Effect of electrolytes and surfactants on the thermoseparation of an ethylene oxide–propylene oxide random copolymer in aqueous solution

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Abstract

The thermoseparation of aqueous solutions of Breox 50 A 1000, an ethylene oxide–propylene oxide 50:50 (w/w) random copolymer, was studied. The cloud-point diagram for Breox in water solution and the effects of electrolytes and surfactants on the cloud-point temperature (CPT) were determined. The Breox concentration in both phases after the thermoseparation was followed with a reversed-phase HPLC method. The effects of separation temperature and additives on phase composition were evaluated. © 1998 Elsevier Science B.V. All rights reserved.

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1. Introduction

The thermoseparating random (statistical) copolymers of ethylene oxide (EO) and propylene oxide (PO), are of increasing interest especially for separation of biomolecules in aqueous two-phase systems [1–7]. These polymer solutions demix into two macroscopic phases when heated above a certain temperature. One of the phases is enriched in the polymer and the other is depleted [1,8].

The EOPO copolymers can form two-phase systems with other polymers, like Reppal or dextran, and salts [1–4,9], suitable for extraction of sensitive biomolecules or particles, such as proteins or membranes. One of the main advantages of using these polymers in aqueous two-phase extraction, is the possibility, after a separation step where the target biomolecule is partitioned to the EOPO rich phase, to heat this phase above the CPT, enabling both polymer recycling and its removal from the target product solution, without any costly separation methods.

Cosolutes, such as salts or surfactants, are often added to enhance the partition of biomolecules in aqueous two-phase extraction. The aim is to take advantage of their strong partition into one of the phases and their ability to exhibit specific interactions, like electrostatic interactions and hydrophobic attractive forces, with the target biomolecule [4,10–12].

The CPT of a thermoseparating polymer/water solution can be altered by the presence of a third component [1,5,7,13–19], which may be often the case in the two-phase system extraction.

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In this work, we studied the thermoseparation of the Breox 50 A 1000 copolymer (EO/PO 50/50% w/w), in the absence and presence of salts and surfactants. The temperature of the thermoseparation can be a limiting factor as, for instance, enzymes are usually thermally deactivated. We have determined the importance of the separation temperature and the effect of the different kinds of salts and surfactants, on the Breox concentration in both phases after phase separation.

2. Experimental

2.1. Polymer and chemicals

Breox 50 A 1000 is a random copolymer consisting of 50% ethylene oxide and 50% propylene oxide (% weight). Its molecular mass (number average) is 3900 Da and it was obtained from International Speciality Chemicals (Southampton, UK).

The salts were of analytical-reagent grade and were purchased from Merck (Darmstadt, Germany). The detergents were from Sigma (St. Louis, MO, USA). Tetrahydrofuran was Chromasolv Riedel-de Haën (Seelze, Germany). Distilled water was used in the preparation of aqueous solutions.

2.2. System preparation

The systems were prepared by weighing the compounds from stock solutions of 50% (w/w) Breox in water, 60% (w/w) Tween 20 (sorbitan monolaurate polysorbate 20), 0.5 M CTAB (cetyltrimethylammoniumbromide) and 0.5 M SDS (sodium dodecyl sulphate) to a total weight of 10 g. After vortex mixing, the phase separation at 50 and 60°C was accomplished by allowing the systems to stand in a water bath for 1 h.

The volumes of the upper and lower phases were read after phase separation, and a sample of each phase was taken with a pasteur pipette. The bottom phases were diluted 5 times to fit into the range of the calibration curve for the HPLC analysis. Two equal systems were prepared for each set of conditions.

Four equal systems composed of only Breox and water of 2 gram total weight were prepared in Eppendorf vials. After phase separation at 60°C, the systems were centrifuged at 6000 g for 3 min. The bottom phase was collected by piercing a hole at the bottom of the Eppendorf and tapping a fraction of the bottom phase into another tube.

2.3. Measurement of cloud-point temperature

The cloud-point measurements were performed in a thermostable bath by immersing the polymer solution in a capped glassed tube, and the temperature was gradually increased by 1°C steps. The CPT was taken at the first sign of turbidity.

2.4. Phase composition determination

A reversed-phase chromatographic method was used for the Breox quantification in both phases [9].

The HPLC system consisted of a combined Merck-Hitachi model L-7100 pump, a L-7200 autosampler with a 100-μl sample loop, a L-4000 UV absorbance detector and a D-2500 Chromato-Integrator. A Lichrocart Lichrospher 100 RP 18 (5 μm) 250×4 mm I.D. column from Merck was used. Assays were performed at 20°C at a flow-rate of 0.5 ml/min. A 20-μl sample was eluted isocratically with tetrahydrofuran–water (30:70, v/v). Detection was at 280 nm.

Calibration curves were performed by eluting reference solutions of Breox in a concentration range of 0.2% to 20% (w/w).

Breox peak was eluted after 13 min. Additives used did not interfere as they had retention times different from that of Breox.

2.5. Partition coefficients

Partition of a solute between two phases is defined by the partition coefficient: $K = C_{top}/C_{bot}$ where C_{top} and C_{bot} are the concentrations of the partitioned solute in the top and bottom phases.

The partition of salts was followed by conductimetric measurements in the top and bottom phases.

3. Results and discussion

3.1. Cloud-point diagram

The cloud-point diagram for the binary system Breox in water is shown in Fig. 1. The curve shape is very typical of this kind of copolymer and is the same as that obtained for UCON 50-HB 5100 [5] which is a less pure ethylene oxide/propylene oxide 50/50 (% w/w) random copolymer. The critical point was 50°C at a Breox concentration between 7 and 15% (w/w).

3.2. Effect of electrolytes and surfactants on the cloud point temperature

We have measured the variation of the cloud point temperature (CPT) as a function of the additive concentration at a constant Breox concentration of 10% (w/w) (Fig. 2).

Ionic surfactants are known to increase the cloud point of nonionic thermopolymers [8]. It was observed that, above a critical surfactant concentration some ionic surfactants can bind cooperatively to nonionic polymers [17]. This phenomenon has been explained [14] by considering the polymer/surfactant specific interaction and the balance of electrostatic repulsive forces by the ionic surfactant head groups on one side, and the attractive forces of the hydrocarbon tails of the added surfactants on the other. The addition of more surfactant increased the electrostatic repulsive forces leading to an increase of the CPT, until the surfactant concentration exceeded the critical micelle concentration in the polymer-surfactant solution, and free micelle forms started to coexist with polymer-surfactant complexes.

Both ionic surfactants analysed lead to an increase of the CPT, with the anionic surfactant SDS having a stronger effect than the cationic surfactant CTAB.

The nonionic surfactant Tween 20 had almost no effect on the CPT. Picullel and Lindman [16] reported that UCON and $C_{12}E_4$ or $C_{12}E_8$ (nonionic surfactants) mixtures have an associative phase separation, meaning that both polymer and surfactant are enriched in the same phase. Conversely, it has also been stated that nonionic polymers and nonionic surfactants do not form complexes at low concentration [18], and that, as a rule, mixtures of nonionic surfactants with nonionic polymers show no strong, mutual effective attraction [16]. The effect of Tween 20 on the CPT is in agreement with the theoretical model for polymer cloud points described in Ref. [5].
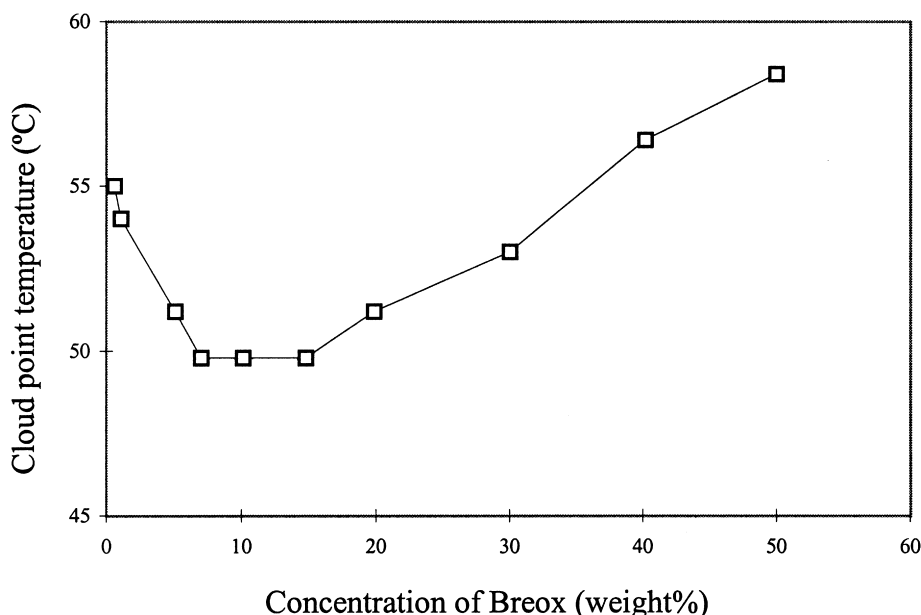

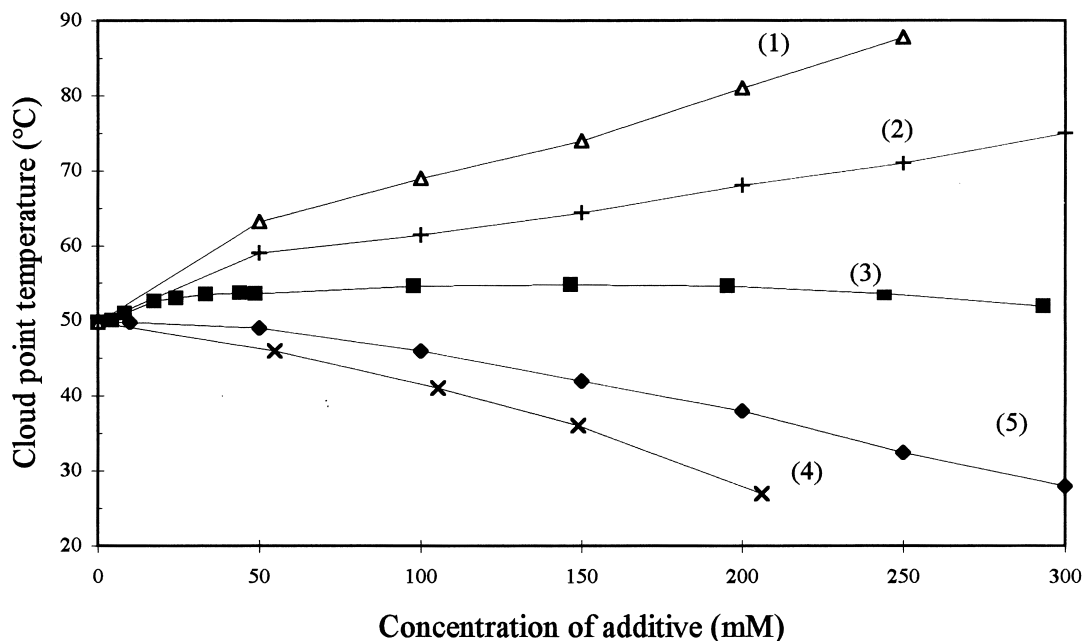Fig. 1. Cloud-point diagram for the system Breox 50 A 1000/water.

Fig. 2. Cloud-point temperature variation of a 10% (w/w) Breox solution with different additives. (1) SDS; (2) CTAB; (3) Tween 20; (4) Na-citrate pH 8; (5) K-phosphate pH 7.

Model calculations show that a substance such as a nonionic surfactant with relatively weak interactions both with water and with the polymer would have no effect or only slightly increase the CPT [5].

The addition of the hydrophilic salts, sodium citrate and potassium phosphate leads to a decrease of the CPT with salt concentration. This salting out behaviour is well known and has been extensively described in the literature [7,10,11].

3.3. Combined effect of surfactants and electrolytes on the cloud-point temperature

In order to study the combined effect of a hydrophilic (or water structure making) electrolyte and a surfactant on the CPT of the nonionic polymer Breox, a potassium phosphate concentration of 100 mM was fixed and the surfactant concentration was changed (Fig. 3), and the surfactant concentration of 100 mM was fixed and the potassium phosphate concentration was changed (Fig. 4). In all these studies, a constant Breox concentration of 10% (w/w) was used.

Fig. 3 shows that for low SDS concentrations, the

CPT decreased to 41°C with the surfactant concentration until 50 mM, in the presence of a salt, and then started to increase, ending up by having converged to the curve without any salt added.

At low surfactant concentrations, there is a screening effect, by the salt ions, of the electrostatic repulsion between the polymer–surfactant complexes induced by the head groups of SDS. Thus the attractive hydrophobic forces, due to the surfactant tails, become predominant, leading to a decrease of polymer solubility. At higher SDS concentrations (>50 mM), the repulsive electrostatic effect dominates even in the presence of a salt, leading to an increase of the CPT [14].

The curve shape of Tween 20 combined with potassium phosphate salt has the same tendency as the Tween 20 curve but at lower CPTs. In this case the salting out of Breox by the hydrophilic phosphate ions was observed.

Fig. 4 presents the curves of 10% (w/w) Breox solution and of 10% (w/w) Breox with 100 mM of surfactant added. A stronger effect of potassium phosphate on the CPT was seen in the case of SDS at low salt concentrations. The electrostatic screening
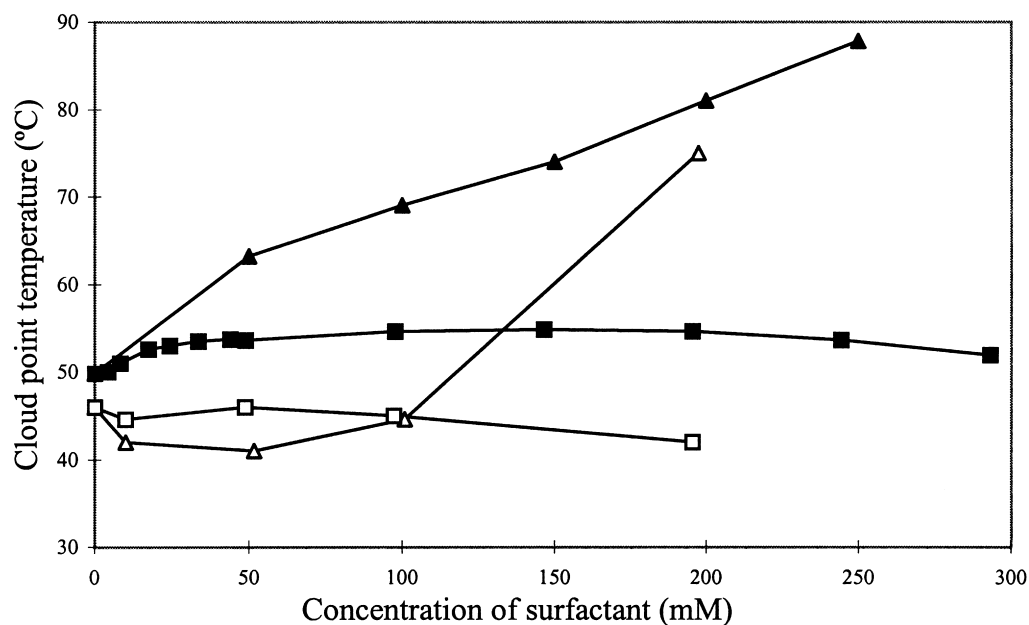
Fig. 3. Effect of surfactants on the cloud-point temperature of a 10% (w/w) Breox solution. Filled symbols are for systems without salt; open symbols are for systems with 100 m*M* K-phosphate buffer pH 7. Squares are for Tween 20 and triangles for SDS.
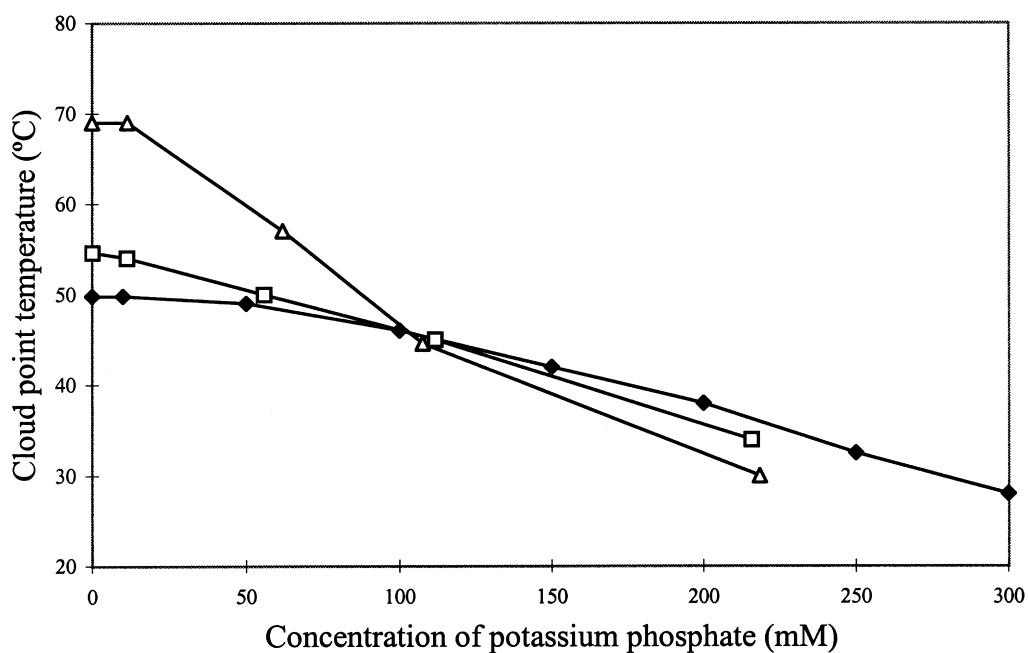

Fig. 4. Effect of K-phosphate pH 7 on the cloud-point temperature of a 10% (w/w) Breox solution. Filled diamonds are for systems without surfactant; open symbols are for systems with 100 surfactant. Squares are for Tween 20 and triangles for SDS.

Table 1
Phase equilbrium composition of Breox solutions. Systems were separated for 1 h at 60°C

Breox$_{initial}$ % w/w	Breox$_{top}$ % w/w	Breox$_{bottom}$ % w/w	Volume ratio
10	2.7±0.1	60±4	8.0
15	4.1±1.1	62±2	5.0
20	2.6±0.3	65±0.5	2.7

induced by the addition of salt has already been explained above. After addition of 100 m*M* of salt, the salting out of both Breox and Breox/surfactant complex was observed in all three curves, with small CPT differences.

3.4. Effects of electrolytes, surfactants and temperature on the phase composition

Top and bottom phase Breox concentrations of different thermoseparating systems at equilibrium are presented in Tables 1–3. Three different Breox concentrations were selected (Table 1). The systems were separated for 1 h at 60°C. The CPT for these systems is 50°C (Fig. 1). All three systems had similar Breox concentrations in the top (3% w/w)

and bottom (60–65% w/w) phases, which is expected as they lie on the same tie line.

Tables 2 and 3 describe the behaviour of 10% (w/w) Breox systems with different additives separated for 1 h at 50 and 60°C, respectively. The conductivity of the systems was measured in order to follow salt partitioning. The conductivity values are shown instead of the partition coefficient, due to the minor interference on the conductivity values observed with addition of Tween 20 and to the small conductivity of the ionic surfactant. A sample of 100 m*M* of SDS, in 10% (w/w) Breox solution had a conductivity of 1.78 mS. The CPTs for these different systems are presented in Fig. 5.

Excluding the case of SDS-composed systems, the phosphate salts partitioned very strongly into the water-rich top phase, and an enhancement of this behaviour with the increase of the temperature was observed. The salt concentration in the bottom phase was fairly constant in all the systems (except the SDS case) at each temperature, being little affected by the amount of salt added and the presence of a nonionic surfactant.

Increasing the salt concentration from 100 to 300 m*M* had a small effect on the top phase Breox content, about 2–3% (w/w) for systems at 50°C and

Table 2
Phase composition and conductivity after thermoseparation at 50°C of different systems composed of Breox and K-phosphate pH 7 salt with and without surfactants. Before thermoseparation all systems contained 10% (w/w) Breox

[KPB] m*M*	[SDS] m*M*	[Tween 20] m*M*	Breox$_{Top}$ % w/w	Breox$_{Bottom}$ % w/w	Conduct.$_{initial}$ mS	Conduct.$_{Top}$ mS	Conduct.$_{Bottom}$ mS	Volume ratio	K$_{Breox}$
100			3.1	41	10.4	15.6	0.49	4.2	0.08
100	100		2.2	33	12.8	17.5	7.83	1.2	0.07
100		100	5.0	33	7.42	10.5	0.31	5.1	0.15
200			2.0	47	19.1	33.0	0.30	4.1	0.04
300			2.1	54	28.1	44.7	0.16	4.5	0.04

Table 3
Phase composition and conductivity after thermoseparation at 60°C of different systems composed of Breox and K-phosphate pH 7 salt with and without surfactants. Before thermoseparation all systems contained 10% (w/w) Breox

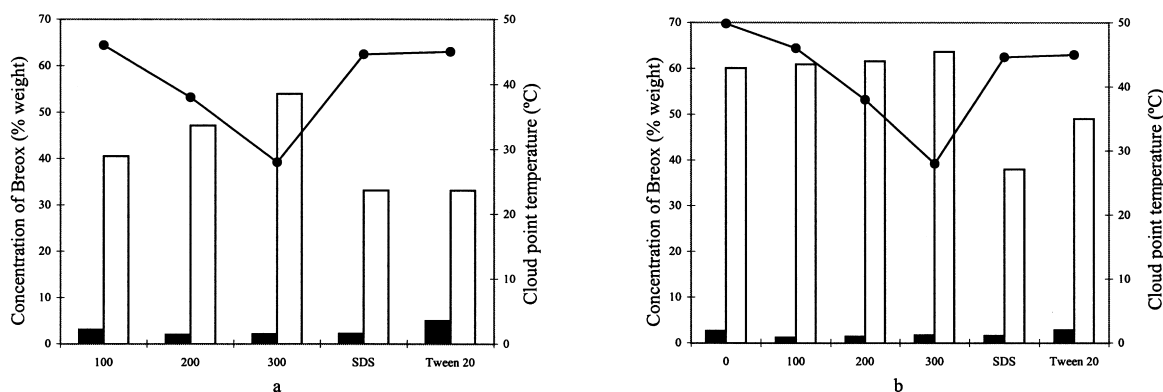| [KPB] m*M*	[SDS] m*M*	[Tween 20] m*M*	Breox$_{Top}$ % w/w	Breox$_{Bottom}$ % w/w	Conduct.$_{initial}$ mS	Conduct.$_{Top}$ mS	Conduct.$_{Bottom}$ mS	Volume ratio	K$_{Breox}$
100			1.2	61	11.0	16.4	0.0094	6.0	0.02
100	100		1.6	38	13.3	17.7	6.45	2.1	0.07
100		100	2.8	49	7.59	12.1	0.019	6.0	0.06
200			1.4	62	19.9	32.2	0.011	5.9	0.02
300			1.7	64	29.6	42.4	0.014	5.9	0.03

Fig. 5. Equilibrium phase composition and cloud-point temperatures of 10% (w/w) Breox systems with different additives separated for 1 h at 50°C (a) and at 60°C (b). Curve with full circles is for the cloud-point temperature. Full bars are for top phase Breox concentration; empty bars are for bottom phase Breox concentration. (0) no additive; (100) 100 mM K-phosphate; (200) 200 mM K-phosphate; (300) 300 mM K-phosphate; (SDS) 100 mM SDS and 100 mM K-phosphate; (Tween 20) 100 mM Tween 20 and 100 mM K-phosphate.

1–2% (w/w) at 60°C. However, for the bottom phases of the systems heated at 50°C, an increase of Breox concentration with the salt added was observed, while at 60°C a rather constant Breox concentration of 60–65% (w/w) was obtained.

At 50°C the increase of the Breox bottom phase concentration for the systems with 100, 200 and 300 mM salt coincided with the decrease of the CPT of these same systems (Fig. 5). This behaviour was not observed at 60°C. In solutions with 10% (w/w) Breox and without surfactants added, the Breox phase concentrations were approximately constant at 60% (w/w) for the bottom phase and 1–2% (w/w) for the top phase (Table 3).

In general, the addition of Tween 20 and SDS to a system of 10% Breox and 100 mM potassium phosphate pH 7, lead to a decrease in the bottom phase Breox content with respect to the same system without the surfactant. The systems that contained SDS were not sensitive to the temperature increase from 50 to 60°C; the same top and bottom phase Breox concentration at both temperatures was observed. Thus, the increment of temperature with respect to the CPT, up to the value of 15°C, has no influence on the Breox phase compositions. The polyelectrolyte-like complex of copolymer/SDS strongly influences the phase behaviour of the aqueous mixture. The top phase composition was equal to the systems without surfactant (2% w/w), while the bottom phase was much lower (approx. 35% w/w).

The volume ratio of this system was smaller than that of all the other systems presented. The copolymer/SDS complex is more hydrophilic which leads to increased water content in the copolymer phase.

The systems with Tween 20 showed a higher Breox content in the top phase than the other systems. This effect was more pronounced at 50°C. The top phase Breox concentration at 50°C was 5.0% (w/w) and at 60°C was 2.8% (w/w) (Tables 2 and 3).

4. Conclusions

The cloud-point temperature of Breox can be increased by the addition of the ionic surfactants SDS and CTAB and decreased by addition of hydrophilic salts. The addition of salts to the ionic surfactant/Breox solution can decrease the CPT of Breox. The increase of surfactant concentration can overcome the electrostatic screening and increase the CPT.

After thermoseparation, the Breox concentration in the top water-rich phase was about 1–3% (w/w), and in the bottom Breox-rich phase was approaching the value of 60–65% (w/w) at 60°C. For the systems composed of the binary mixture Breox/water or with salt added, the difference between the separation

temperature and the CPT up to 10°C interferes with the partition of Breox between the phases.

The presence of the surfactants SDS and Tween 20 decreases the bottom phase Breox concentration, and Tween 20 also increases the top phase Breox concentration.

Acknowledgements

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