for the Pluronic L64/dextran T500/water system at 5 and 40 °C with tie lines. Concentration is in weight percent.

previous investigations and are given in Table 2. Thus, no interaction parameters were adjusted in this study.

Results and Discussion

Ternary Phase Diagrams. Three phase diagrams of aqueous three-component systems containing dextran T500 as one polymer component and a Pluronic block copolymer as the other polymer component are shown in Figures 2–4. As mentioned above, the Pluronic block copolymers used were L64, P105, and F68. In all three cases phase separation occurred at sufficiently high polymer concentration. Two isotropic phases were obtained; each phase contained a high concentration of one of the polymers, characteristic of a segregative phase separation. The lower phase was dextran enriched, and the upper phase was enriched with Pluronic block copolymer. Tie lines between the two phases in equilibrium are given at two (three for the Pluronic F68 phase diagram) different temperatures. The experi-
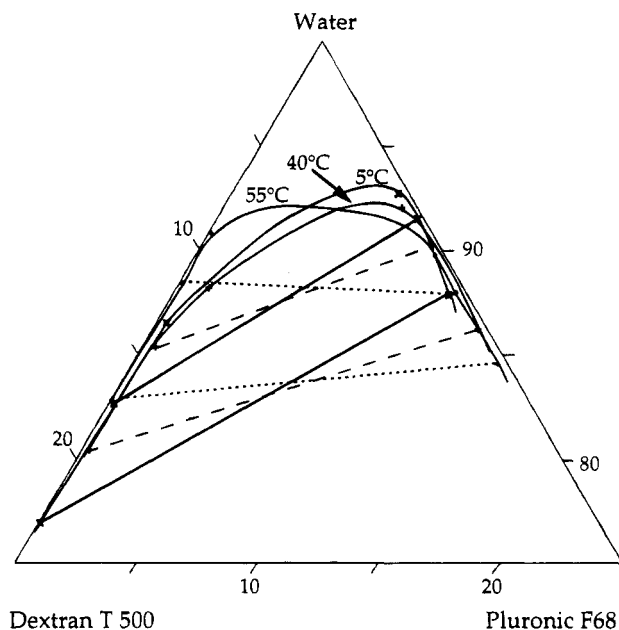
Figure 4. Experimental phase diagram for the Pluronic F68/dextran T500/water system at 5 (solid tie lines), 40 (dashed tie lines), and 55 °C (dotted tie lines). Concentration is in weight percent.
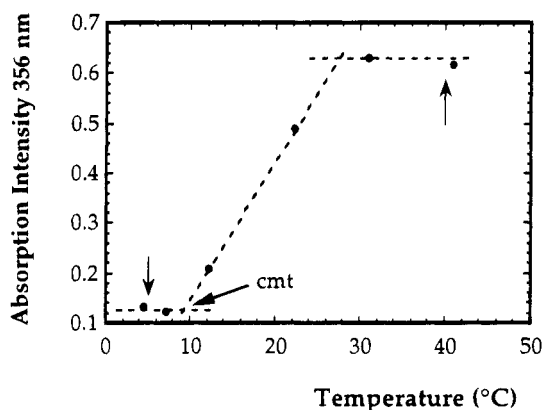

Figure 5. Absorption intensity at 356 nm at various temperatures of a 10 wt % aqueous solution of Pluronic P105 block copolymer containing a DPH probe. The vertical arrows show the temperatures 5 and 40 °C at which the phase diagrams were measured. The location of the cmt is also indicated.

mental phase diagrams at the lower temperature, 5 °C, resemble those for the PEO/dextran/water system, whereas the distribution of water is the opposite at the elevated temperature, i.e., more water in the dextran phase.

Before interpreting the phase diagram results, it is important to confirm that the applied temperatures are correctly chosen. According to data from a detailed cmt and cmc investigation of 12 different Pluronic block copolymers (including the three Pluronic polymers used in this work) performed by dye solubilization using DPH as the spectroscopic probe,[21] a temperature of 40 °C should be sufficiently high at the present polymer concentrations to induce self-association of the Pluronic polymers into micelles. To confirm that the Pluronic-rich phases obtained at 5 and 40 °C mainly contain Pluronic polymers in the unimer and micellar state, respectively, the same dye solubilization technique was used. Figure 5 shows the maximum absorbance peak for DPH in hydrophobic surroundings (356 nm) as a function of temperature for a 10 wt % P105 solution (a

typical Pluronic polymer concentration in the Pluronic-rich phase; cf. Figure 2). Arrows in Figure 5 show the positions of 5 and 40 °C, the temperatures at which the phase diagrams were determined. The temperature at the first break of the absorption intensity has been identified as the critical micelle temperature,[21] and for this Pluronic P105 concentration we estimate the cmt to be 9 °C. As shown in Figure 5 and frequently reported, the temperature transition range of the micellization of Pluronic polymers is broad, ca. 15 °C.

Our obtained cmt of 9 °C at 10 wt % is in agreement with the decreasing trend from 30 to 20 °C at 0.025–2.2 w/v % found by Alexandridis et al. using the same method.[21] Thus, from the absorbance measurements given in Figure 5, we conclude that Pluronic P105 polymers in the Pluronic-rich phase at 5 °C mainly exist in a unimeric state and at 40 °C they are self-associated into micelles. The cmt's of the aqueous solutions of Pluronic L64 and F68 at 10 wt % are 23 and 33 °C, respectively,[21] and we can thus expect these Pluronic polymers to be in a unimeric state at 5 °C and micellized at 40 °C, although the micellization of Pluronic F68 might not be completed considering the broad transition range.

The origin of the micellization process for Pluronic block copolymer is general and well-known.[12,16,20,31–33] When increasing the temperature, the solubility of PO (and also EO) in water decreases. Above some temperature, the cmt, the free energy of the system is reduced by forming polymer micelles consisting of an essentially hydrophobic PO core surrounded by hydrated EO segments, as compared to single polymer chains in solution. In the unimeric state, the PO segments are likely to also be shielded by the EO segments, but less efficiently as compared to the micellar state (see discussion below).

Similar temperature-dependent micellization is observed for an aqueous solution of nonionic surfactants containing EO groups.[34] Moreover, at sufficiently high temperature, both aqueous solutions of Pluronic polymers and nonionic surfactants as well as aqueous solutions of nonmicellar-forming EO-containing polymers as PEO and EO–PO random copolymer phase separate into two phases, one containing almost only water and the other phase enriched in the chain molecule. This phase separation temperature is known as the cloud point temperature and depends on the molecular structure of a polymer (or surfactant) and (only slightly) on the concentration.

Returning to the phase diagrams in Figures 2–4, the temperature has a very strong effect on the composition of the two phases in the two-phase region. The effect is more pronounced in systems containing Pluronic L64 and P105 than for the Pluronic F68 containing system. We will return to this difference below. From the changes of the direction of the tie lines, we infer that the temperature increase from 5 to 40 °C promotes a transfer of water from the Pluronic-rich phase to the dextran-rich phase. At both temperatures we have a strong segregative phase separation; i.e., the two kinds of polymers tend to distribute themselves in different phases. For example, Figure 2 shows that for a system with an overall composition of (7, 7, 86) (% Pluronic, % dextran, % water), the composition of the Pluronic and dextran phases becomes at 5 °C (8, <1, 92) and (1, 17, 83) respectively, and at 40 °C (18, <1, 82) and (<1, 8, 91), respectively.

The presence of dextran in the aqueous Pluronic polymer solution should probably reduce the cmt of the

Pluronic polymer as compared to the case of no dextran present. We have not investigated this effect. But we anticipate it to be unimportant in the Pluronic-rich phase, since the Pluronic polymer concentration exceeds the dextran concentration by more than 1 order of magnitude. In the dextran-rich phase, however, the effect should be larger. But at 5 °C, we still believe that we have essentially unimers, since the Pluronic polymer concentration is far below the estimated cmc (if it at all forms micelles) at that temperature and the effect of dextran should not be sufficient.

Qualitatively, the drastic change in the phase diagram with temperature is in agreement with the Flory—Huggins theory, if the Pluronic micelles at 40 °C are modeled as a large single polymer. The tie lines and the shape of the binodal curve change in the way that is expected from an increase of the molecular weight.[35,36] However, the water concentration at the critical point does not increase as the temperature is increased. According to the Flory—Huggins theory, an increased degree of polymerization leads to a lower polymer concentration needed to cause phase separation, due to a smaller entropic gain in mixing the two polymers.[37,38] This discrepancy between Flory—Huggins theory and the experimental findings in this case can be found in the fact that a micellization process cannot strictly be treated as a polymerization process (see further below).

For the ternary system with Pluronic F68, the temperature increase from 5 to 40 °C causes only minor changes of the phase diagram (Figure 4). However, a further increase to 55 °C (i) shifts the binodal curve in a similar way and (ii) alters the direction of the tie lines, but to a lesser extent, as for the other two systems at 40 °C. The more hydrophilic character of Pluronic F68 leads to the largest cmt of the three Pluronic polymers considered, 33 °C at 10 wt %, and the small difference of the phase diagrams between 5 and 40 °C is likely due to incomplete micelle formation. We recall that the cmt denotes the temperature where micelles start to form, whereas the phase equilibrium is more likely to be controlled by the fraction of polymers being micellized. According to Figure 5, full impact of the micellization on the phase diagram is not expected before 15—20 °C above the cmc, in reasonable agreement with the observation given in Figure 4. The smaller change of the direction of the tie lines observed for the Pluronic F68 system at 55 °C could be attributed to its lower PO content (see Table 1) and/or to the temperature not being sufficiently high.

Thus, we have experimentally found that the ternary phase diagrams of Pluronic/dextran/water at 5 °C resemble those for the PEO/dextran/water system, but as the temperature is increased, (i) the phase diagrams undergo drastic changes and (ii) unimeric Pluronic polymers start to self-associate into micelles. In the following subsection, we will by model calculations strengthen our arguments that it is the micellization which causes the changes in the phase diagrams.

Model Calculations. Phase diagrams of the Pluronic/dextran/water system have been calculated at different temperatures using the previously presented Flory—Huggins mean-field lattice theory extended with a model for polymers possessing internal states.

Earlier binary phase diagram calculations involving EO- and PO-containing polymers such as Pluronic polymers and EO—PO random copolymers showed that these polymers could be modeled as EO- and PO-containing copolymers using interaction parameters for
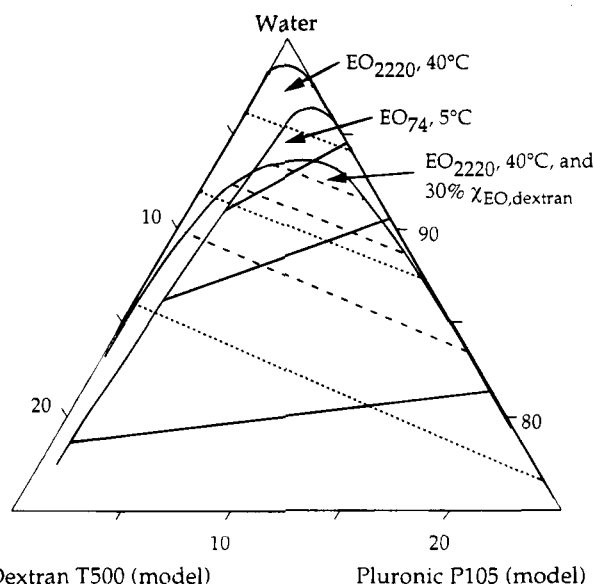

Figure 6. Calculated phase diagram for the Pluronic P105/dextran T500/water system at 5 and 40 °C with tie lines. Pluronic P105 was represented at 5 °C (unimeric state) by $(EO)_{74}$ and at 40 °C (micellar state) by $(EO)_{2220}$, and dextran T500, by a homopolymer with 3080 segments. Interaction parameters are according to Table 2 (solid and dotted tie lines). Results obtained with reduced EO—dextran interaction parameters (see text) are also given (dashed tie lines). Concentration is in volume percent.

EO and PO segments established from simpler systems such as aqueous solutions of corresponding homopolymers.[25,26,39]

The similar approach of modeling the Pluronic polymer in its unimeric state, i.e., as $(EO)_x(PO)_y(EO)_x$, gave different slopes of the tie lines and a too large two-phase region (data not shown) as compared to the experimental findings at 5 °C. These calculated results at 5 °C displayed a phase behavior similar to the experimental high-temperature behavior; e.g., the highest water content occurred in the dextran-rich phase. This implies that the model of the unimeric Pluronic polymer is too hydrophobic. One obvious explanation is that the more hydrophobic PO segments have too much water contact in the homogeneous system with a mean-field description as compared to reality. Thus, we have indirect evidences that PO segments of the Pluronic polymers are partly shielded from water in its unimeric state.

In order to retain the simple description by using a homogeneous system with the Flory—Huggins theory with its random-mixing approximation and gain some further insight, we have modeled the Pluronic polymer as $(EO)_{2x}$, i.e., $y = 0$ and ignoring the existence of the PPO block. Of course, some value of $y > 0$ would have been more appropriate, but we have refrained from such fitting of y. By this drastic simplification of the Pluronic polymer in the unimer state, the Pluronic/dextran/water system now becomes the same as the frequently studied PEO/dextran/water system.[1,2]

Figure 6 (solid tie lines) shows results from such a calculation for the Pluronic P105/dextran/water system at 5 °C. The Pluronic polymer is modeled as a homopolymer with 74 EO units, i.e., $x = 37$ which is the number of EO units in a PEO block of Pluronic P105. The dextran molecule with molecular weight 500 000 was in all calculations modeled as a linear homopolymer with 3080 monomer units. Note that all concentrations in the calculated phase diagrams are in volume percent-
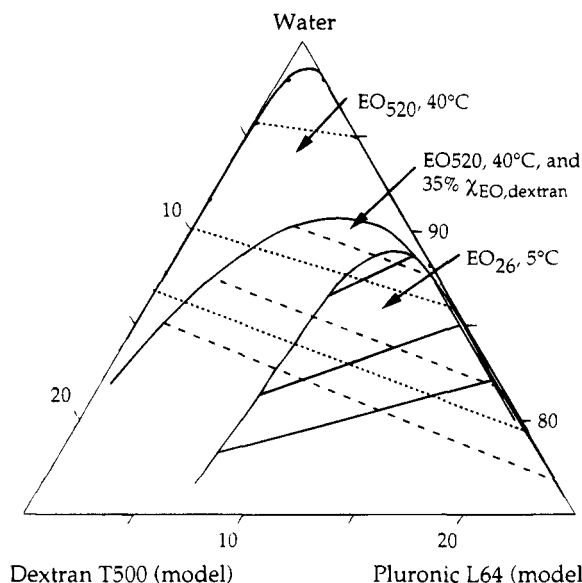
Figure 7. Calculated phase diagram for the Pluronic L64/dextran T500/water system at 5 and 40 °C with tie lines. Pluronic L64 was represented at 5 °C (unimeric state) by $(EO)_{26}$ and at 40 °C (micellar state) by $(EO)_{520}$, and dextran T500, by a homopolymer with 3080 segments. Interaction parameters are according to Table 2 (solid and dotted tie lines). Results obtained with reduced EO−dextran interaction parameters (see text) are also given (dashed tie lines). Concentration is in volume percent.

age, whereas weight percentage is used in the experimental phase diagrams. This difference is not important when comparisons are made in a qualitative way between experimental findings and model calculations. The comparison between the results from the model calculations in Figure 6 and the experimental data at 5 °C in Figure 2 gives a very good agreement with respect to both the shape of the binodal curves and the slope of the tie lines. In both cases the two-phase region is shifted toward the Pluronic/water axis and the water content is largest in the Pluronic phase.

Regarding the modeling of the micellar state, we have used the same simple approach as for the unimeric state. Thus, the shielding of the PO segments from water and dextran by forming a hydrophobic micellar core is achieved by using a PEO homopolymer in the random-mixing model system. As discussed earlier, the micellization as such was represented by multiplying the number of segments with the aggregation number, N_{agg}. For Pluronic P105, $N_{agg} = 30$ was used. Figure 6 (dotted tie lines) also shows the results of such a model calculation of the Pluronic P105/dextran/water system at 40 °C. The main location of the binodal and the directions of the tie lines are in good agreement with those experimentally found; cf. Figure 2. However, the calculated critical point occurs at a too high water content as compared with experimental results (which was also the case at 5 °C but less prominent).

To ensure that the temperature increase by itself, without any self-association, does not cause the drastic change on the tie lines, a calculation at higher temperature with 74 EO groups was performed (result not shown). This resulted only in small change of the tie lines, as compared to the results at 5 °C, which amplifies the statement that the micellization process as such, which of course is a result of the temperature increase, causes the drastic changes of the phase diagrams.

In Figure 7 (solid tie lines) results from similar calculations for the Pluronic L64/dextran/water system

at 5 °C are displayed. Qualitatively, the same phase separation behavior occurs as experimentally found at the same temperature; i.e., the Pluronic-rich phase has a higher water content than the dextran-rich phase, and the binodal merges with the Pluronic/water axis. Still, some differences remain. For example, when decreasing the total water concentration in the system, we do not achieve the strong segregation effect observed in Figure 3. One possible explanation for this disagreement is that the Pluronic L64 polymer is too short to hide its PO segments from water and dextran contacts. Since the PO−dextran interaction is more repulsive than the EO−dextran interactions,[40] an inclusion of some PO−dextran contacts would lead to strong segregation.

Figure 7 (dotted tie lines) shows the phase diagram of Pluronic L64/dextran/water at 40 °C where the Pluronic L64 micelles are modeled in the same way as in the case of Pluronic P105 but with an aggregation number $N_{agg} = 20$.[32] Again, the two-phase region is too large as compared with the experimental one given in Figure 3. Nevertheless, the slopes of the tie lines agree with those experimentally determined, indicating that also the micellization of L64 can be reasonably modeled by this simple approach.

Thus, we have obtained fair agreements between the experimental and calculated phase diagrams at 5 °C, but the calculated two-phase regions at 40 °C are too large. This finding is consistent with the basic assumptions of the Flory−Huggins theory and the presence of self-aggregated polymers at the higher temperature. The reason is that the random-mixing assumption in the Flory−Huggins theory implies that there are no spatial polymer density correlations. But when micelles are formed, we obviously have large density correlations. Besides that the PO segments form the core of the micelles, EO segments form an outer layer of the micelles, and hence a substantial amount of the EO groups are shielded from having dextran contacts. The effective EO concentration felt by the dextran polymers is hence reduced, making the extension of the two phase region less developed. Hence, the treatment of the self-aggregation as a polymerization process in the Flory−Huggins framework leads to an overcount of exposed polymer segments.

A simplistic way to improve the description, still using the Flory−Huggins theory, is to reduce the interaction parameters between dextran and two EO states, since the enthalpic contribution from the interactions between the two polymers takes the form $\phi_{EO}\phi_{dextran}\chi_{EO,dextran}$, where $\chi_{EO,dextran}$ represents the EO−dextran interaction parameter averaged over the EO states. A reduction of $\chi_{EO(polar),dextran}$ and $\chi_{EO(nonpolar),dextran}$ (same for both) by ca. $^2/_3$ gave good agreement between the calculated phase diagrams shown in Figures 6 and 7 (dashed tie lines) and the experimental ones in Figures 2 and 3. The use of the reduced interaction parameters leads to a higher total polymer concentration necessary to induce phase separation, thus improving the position of the critical point of the binodal curve. Thus, the calculations indicate that only ca. one-third of the EO segments have full random-mixing interaction with dextran segments. (In this discussion we have neglected the reduced number of EO−water contacts.)

We have previously calculated the radial EO and PO density profiles of micelles formed by Pluronic triblock copolymers in an aqueous solution by using a lattice model for heterogeneous systems.[19] The center of the micelle was found to consist of mainly PO segments,

whereas the outer layer (corona) was composed of EO and water, the water content increasing with the radius (cf. Figure 11 of ref 19). It is reasonable that a large fraction of the EO segments are shielded from dextran contact. The dextran segments predominantly interact with those EO groups localized in the outer part of the micelle corona, whereas further in where the concentration of EO groups becomes higher, the dextran concentration is expected to be small. A first estimate of the local dextran concentration and hence the shielding could be obtained by using the local EO concentration and the PEO/dextran/water phase diagram.

Finally, the effect of using two polymers with large differences in the degree of polymerization, often called a asymmetric polymer system, is clearly illustrated in Figure 7 (5 °C). In such systems, the two-phase area is shifted to the low molecular weight polymer/solvent axis, due to the fact that only a small concentration of the high molecular weight polymer is enough to induce phase separation.[35,36]

Conclusions

Phase diagrams have been determined for the PEO–PPO–PEO/dextran T500/water system at two temperature regions corresponding to the unimer (monomolecular) and micellar state of the Pluronic block copolymers for three different Pluronic polymers. The slopes of the tie lines change in a drastic way with increasing temperature, indicating that the micellization process has a strong effect on the phase behavior. Qualitative agreement was obtained between experimental findings and theoretical calculations using a Flory–Huggins lattice model extended to internal degrees of freedom of EO groups. It was found that consistent results were obtained in both the unimeric and micellar states, provided the Pluronic polymers were modeled as PEO homopolymers rather than PEO–PPO–PEO block copolymers. This implies that the PO segments have a restricted contact with water and dextran, also in the unimeric state. It was also concluded from the calculations that it is mainly the micellization process, triggered by the temperature increase, that causes the drastic changes of the slope of the tie lines and not the temperature increase as such.

The use of self-associating polymers in aqueous solution makes it possible to improve the partitioning of hydrophobic proteins due to the creation of a hydrophobic region. In the case where the self-association is strongly temperature dependent, as in the case of Pluronic polymers, temperature variation should be a convenient way of controlling the partitioning as well as improving the separation of the phase polymer from the target protein. Such investigations are currently in progress.

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