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ORIGINAL CONTRIBUTION

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Effect of solute hydrophobicity on phase behaviour in solutions of thermoseparating polymers

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Abstract Two-phase systems consisting of a polymer rich phase and polymer depleted phase, where the polymer is either ethyl(hydroxy ethyl)cellulose (EHEC) or Ucon (a random copolymer of ethylene oxide and propylene oxide), have been studied. Both of these polymers can be separated from an aqueous solution by either temperature increase or addition of cosolutes. The polymers are thermoseparating and phase separate in water solutions at the cloud point temperature. Two types of EHEC have been studied: one with a cloud point at 60 °C and the other at 37 °C. The Ucon polymer used in this study has a cloud point at 50 °C. Ternary phase diagrams of polymer/water/cosolute systems have been investigated. When a strongly hydrophilic or hydrophobic cosolute is added to an EHEC- or Ucon–water solution, a phase separation occurs already at, or below, room temperature. As cosolutes, hydrophobic molecules like phenol, butyric and propionic acid, and hydrophilic molecules like glycine, ammonium acetate, sodium carboxylates (acetate to valerate), were studied. The polymer rich phase formed when mixing polymer, water and cosolute was strongly enriched or depleted with hydrophobic or hydrophilic cosolutes, respectively. The two phase region increased for propionic acid, butyric acid and phenol as a result of increased cosolute hydrophobicity. The opposite occurred in the series sodium acetate, sodium butyrate and sodium valerate. The effect of temperature on the phase behaviour has also been investigated. Model calculations based on Flory–Huggins theory of polymer solutions are presented, in form of a phase diagram, which semiquantitatively reproduce some experimental results.

Key words Polymer – thermoseparation – aqueous two-phase systems – temperature-induced phase separation

Introduction

Thermoseparating polymers are polymers which have a decreased solubility at elevated temperatures. The temperature where the polymer starts to separate from the solution is called the cloud-point temperature (CPT) or the lower critical solution temperature (LCST) [1, 2]. Many of the thermoseparating polymers contain ethylene oxide groups (EO), e.g. PEG (polyethylene glycol) [1], Ucon (random copolymer of ethylene oxide and propylene oxide (= PO)) [3–7], EHEC (ethyl (hydroxyethyl) cellulose) [8] and poly (oxyethylene) surfactants [9]. There are also polymers which do not contain EO groups, e.g. poly (vinylcaprolactam) and poly(N-isopropyl acrylamide) [10], which display a CPT in water.

An aqueous two-phase system is formed with a thermoseparating polymer in water at temperatures above the CPT for the polymer. Two aqueous phases are formed where one is almost pure water and the other is an aqueous polymer phase [5, 7]. The top-phase water concentration minus the bottom-phase water concentration is usually 20–80 wt%, depending on the polymer hydrophobicity and the phase-separation temperature [5]. The temperature for phase separation is dependent of the polymer composition and molecular weight. With EO–PO copolymers the CPT is determined by EO/PO ratio [6].

Thermoseparating polymers can be used together with dextran or hydroxypropyl starch in polymer–polymer aqueous two-phase systems [4, 6, 11]. In such systems, the phase separation is due to an effective repulsion between the two polymers. This repulsion is caused by the difference in hydrophobicity between the two polymers, the EO-containing, thermoseparating polymer being more hydrophobic than the dextran or the hydroxypropyl starch polymers. PEG-dextran aqueous two-phase systems are used for separation of biomolecules [12, 13].

A protein separation technique has been developed in systems with thermoseparating polymer and dextran or hydroxypropyl starch where the target protein after extraction to the phase containing the thermoseparating polymer (Ucon), could be separated from the polymer by a temperature increase [4, 6, 11]. Steroids have been separated in similar aqueous phase systems [14, 15].

New two-phase separation systems for biomolecules have been studied where only one polymer, a thermoseparating polymer, is used in water solution. In these separation systems the biomolecules are partitioned between a polymer-rich phase and a water-rich phase. This two-phase system obtained above the polymer CPT is usually not suitable for partitioning of macromolecules, such as proteins, to the polymer phase, due to the lower entropy of mixing and the reduced possibility to solvate the hydrophilic groups of the proteins in the polymer-rich phase compared to the water-rich phase. These factors favour an exclusion of the macromolecules from the polymer-rich phase [4, 6, 11]. However it has been recently shown that a highly positively charged polypeptide, composed of lysine and tryptophan, can be quantitatively partitioned to the Ucon polymer-rich phase or to the water-rich phase if the salts $NaClO_4$ or Na_2SO_4, respectively, are added to the system [16]. The extreme partitioning of the amphiphilic polypeptide to the Ucon-rich phase, and the quantitative reextraction of the biopolymer to the water phase with the change of salt composition, facilitates the use of a small volume of the polymer-rich phase in an extraction procedure. Small, water-soluble and hydrophobic molecules, however, can be strongly partitioned to such polymer-rich phases. The partitioning of amino acids has recently been studied in the Ucon–water two-phase system [7].

In this work we study how the phase behaviour of two types of thermoseparating polymers in water solution, EHEC and Ucon, is affected by addition of low-molecular weight solutes with different hydrophobicities. Depending on hydrophobicity a significant change of the cloud point is observed [5]. The solute will also partition between the polymer-rich phase and the water phase. The effects are determined by polymer–solute and water–solute interactions. The understanding of these effects form the basis for design of separation systems for biomolecules.

Theory of thermoseparating polymers

A general property of molecules in solution is increased solubility upon temperature increase. For small molecules there are few exceptions from this rule (nicotine in water is a well-known exception [17, 18]). However, for large molecules like polymers there are many examples of a decreased solubility in a solvent at elevated temperatures [1]. This phenomenon can occur both in polar and nonpolar solvents [1, 2]. The LCST found in nonpolar systems (e.g. polystyrene–acetone or polystyrene–cyclohexane) is obtained at temperatures close to the critical temperature of the solvents [19, 20]. The difference in thermal expansions of the components is believed to cause the phase separation [1, 19, 20]. The LCST found in polar solvents is, however, due to other effects [1].

Studies of thermoseparating polymers in different polar solvents have been done [21, 22]. Thermoseparating phenomenon of the EO-containing polymers has been studied in water [1, 5] and formamide solutions [22]. Water and formamide have strong hydrogen-bonding capacity. The most common explanation of LCST in polar solvents in the literature is the breaking of the hydrogen bonds between the solvent and the polymer segments. This theory has been modelled by Goldstein [23].

A different theory which explains the LCST of the EO-containing polymers is explained by an increased polymer segment hydrophobicity at elevated temperatures due to conformational changes in the monomer [24]. It is based on Flory–Huggins theory of polymer solubility [25]. The EO segment is polar when the C–C and C–O are in the gauche and anti conformation, respectively. The polar and hydrophilic forms of the monomers are enthalpically favoured. At higher temperatures the nonpolar forms dominate since they are entropically favoured (i.e. they are numerous). The addition of a cosolute affects the ratio of the polar and nonpolar forms of the monomer. Hydrophilic, inorganic salts like Na_2SO_4 cause a decrease of the CPT [26]. Hydrophobic but still water-soluble substances like butyric acid cause a decrease of the CPT at low or moderate concentrations. At high concentrations such substances elevate the CPT [5]. Amphiphilic compounds like sodium dodecyl sulfate (SDS) usually prevent phase separation since these molecules have an attractive interaction with both water and polymer [8]. The changes of CPT upon addition of hydrophobic, hydrophilic and amphiphilic cosolutes in an aqueous Ucon system have been qualitatively reproduced with our model calculations [27].

Calculations of phase behaviour of thermoseparating polymers, according to the described model above, have been done. In this model effective pairwise interaction parameters w_{nk} (J/mol) are introduced for the components n and k. The expression of the enthalpy of mixing for the thermoseparating polymer–water solution is:

$$\Delta H_{\mathrm{mix}} = n_{\mathrm{tot}}\,[\Phi_1\Phi_2(Pw_{1\mathrm{p}} + (1-P)\,w_{1\mathrm{u}}) + \Phi_2^2(P(1-P)w_{\mathrm{pu}} + (1-P)^2 w_{\mathrm{uu}}/2)]\ . \qquad (1)$$

n_{tot} is the total amount of moles of solvent and polymer segments in the system. Φ_1 and Φ_2 are the molar fractions of water and polymer monomers in the solution. The subscripts p and u stand for polar and unpolar segment conformation, respectively. P is the probability of polar conformation.

The entropy is

$$\Delta S_{\mathrm{mix}} = -\,n_{\mathrm{tot}}\,R\,[\Phi_1 \ln \Phi_1 + (\Phi_2/M_2)\ln \Phi_2 + \Phi_2(P\ln P + (1-P)\ln((1-P)/f))]\ . \qquad (2)$$

M_2 is the number of monomer units in the polymer chain. The parameter f is the ratio between the number of possible nonpolar conformations and the number of possible polar conformations of the polymer segment. In this model f is put equal to 8. With this value a calculated Ucon–water-phase diagram resembles the experimental one. The real f value has not been determined experimentally, but quantum mechanical calculations of small molecules consisting of ethylene oxide units support a f value larger than one [28]. For a three-component system the contribution from the third component (index 3) which is added to the enthalpic term (Eq. (1)) is

$$n_{\mathrm{tot}}\,[\Phi_1\Phi_3 w_{13} + \Phi_3\Phi_2(Pw_{3\mathrm{p}} + (1-P)w_{3\mathrm{u}})]\ . \qquad (3)$$

The corresponding contribution to the entropy expression (Eq. (2)) is

$$-\,n_{\mathrm{tot}}R\,[(\Phi_3/M_3)\ln \Phi_3]\ . \qquad (4)$$

M_3 is the polymerization degree of the cosolute. The free energy of mixing for the solution of the polymer in water is then

$$\Delta G_{\mathrm{mix}} = \Delta H_{\mathrm{mix}} - T\Delta S_{\mathrm{mix}}\ . \qquad (5)$$

By minimizing Eq. (5) for a given composition and P it is possible to calculate the phase compositon for the system. The used interaction parameters w_{nk} are effective since the interaction between the same components has been put to zero (the effective interaction can be calculated as $w_{nk}^{\mathrm{eff}} = w_{nk} - 0.5\,(w_{nn} + w_{kk})$). However, the w_{uu} parameter is nonzero in order to describe the equilibrium between the high- and low-temperature state of the polymer. This means that $w_{\mathrm{iu}}^{\mathrm{eff}} = w_{\mathrm{iu}} - 0.5w_{\mathrm{uu}}$. It is the w_{iu} parameters that are used in Eq. (5) but the $w_{\mathrm{iu}}^{\mathrm{eff}}$ that are presented in Table 1. For more details see Refs. [5, 24].

Experimental section

Materials

Polymers: The polymers used were Ucon 50-HB 5100, EHEC CD-101-90 and EHEC 230 G. Ucon 50-HB 5100 is a random copolymer of equal amounts of ethylene oxide and propylene oxide. The molecular weight M_{w} is 4000. It was obtained from Union Carbide, New York. The ethyl(hydroxyethyl) cellulose polymers were supplied by Akzo Nobel AB, Stenungsund, Sweden. The EHEC CD-101-90 has an average degree of substitution of ethyl and ethylene oxide groups per anhydro glucose unit of 1.7 and 0.7, respectively. For EHEC 230 G the corresponging values are 0.8 and 0.8, respectively. The CPT for 1 wt/wt % aqueous solution of EHEC CD-101-90 is 37 and 65 for EHEC 230 G. The molecular weight M_{w} is about 50 000 for EHEC CD-101-90. The molecular weight of EHEC 230 G is between 50 000–100 000. EHEC was filtered with an ultrafiltration unit (Filtron Crop., Clinton, MA) to remove the small amount of NaCl in the product.

Chemicals: Sodium acetate, phenol propionic acid, sodium valerate and sodium butyrate were purchased from Merck, Dramstadt, Germany. Butyric acid and sodium propionate were purchased from Fluka AG, Buchs, Switzerland. All chemicals were of analytical grade. Millipore water was used in all water solutions.

Methods

The phase boundaries of the investigated two-phase system were established by determining the CPT for given composition. The CPT measurement was performed as follows: A 50 ml glass tube with the weighted compounds was put in a small waterbath and the solution was stirred with a magnetic stirrer. The total weight of the solution was 15 g. The waterbath was heated with a rate of 2°/min. The temperature was measured with a mercury thermometer which was immersed in the solution. The CPT was taken as the first visual sign of turbidity. Tie lines in the diagrams were determined by analyzing the cosolute concentration in the top and bottom phases. The Ucon polymer was determined by refractive index measurements. The phenol concentration was determined by spectrophotometric measurement at 270 nm. Sodium acetate was determined by titration with hydrochloric acid. Tie lines in EHEC containing systems were not determined since it was not possible to separate a well-defined EHEC-rich phase. The high viscosity of EHEC solutions makes it unpractical to work at EHEC concentrations higher than 3–4% (wt/wt).

Results

At room temperature the thermoseparating polymer is completely miscible with water. The thermoseparating polymers phase-separate from water upon heating the solution to the cloud-point temperature (CPT). The less hydrophobic the polymer is the higher is its CPT. The lowest CPT in aqueous binary solutions of the investigated polymers in this work was 37 °C, obtained for EHEC CD-101-90. However, upon addition of a cosolute, phase separation for a thermoseparating polymer can be induced at different temperatures, depending on type and concentration of cosolute. The phase diagrams shown in Figs. 1–5 were determined at 22 °C. In two-phase systems where the cosolute was a carboxylic acid or phenol, the polymer and the cosolute were enriched in the bottom phase. In corresponding systems with the hydrophilic cosolutes sodium carboxylates, glycine and ammonium acetate, the polymer was enriched in the top phase and the cosolute in the bottom phase. Though no tie-lines were determined in the EHEC-containing systems, a strong enrichment of EHEC to one of the phases was indicated by the large viscosity difference between the phases.

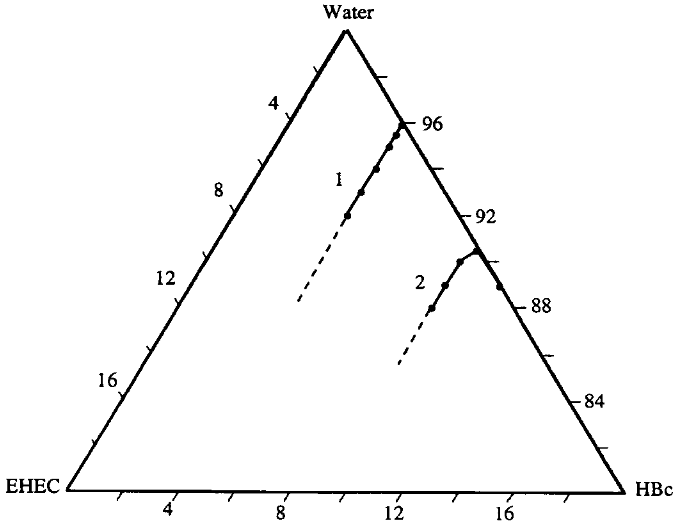

Fig. 1 Phase diagrams for aqueous solutions of butyric acid and (1) EHEC CD-101-90 or (2) EHEC 230 G, Temperature: 22 °C. Concentrations in wt/wt%

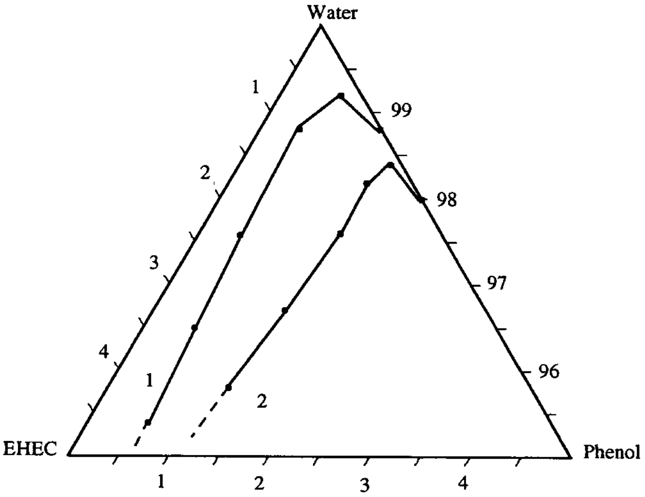

Fig. 2 Phase diagrams for aqueous solutions of phenol and (1) EHEC CD-101-90 or (2) EHEC 230 G. Temperature: 22 °C. Concentrations in wt/wt%

Effect of polymer hydrophobicity on the ternary system: EHEC/water/hydrophobic cosolute

In Fig. 1 the phase diagrams of two types of EHEC-polymers in water–butyric acid systems are shown. Line 1 is the binodal of the EHEC CD-101-90 containing system. Line 2 is the corresponding binodal for EHEC 230 G. Butyric acid decreases the CPT of both polymers. The more hydrophilic EHEC 230 G gives a smaller two-phase region than EHEC CD-101-90. At room temperature 1–2% (wt/wt) EHEC 230 G is completely miscible with butyric acid and water at butyric acid concentrations higher than 43% (wt/wt). For EHEC CD-101-90 this occurs at butyric acid concentrations higher than 49% (wt/wt).

Figure 2 is a similar phase diagram with phenol instead of butyric acid as cosolute, By increasing the hydrophobicity of the cosolute a larger two-phase region is obtained for both polymers, which indicates that phenol has a stronger attractive interaction than butyric acid with the polymer. Again the system with EHEC 230 G has a smaller two-phase region than the corresponding system with EHEC CD-101-90.

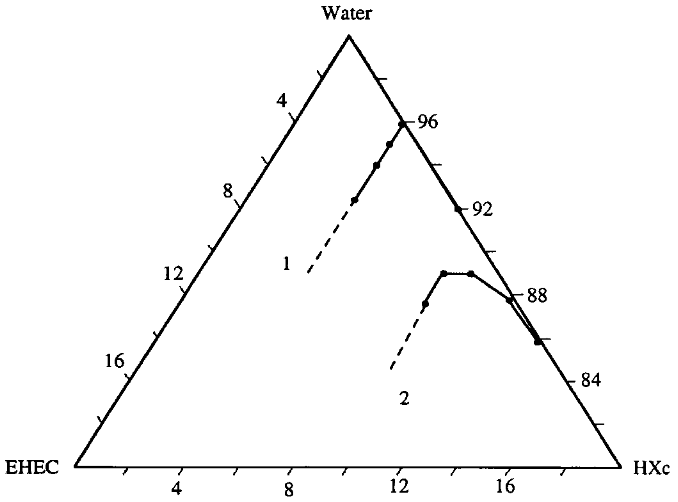

Fig. 3 Phase diagrams for aqueous solutions of EHEC and butyric acid (1) or propionic acid (2). EHEC = EHEC CD 101-90. Temperature: 22 °C. Concentrations in wt/wt%

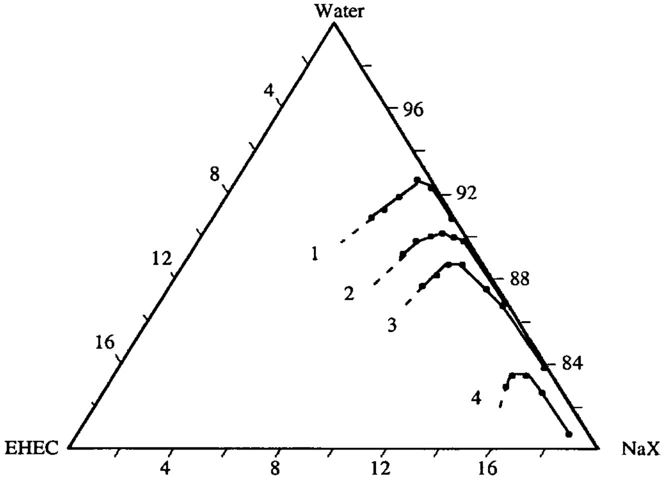

Fig. 4 Phase diagrams for aqueous solutions of EHEC and a sodium carboxylate. Cosolute type: sodium acetate (1), sodium propionate (2), sodium butyrate (3) and sodium valerate (4), respectively. EHEC = EHEC CD 101-90. NaX = sodium carboxylate. Temperature: 22 °C. Concentrations in wt/wt%

Effect of cosolute hydrophobicity in the system: EHEC/water/cosolute

The effect of carboxylic acids (propionic and butyric acid) with different hydrophobicities on the phase behaviour of EHEC CD-101-90 is presented in Fig. 3. The two-phase area is decreased when the hydrophobicity of the carboxylic acid is decreased. At room temperature no phase separation was observed when the cosolute was acetic acid.

In Fig. 4, a phase diagram with four binodals is shown for the system EHEC-CD-101-90, water and sodium carboxylate. The hydrophobicity of the carboxylates increases with increasing number of methylene groups. The two-phase region is successively reduced when acetate is replaced by propionate and butyrate. The size of the two-phase region is substantially reduced with sodium valerate as cosolute.

Polymer/solvent miscibility for solvents with different polarities

Miscibility of the EHEC polymers for some different polar solvents at room temperature is shown in Table 1. Water and short carbon chain carboxylic acids are good solvents for the EHEC polymers, while less polar solvents like the *n*-alcohols and acetone are poor solvents. Swelling of the EHEC polymers was observed in the alcohol solutions and the swelling was stronger for the more hydrophobic EHEC CD 101-90 compared with EHEC 230 G. The Ucon polymer was completely miscible for all solvents listed in Table 1.

Effect of cosolute hydrophobicity in the system: Ucon/water/cosolute

Phase diagrams with Ucon instead of EHEC have been done for comparison with the EHEC containing system. The Ucon solutions have a fairly low viscosity which facilitates the separation of the phases in the two-phase region. Furthermore, Ucon forms liquid phases which are well defined and easily handled. These properties makes it possible to accurately determine tie-lines in the Ucon containing systems.

Table 1 Solubility of EHEC in different solvents. Concentration of EHEC in the systems: 2.5–3 wt %. Temperature; 22 °C

Solvent	EHEC CD-101-90	EHEC 230 G
Water	Soluble	Soluble
Acetic acid	Soluble	Soluble
Propionic acid	Soluble	Soluble
Butyric acid	Soluble	Soluble
Valeric acid	Swells[a]	Swells[a]
Methanol	Swells[a]	Swells
Ethanol	Swells	Swells
Propanol	Swells	Swells
Butanol	Swells	Insoluble
Acetone	Insoluble	Insoluble
DMSO	Soluble	Soluble

[a] Very strong swelling, i.e., the swollen polymer occupied 20–30 times the volume of the pure polymer solid. In the other cases it swelled 2–5 times the volume of the pure polymer solid. DMSO = dimethyl sulfoxide.

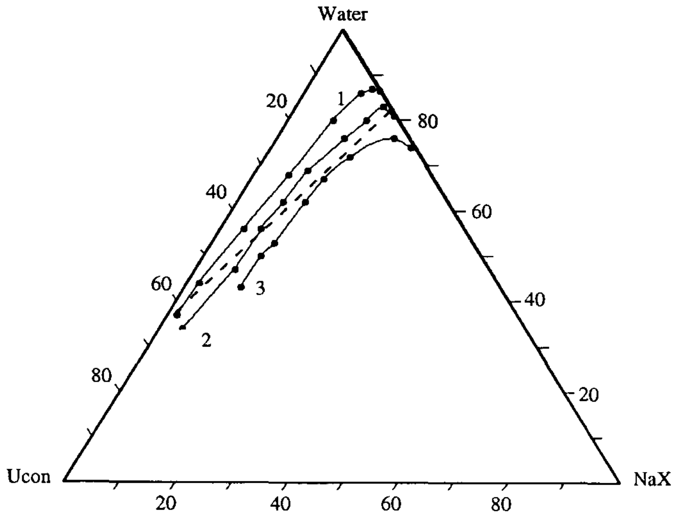

Fig. 5 Phase diagrams for aqueous solutions of Ucon and sodium carboxylate. Cosolute type: sodium acetate (1), sodium propionate (2), sodium butyrate (3), respectively. Nax = sodium carboxylate. Temperature: 22 °C. Concentrations in wt/wt%. Tie-line (---) for the sodium acetate containing system

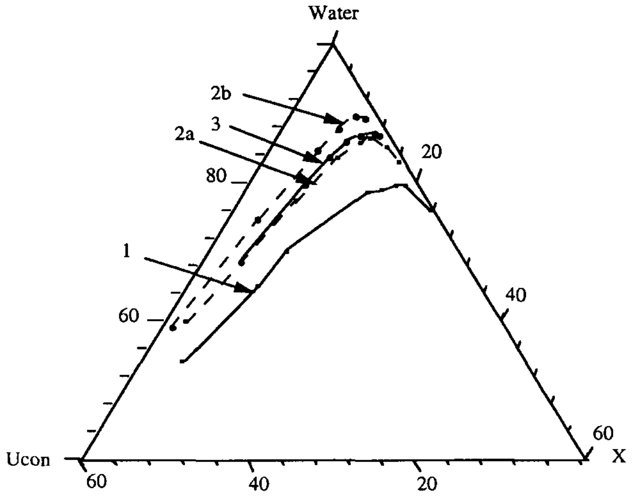

Fig. 6 Phase diagrams for aqueous solutions of Ucon and hydrophilic cosolute. Cosolute type: ammonium acetate (1) binodal at 22 °C, glycine (2a) binodal at 22 °C, glycine (2b) binodal at 30 °C, sodium acetate (3) binodal at 22 °C. X is the cosolute. Concentrations in wt/wt%

In Fig. 5 the phase diagram for the Ucon–water–sodium carboxylate system at 22 °C is shown. The area of the two-phase region decreases in the same order as in the EHEC-containing systems in Fig. 4. However, the one-phase region has, for all carboxylates, increased compared with the corresponding EHEC systems in Fig. 4. In the concentration regime investigated (0–40% sodium carboxylate), sodium valerate does not form a two-phase system with Ucon and water at room temperature. The tie-line in the sodium acetate containing system shows partitioning of acetate to water indicating the strong repulsion between Ucon and sodium acetate.

The phase diagram for the Ucon/water/X system, where X is glycine, sodium acetate or ammonium acetate, is shown in Fig. 6. These hydrophilic solutes are all enriched in the water phase. Glycine and sodium acetate have almost the same binodal at 22 °C, while that of ammonium acetate has a larger one-phase region. A comparison between glycine and ammonium acetate is interesting, since they contain relatively similar chemical groups in their molecular structure. The binodal isotherms 2a and 2b for glycine at 22 and 30 °C, respectively, are also shown. The expansion of the two-phase region at higher temperature was also observed for the two other solutes (not shown).

Figure 7 shows the phase diagram for the Ucon–phenol–water system. Phenol is not completely miscible with water under 66 °C. The tie lines show that the addition of Ucon to the phenol–water system causes a depletion of phenol in the water-rich phase. Phenol is strongly enriched in the Ucon phase. The lowest concentration of phenol in the two-phase region is 0.5% (wt/wt) at 22 °C. This is about the same value as in the EHEC-CD-101-90 containing system in Fig. 2. The isotherms of the phase boundaries show how the two-phase region is moved towards the Ucon–water line at higher temperatures. This effect was also observed in the other phase systems (Figs. 1–6). The region close to the phenol corner has not been fully investigated.

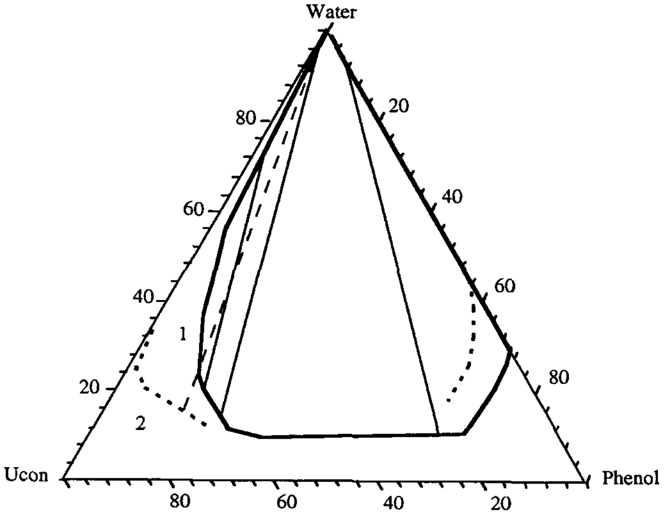

Fig. 7 Phase diagram for Ucon, water and phenol. Binodal isotherms 1 (–) and 2 (- - -) are at 22 °C and 60 °C, respectively. Concentrations in wt/wt%

Discussion

The increase of the two-phase region when a hydrophobic cosolute is present in systems with the more hydrophobic EHEC compared with the less hydrophobic EHEC (Figs. 1 and 2), can be understood as a more effective, attractive interaction between the cosolute and EHEC in water when the polymer hydrophobicity is increased. The effective interaction is the difference between the water–solute interaction and polymer–solute interaction. This implies that the polymer-solute interaction is attractive or only weakly repulsive and that the water–solute interaction is repulsive. An increased hydrophobicity of the cosolute also increases the effective attraction to the polymer (Fig. 3). By an increase of cosolute or polymer hydrophobicity, a stronger attraction is obtained between the polymer and the cosolute. Similar results were obtained in an earlier investigation on Ucon/water/carboxylic acid two-phase systems [5] and from the literature [29] where the CPT in a EHEC/water/alcohol system was shown to be lowered with increasing hydrophobicity of the alcohol.

The results from Table 1 indicate that EHEC is soluble in highly polar solvents such as water, DMSO and short-chain carboxylic acids, while the low polarity *n*-alcohols cannot solubilize EHEC. Valeric acid is the first of the carboxylic acid series in which the polymer is no longer completely soluble. EHEC is not completely soluble in any of the investigated *n*-alcohols. The carboxylic acids have higher hydrogen bonding capacity than the *n*-alcohols, and this can facilitate the solubilization of EHEC.

The reduction of the two-phase region when the hydrophobicity of the carboxylate ion is increased (Fig. 4), is a consequence of a diminished repulsive interaction between the cosolute and the polymer. As the carbon chain of the carboxylate ion increases, the amphiphilic character of the sodium carboxylate will increase. It is known that strongly amphiphilic molecules such as ionic surfactants, tend to stabilize one-phase systems. SDS in EHEC–water solutions displays this effect. However, if a small amount of salt is added to the EHEC/SDS solution a phase separation occurs easily even at low temperatures [8].

The hydrophilic solutes glycine, sodium- and ammonium acetate display a typical "salting-out effect" on the thermoseparating polymers, in the same way as many inorganic salts. The explanation of the larger two-phase region for glycine at 30 °C compared to 22 °C (Fig. 6) can be understood with our model, where the polymer gets more hydrophobic at higher temperatures and consequently the repulsion between the hydrophilic cosolute and the polymer is increased. The comparison of the phase diagrams of glycine and ammonium acetate is interesting from a fundamental theoretical point of view. Both substances have very similar chemical groups in the molecule. However, this does not mean that the solvent–solute interaction is the same for these compounds. The solvation energy for ammonium acetate (-750 kJ/mol [30]) is more negative than for glycine (calculated to -326 kJ/mol [31]) since the two free ions can solvate more water molecules than the single zwitterionic molecule. This would also imply that ammonium acetate is more hydrophilic than glycine and consequently has a larger tendency than glycine to salt-out the polymer. The experimental results show the opposite behaviour which means that the entropic contributions to the mixing free energy, which favour mixing, are larger for ammonium acetate than for glycine (there are more ways to place the two ions (ammonium and acetate) than the zwitterionic glycine, in the solution).

The difference in two phase area between sodium acetate and ammonium acetate (Fig. 6) can be understood as a difference in effective cation hydrophilicity, the ammonium being relatively less hydrophilic than sodium. A similar phase behaviour was also obtained in PEG/salt system where Na_2SO_4 caused two-phase formation at much lower concentration than $(NH_4)_2SO_4$ [26]. The ion hydrophilicity can be related to the ion size [30], where ions with low charge/surface ratio are less hydrophilic and have more positive solvation energy [7, 30]. These ions are often referred to as "chaotropic" [32]. Ions with relatively low hydrophilicity have been shown to partition to the Ucon-rich phase in Ucon–water phase systems [7], and to increase the stability of one-phase aqueous systems containing ethylene oxide–propylene oxide block copolymers [22]. Ions with relatively low hydrophilicity have also been shown to adsorb at the nonionic ($C_{12}E_5$) surfactant monolayer in bicontinuous microemulsions, in contrast to more hydrophilic ions which desorb [33]. These observations indicate that less hydrophilic ("chaotropic") ions have preference for ethylene oxide containing polymers in water solutions.

The strong partition of phenol to the Ucon-rich phase in Fig. 7 and the large two-phase region formed in EHEC/water/phenol systems (Fig. 2) indicate a strong attractive interaction between phenol and the ethylene oxide groups. The solubilization of phenol in polyethoxylated nonylphenol micelles has been studied [34], where it was shown that a poly(ethylene oxide) tail of 20–30 ethylene oxide groups could solubilize about five phenol molecules. It has also been found that micelles of poly(ethylene oxide) and poly(propylene oxide) block copolymers strongly prefer to solubilize aromatic, instead of aliphatic compounds [35] in a two-phase system consisting of a hydrocarbon phase and an aqueous solution of the block copolymers. This indicates that the aromatic compounds have strong attractive interaction with ethylene oxide units. The change of the two-phase region with the temperature

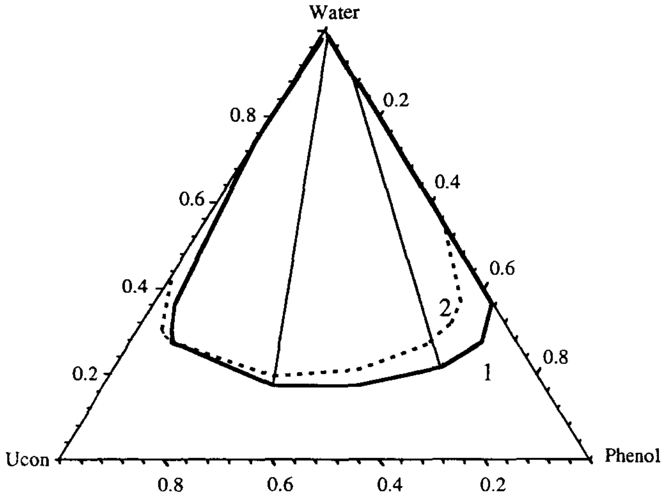

Fig. 8 Calculated phase diagram for Ucon, water and phenol. Binodal isotherms 1 (—) and 2 (- - -) are at 22 °C and 60 °C, respectively. U = Ucon 50 HB 5100. P = phenol. Concentrations in volume fraction. The polymerization degree of Ucon and phenol is 217 and 5, respectively. Interaction parameters are presented in Table 2

Table 2 Interaction parameters in J/mol. All parameters are effective. Indices l, *p*, *u* and 3 refer to components water, polar- and unpolar form of polymer segment, and phenol, respectively

w_{lp}	546
w_{lu}	6188
w_{l3}	2950
w_{pu}	440
w_{p3}	− 2000
w_{uu}	10242
w_{u3}	− 3121

increase, shown in Fig. 7, can be understood as follows: The solubility of phenol in water increases with temperature. At the phenol-rich region of the phase diagram, the difference in hydrophobicity of the phases is mainly governed by water and phenol, and this difference decreases with increasing temperatures and thereby facilitates an increased partitioning of polymer to the water-rich phase. In the Ucon-rich region, the difference in hydrophobicity between the phases increases with temperature which leads to a stronger partitioning of phenol to the polymer-rich phase.

The observed phase behaviour in Fig. 7 can be qualitatively reproduced with our model. Calculated phase diagrams of the Ucon/phenol/water system at 22 and 60 °C are shown in Fig. 8. The concentration of the components is expressed in volume fractions. The polymerization degree of polymer and phenol is 217 and 5, respectively. These values are obtained by dividing the molecular weight of the polymer and the phenol with that of water. The reason for this is to compensate for the size differences between the components. The values of the interaction parameters have been obtained by fitting them until the calculated phase diagram resembles the experimental one. The parameters for Ucon–water and phenol–water interactions are fitted according to the phase behaviour of the respective phase systems. For the three-component system, only the phenol–Ucon parameter is fitted, since the parameters for the two-component systems above are retained.

The used parameters have only a qualitative significance and cannot be taken too literally. The calculated phase diagram is not very sensitive to the absolute values of the parameters, but rather to their relative difference. The values of the parameters reflect the component hydrophobicity or polarity. The polarity of an uncharged dipolar component can be related to the quotient (μ/V), where μ is the dipole moment and V the molecular volume of the component (for the importance of this quotient for phase separation of the thermoseparating polymers see Ref. [22]). If the difference of polarity between two components is sufficiently high they will not mix. Since the polarity of phenol and the Ucon polymer is much lower than water they will have repulsive (positive) interactions with the water. Furthermore, since phenol and Ucon polymer are miscible, they have an attractive interaction to each other. For a more hydrophobic and less polar component like cyclohexane the miscibility with the Ucon polymer is lower. The values of the interaction parameters does not represent "real" physical values but rather the qualitative chemical nature (in terms of hydrophobicity) of the components. The used interaction parameters are listed in Table 2. For similar model calculations of tryptophan and hydrophobic peptides in thermoseparated Ucon/water systems see Refs. [7, 16], respectively.

The calculated phase diagrams (Fig. 8) show qualitatively the same phase behaviour as the experimental phase diagrams (Fig. 7). The experimental diagrams in Figs. 1–6 have not been calculated, since more parameters have to be introduced, allowing proton-transfer equilibria of the weak acids. In that case one has to assume different pK_a values of the acids in the water-rich and the polymer-rich phases. This is outside of the scope of this work. However, a phase diagram of PEG/water/glucose has been calculated in the context of the Karlström model by Sjöberg et al. [36]. This phase diagram qualitatively resembles the diagrams in Fig. 5. Thus, glucose has also the "salting-out" property as the cosolutes in Fig. 5.

Conclusions

The thermoseparating polymers Ucon 50 HB-5100, EHEC CD-101-90 and EHEC 230 G form aqueous two-phase systems with hydrophobic substances such as propionic acid, butyric acid and phenol at 22 °C. Phase separation is also obtained and semiquantitatively reproduced in model calculations using a theoretical model based on the Flory–Huggins polymer solution theory.

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