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Investigation of surfactant alkyl chain length and counterion effects on the thermogelling EHEC system

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Abstract

Ethylhydroxyethylcellulose (EHEC) forms a reversible thermogelling system in water in the presence of ionic surfactants. In an effort to minimise the amount of ionic surfactant needed to produce a thermogelling system with timolol in salt form, we have examined in detail the system with respect to the alkyl chain length and counterion of the ionic surfactant. Two salts of timolol were also investigated, timolol (hydrogen)maleate and timolol chloride. It was found that in order to form a gel with a small amount of ionic surfactant, a number of criteria have to be fulfilled, e.g., (i) the ionic drug should typically be a co-ion to the ionic surfactant, (ii) the counterion of the drug and the ionic surfactant should preferably be inorganic and with a low polarisability and (iii) the ionic surfactant should have a low CMC but a Krafft temperature not higher than room temperature.

Keywords: Thermal gelation; Ethyl(hydroxyethyl)cellulose; Ionic surfactant; Timolol; Phase behavior

1. Introduction

Semi-dilute aqueous solutions of a certain, rather hydrophobic type of the nonionic cellulose derivative EHEC (ethyl(hydroxyethyl)cellulose) have been shown to exhibit thermogelling properties in the presence of ionic surfactants (Carlsson et al., 1990a,b). The sol-gel transition may occur at temperatures around 35° C, making the system interesting from a drug delivery point of view (Lindman et al., 1991, 1993; Rydén and Edman, 1992a,b). In a previous paper, it was shown that timolol maleate, a potent β-blocker, could be included in the thermogelling EHEC system at a concentration found in commercial eye-drops, which points towards one potential application (Lindell and Engström, 1993). The system was also seen to be very sensitive to high (internal) ionic strengths, but it has been shown that gelation can occur readily in physiologically saline solutions (Lindman et al., 1993).

One drawback of the system, from a toxicological aspect, is the need for an ionic surfactant, which may cause irritation problems. Other results indicate, however, that the effect of the surfactant could possibly be reduced when administered in the EHEC system as compared to an aqueous solution, due to the strong interaction between the surfactant and the EHEC polymer which leads to a very slow release of the surfac-

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tant from the vehicle (Lindman et al., 1993). Nevertheless, the reduction of the amount of ionic surfactant needed in order to form a thermogel will most probably increase the potential of the system in drug delivery.

The objective of this work was therefore to investigate the gelling behaviour as a function of the chain length and counterion of one ionic amphiphile used, as well as the effects of the salt form of the drug.

2. Materials and methods

The EHEC sample used was obtained as a gift from Berol Nobel (Stenungsund, Sweden). It was designated DVT 88001 and according to the manufacturer the average substitution degree of ethyl groups (DS_{ethyl}) was 1.7 and the molar substitution degree of ethylene oxide groups (MS_{EO}) was 1.0 per anhydroglucose unit. The viscosity average molar mass for the sample was 100 000.

The ionic surfactants dodecyltrimethylammonium bromide (DoTAB, Sigma), tetradecyltrimethylammonium bromide (TTAB, Sigma), hexadecyltrimethylammonium bromide (CTAB, Sigma), hexadecyltrimethylammonium chloride (CTAC, Tokyo Kasei), octadecyltrimethylammonium bromide (OTAB, Fluka), octadecyltrimethylammonium chloride (OTAC, Tokyo Kasei) and sodium dodecyl sulfate (SDS, BDH), all of high purity (95% or better), were used as received.

The hydrochloride salt of timolol (TCl) was prepared from timolol maleate according to a method described as the initial step in the synthesis of timolol prodrugs (Bundgaard et al., 1986). The purity was confirmed by the melting point (m.p.) 130–134° C of the white crystalline salt as well as by the recording of a (500 MHz) ^{1}H-NMR spectrum. Timolol (hydrogen)maleate (TM), the monosodium salt of maleic acid (NaM) and sodium chloride (NaCl) were purchased from Sigma and used without further purification.

2.1. Sample preparation

EHEC aqueous solutions containing 1.0% (or 2.0%) (w/w) polymer, were prepared by adding hot doubly distilled water to the polymer while agitating with a magnetic stirrer to disperse the polymer in the medium so as to avoid formation of insoluble lumps. The dispersions were then put in a cool room (+5° C) with continuous stirring for at least 24 h to ensure complete dissolution and the resulting clear solutions were stored in a refrigerator until use. These solutions were equilibrated at room temperature (20–22° C) before sample preparation. In cases where salt was to be included in the system, it was first dissolved in the EHEC stock solution. Calculated amounts of (solid) ionic surfactant and EHEC/(salt) solution were weighed into glass tubes, sealed with Teflon coated screw-caps and thoroughly mixed with a Whirlimixer™ shaking machine. All samples were then equilibrated at room temperature for at least 24 h, or until no detectable change in the phase behaviour could be observed.

2.2. Phase studies

Phase maps were obtained in the temperature range of 20–80° C. The samples were equilibrated in a block thermostat (Grant BT3) for 2 h at each temperature before investigation and with an increase of not more than 5° C per step. The phase behaviour was studied by visual observation and the macroscopic fluidity was estimated to define regions with the thermogelling property. Most of the samples were checked several times after differing storage times to confirm equilibrium conditions and detect any hysteresis effects.

3. Results

3.1. Phase behaviour of the EHEC system

EHEC belongs to a class of nonionic polymers with reduced solubility in water at elevated temperatures. The EHEC quality used in this study has a cloud point (CP) of about 30° C, visually taken as the temperature where the sample begins to scatter light and thus is transformed from a perfectly clear to a bluish, turbid solution by eye. This means that a 1% (w/w) aqueous solution of EHEC will separate to a white, more or

less brittle polymer-rich phase and an almost clear water-rich phase, if left to stand, at temperatures slightly above the clouding temperature.

In order to clarify the discussion of the phase behaviour, we will shortly present the characteristic features of the EHEC phase map. The notation phase map, instead of phase diagram, is used to emphasise that we are dealing with a multicomponent system, EHEC being a particularly heterogeneous substance in many respects, e.g., as regards molecular weight as well as degree and position of substitution. The maps presented in this work also differ from ordinary phase diagrams in the sense that we have 'locked' the composition of some of the components (polymer, water and salt) and investigate the effects of the addition of ionic surfactant on a molal basis, i.e., mmol surfactant added per kg of polymer/(salt) solution. We will use the phase maps of the 1.0% (w/w) EHEC aqueous solution with different amounts of timolol maleate (0, 0.10, 0.20, and 0.30% (w/w)) and the cationic surfactant CTAB, shown in Fig. 1a–d, as reference to illustrate the general features of the thermogelling EHEC/ionic surfactant system.

The temperature and surfactant concentration are represented on the vertical and horizontal axes, respectively. The filled line is to be consid-

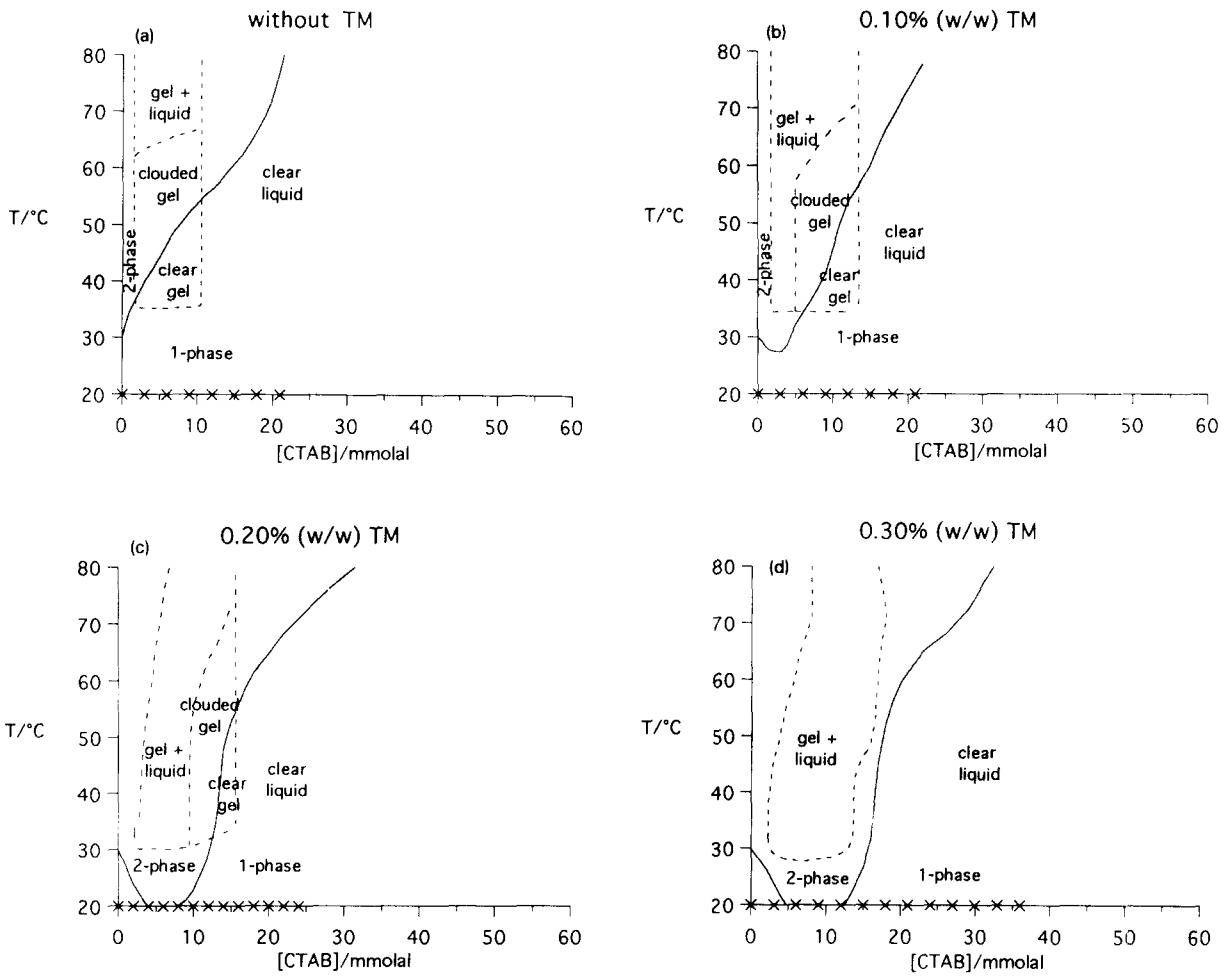

Fig. 1. Phase maps of the aqueous system 1.0% EHEC and different concentrations of timolol maleate (TM) with CTAB as ionic surfactant (symbols are explained in section 3).

ered as a cloud-point curve or, more correctly, as the turbidity boundary, below which all samples are perfectly clear and consist of only one phase. Above this line the samples either scatter (visible) light or are macroscopically phase separated. The dotted lines indicate the domain (with respect to temperature and surfactant concentration) where the most pronounced viscosity enhancement is found. This can further be divided into (at the most) three parts depending on the visual appearance of the samples; 'clear gels' formed below the turbidity line, 'clouded gels' which are not macroscopically phase separated but scatter light and 'gel + liquid' where the gel coexists with a very low-viscous water-rich phase. The relative volume of the gel phase is here seen to decrease with increasing temperature (syneretical behaviour) and decreasing surfactant concentration. The viscosity of samples outside but near the dotted area can also be substantially enhanced, indicating a gradual changeover, but the thermogelling property is then lost with increasing surfactant concentration and the samples at the far right become even less viscous than the polymer solution without surfactant at room temperature.

We can now discuss the appearance of the phase maps of the first system in this investigation.

3.2. 1% EHEC, increasing amount of TM with CTAB

If we begin by looking at the behaviour without any addition of surfactant, i.e., at the intercept of the temperature axis, it can be seen that the cloud-point (CP) is similar (30° C) in the four maps, implying that the drug (TM) alone does not influence the phase behaviour of the surfactant-free system at these low concentrations. The situation becomes much more different when increasing amounts of CTAB are present. In the salt-free case (no TM), shown in Fig. 1a, the surfactant addition gives rise to a monotonic increase of the CP with increasing amount of surfactant. In the three other maps (Fig. 1b–d), an initial depression of the turbidity boundary at low surfactant concentrations is clearly seen with increasing amounts of TM. The line goes through a

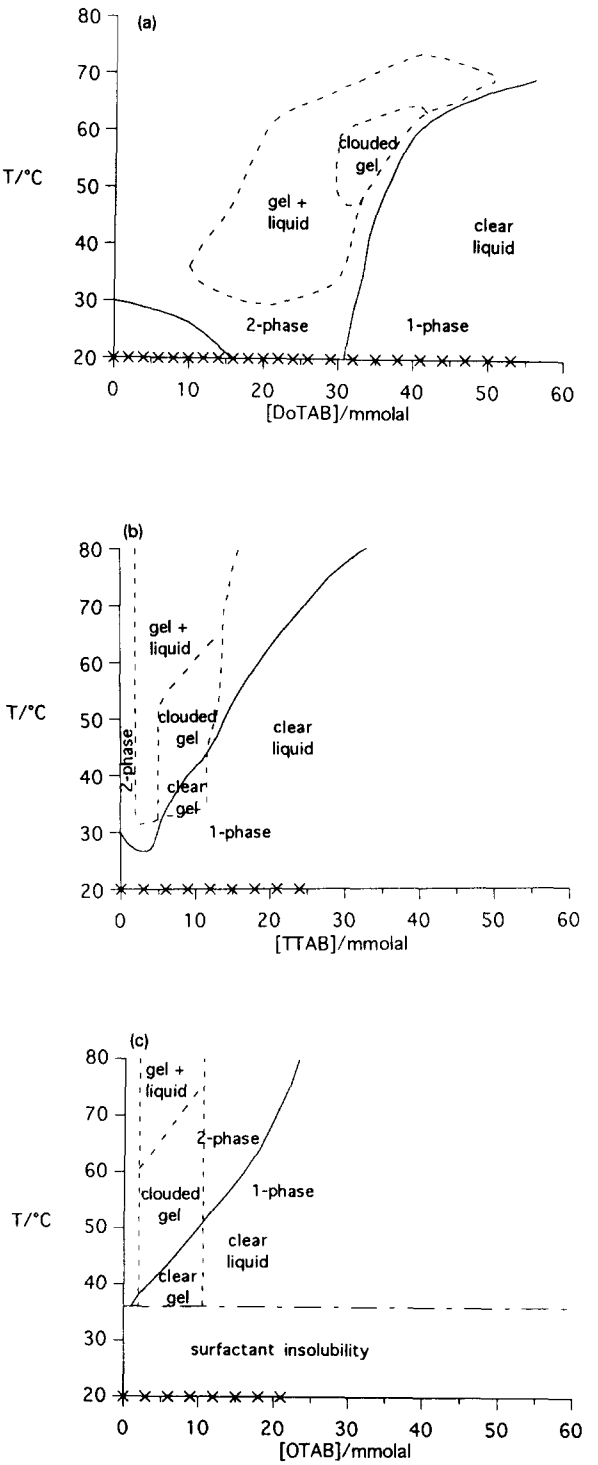

Fig. 2. Phase maps of 1.0% EHEC with surfactants of different alkyl chain length; DoTAB (a), TTAB (b) and OTAB (c).

minimum and then returns to the increasing shape seen without salt. This so-called synergistic effect will be discussed later. As for the domain with the most pronounced gels, this is also shifted slightly to higher surfactant concentrations with increasing amounts of drug but to a smaller extent, leading to obvious problems from the formulational point of view. We would like to incorporate a substantial amount of the drug (TM) and keep the desired phase and gelation behaviour, using as little ionic surfactant as possible. We are thus screening for a composition which gives a low-viscosity, thermodynamically stable and clear solution at room temperature and a homogenous gel at body temperature. We have chosen to increase this demand to an optically clear gel to ensure optimal stability at elevated temperatures as well. This behaviour is seen with CTAB concentrations of 2.0–10.5 mmolal without TM, 7.5–13 mmolal with 0.10% (w/w) TM and 13.5–15.5 mmolal with 0.20% (w/w) TM. The property is lost if the drug amount is increased to 0.30% (w/w), as the thermal gelation only occurs in phase-separated samples.

From these findings we can deduce that in order to create an acceptable thermogelling formulation with 1.0% (w/w) polymer and CTAB as the ionic surfactant, about 0.20% (w/w) TM is the maximal amount that can be incorporated and the surfactant concentration then needed is 13.5 mmolal.

3.3. Effects of the surfactant alkyl chain length

Phase maps of 1.0% (w/w) EHEC without TM and the three alkyltrimethylammonium bromides DoTAB, TTAB and OTAB are shown in Fig. 2a–c. These surfactants are CTAB analogues with 12, 14 and 18 carbons in the alkyl chain, respectively. In comparison with the map in Fig. 1a, it is clearly demonstrated that these surfactants are inferior to CTAB to meet up to the demands mentioned above. The DoTAB map exhibits a very broad initial CP depression (even without salt) and more than 30 mmolal surfactant is needed to re-establish a clear solution at 20° C. The gel domain is moreover totally separated from the monophasic areas, making it impossible to accomplish the desired properties at any surfactant concentration. In the TTAB case, the map is strikingly similar to CTAB with 0.10% (w/w) TM (Fig. 1b). A minor initial depression of the turbidity boundary is seen, and at 37° C, clear gels are found with surfactant concentrations between 8 and 11.5 mmolal. Another difficulty appears with OTAB since this surfactant has a lowest solubility temperature (Krafft point) exceeding 30° C (Kaneshina and Yamanaka, 1990).

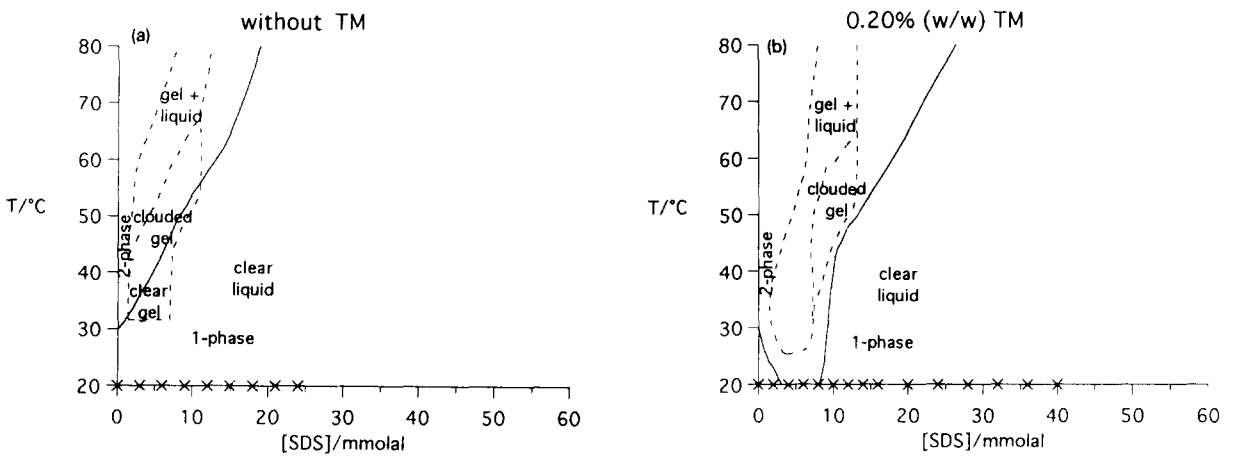

Fig. 3. Phase maps of 1.0% EHEC with the anionic surfactant SDS; without TM (a) and with 0.20% TM (b).

Table 1
Critical micelle concentration (CMC) for the surfactants in aqueous solution [a]

Surfactant	DoTAB	TTAB	CTAB	OTAB	CTAC	OTAC	SDS
CMC (mM)	14.5	3.6	1.0	0.3	1.3	0.4	8.3
T (° C)	25	30	25	23 [b]	30	25	25

[a] CMC values taken from Mukerjee and Mysels (1971).
[b] Inconsistent with the Krafft temperature reported by Kaneshina and Yamanaka (1990).

For practical reasons, it cannot be used in formulations, but we wished to investigate the behaviour above this temperature in order to compare the relative position of the turbidity boundary to the gel domain. The samples had to be equilibrated at 37° C for at least 1 week to complete the surfactant dissolution. The resulting reduced map is almost superimposable on the salt-free CTAB system, exhibiting clear gels in the same surfactant range. Phase maps including 0.20% (w/w) TM with these surfactants (not shown) were also determined to rule out any deviations from the salt effects observed in the CTAB system. It was here seen that the presence of TM was even more detrimental to the DoTAB and TTAB systems, as no homogenous gels could be detected and the salt had no positive effect on the OTAB solubility. The gelation capacity of this surfactant series will later be discussed in relation to the critical micelle concentrations (CMC), listed in Table 1.

3.4. Comparison with an anionic surfactant

Phase maps of 1.0% (w/w) EHEC, without TM (a), and including 0.20% (w/w) TM (b), with sodium dodecyl sulfate (SDS) as surfactant are shown in Fig. 3a–b. If these are compared to the corresponding maps with CTAB (in Fig. 1a and c) we can see many similarities and a few differences. The shapes and positions of the turbidity boundaries are almost identical, with an instantaneous increase without TM and a pronounced initial minimum at low surfactant concentrations with 0.20% (w/w) drug present. The domain with the most enhanced viscosity, on the other hand, is narrower with respect to the surfactant concentration interval giving rise to clear gels at 37° C (3.5–7 mmolal compared to 2–10.5 mmolal with CTAB) without drug, and fully separated from the optically clear area when 0.20% (w/w) drug is included. The shape of this domain is also somewhat tilted towards higher SDS concentrations at more elevated temperatures. The gels formed with SDS seem, moreover, macroscopically stiffer than with CTAB at corresponding temperatures but are more disposed to syneresis.

3.5. Effects of the salt type

In order to estimate possible contributions from the particular type of salt used (inorganic/organic, co- or counterion) to the phase and gelation behaviour, we have also obtained phase maps with sodium chloride (NaCl), sodium (hydrogen)maleate (NaM) and timolol chloride (TCl) at molar concentrations corresponding to 0.20% (w/w) TM, i.e., 4.6 mM, for the 1.0% (w/w) EHEC system with CTAB and SDS. We have chosen to summarise these maps in Table 2, as they do not substantially differ from those already shown with TM and CTAB (Fig. 1c) or SDS (Fig. 3b) to more than the individual and relative shifts of the turbidity boundary and the domain with most pronounced viscosity enhancement. The surfactant concentration intervals exhibiting clear gels at 37° C are listed. The results indicate apparent effects related to the type of salt used. Let us first consider the system with the cationic surfactant (CTAB). It is here seen that additions of TCl or NaCl

Table 2
Surfactant concentration interval exhibiting clear gels at 37° C in systems with 1.0% (w/w) EHEC, and different additives at the concentration of 4.6 mM, for CTAB and SDS

Additive	Concentration range (mmolal)	
	CTAB	SDS
– [a]	2–10.5	3.5–7
NaCl	8.5–13	5–8.5
NaM	10–13	5–8.5
TM	13.5–15.5	– [b]
TCl	8–13	9–10.5

[a] Without addition of salt.
[b] No clear gels detected.

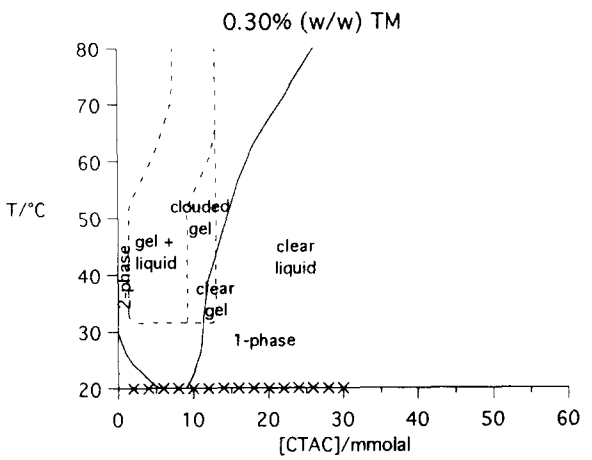

Fig. 4. Phase map of 1.0% EHEC with 0.30% TM and CTAC as ionic surfactant.

show very similar influences on the behaviour – an effective shift and narrowing of the clear gel interval to 8(8.5)–13 mmolal surfactant, whereas NaM and TM seem to affect the system to a greater extent. This order is changed for the system with the anionic surfactant (SDS), where NaM and NaCl show identical shifts of the interval to 5–8.5 mmolal surfactant and the two timolol salts exhibit stronger effects; clear gels between 9 and 10.5 mmolal SDS with TCl and no clear gels with TM.

3.6. Effect of surfactant counterion

A phase map of 1.0% (w/w) EHEC with 0.30% (w/w) TM and the cationic surfactant hexadecyltrimethylammonium chloride (CTAC) is shown in Fig. 4. The surfactant only differs from CTAB with respect to the counterion. This exchange has a noticeable effect on the phase behaviour in our model system. If we compare with the CTAB system with 0.30% (w/w) TM in Fig. 1d, it is clearly seen that CTAC has a more favourable influence. The turbidity boundary is shifted to a less extent and slightly overlaps the domain with enhanced viscosity, making it possible to obtain clear gels at 37° C in a narrow range between 11.5 and 13 mmolal CTAC, which is even lower than for CTAB with 0.20% (w/w) drug. We have also established a phase map with 0.30% (w/w) TM and octadecyltrimethylammonium chloride (OTAC). The effect on the phase behaviour was identical to that observed with CTAC, giving clear gels between 11.5 and 13 mmolal surfactant, implying, as with the bromide series, no further gain in lengthening of the alkyl chain. No problems related to surfactant insolubility could be detected since the chloride salt has a lower Krafft temperature (Kodama and Seki, 1983).

4. Discussion

4.1. General considerations

One reasonable explanation for the clouding phenomenon based on temperature-dependent conformational changes in the ethylene oxide (EO) segments of the polymer has been proposed by Karlström (1985). From statistical-mechanical and quantum-chemical calculations it is suggested that polar conformations of the polymer chain are most abundant at low temperatures, leading to a more favourable polymer-water interaction, whereas less polar conformations are entropically favoured at higher temperatures, resulting in an effectively weaker overall polymer-water interaction. This model has been successfully applied to predict the phase behaviour of aqueous EHEC systems (Karlström et al., 1990; Zhang et al., 1992, 1994).

The interactions of EHEC with surfactants and other cosolutes in semidilute aqueous solutions have been subjected to extensive investigations (Carlsson, 1989). The polymer interacts strongly with ionic surfactants which is manifested, for example, as an increased solubility, i.e., CP, when surfactant is added to the solution. This behaviour is drastically changed as small amounts, typically in the mmolar range, of electrolytes are present. Addition of ionic surfactant then leads to a pronounced CP depression at low surfactant concentrations. This effect has been denoted synergistic, whereas the electrolytes alone do not affect the polymer solubility at such low concentrations and the surfactant would have caused a more or less immediate increase in CP,

if no other cosolutes were present (Carlsson et al., 1986; Karlström et al., 1990). One way to rationalise this effect is to consider the interaction between polymer and ionic surfactant as divided into two parts. As the surfactant 'binds to' or is closely associated with the polymer, it introduces both hydrophobic (the hydrocarbon tail) and hydrophilic (the charged polar head) groups, resulting in a delicate balance between attractive hydrophobic and repulsive electrostatic forces. The hydrophobic effect can dominate if the electrostatic interactions are partly screened by the addition of salt, or even by high free surfactant and counterion concentrations, as in the case of surfactants with higher critical micelle concentration (CMC). A complementary explanation for this phenomenon comes from considerations on analogy with aqueous mixtures of nonionic polymer and polyelectrolyte. Phase separation in such a system is unfavourable due to a resulting loss of mixing entropy of the counterions, since these would have to accompany the polyelectrolyte in the separated phase, due to the condition of electroneutrality. This strain is then lost as extra salt is introduced (Piculell and Lindman, 1992). The determined ternary phase diagrams of EHEC/SDS/H_2O, also with addition of NaCl, demonstrate an associative phase separation of polymer and surfactant (Zhang et al., 1992).

A fruitful approach to consider the polymer-surfactant interaction in systems of nonionic polymer and ionic surfactants is to analyse the effect of the polymer on the surfactant micellisation (Lindman and Thalberg, 1993). The surfactant binding to the polymer is usually very cooperative, with a quite well-defined onset of binding, called the critical association concentration (CAC). Characterisation of such systems by different techniques demonstrates that the bound surfactant molecules form aggregates which resemble conventional micelles (Cabane and Duplessix, 1985; Zana et al., 1985). Investigations of the EHEC system have shown a strong cooperative binding of ionic surfactant to the polymer at concentrations lower than the CMC. This CAC value was moreover seen to decrease with increasing temperature, implying an increased attraction between surfactant and EHEC at elevated temperatures. The unusual temperature dependence was explained as an effect of conformational changes of the polymer, making it more hydrophobic (Carlsson et al., 1989a,b).

A possible mechanism for the thermal gelation based on this cooperative association of micelle-like clusters of ionic surfactant molecules to the polymer has been proposed (Carlsson et al., 1990a,b). These may then act as physical cross-links between hydrophobic segments in different polymer chains, leading to network formation and a consequent viscosity enhancement. The temperature effect can be considered as induced by the temperature dependent polarity of the polymer chain – serving as a better 'nucleus' for surfactant aggregation at higher temperatures. More recent investigations have demonstrated that, in addition to the CAC, the micelle size and degree of counterion binding are markedly reduced with increased temperature (Zana et al., 1992; Kamenka et al., 1994). In an optimised system, a temperature increase could therefore lead to the formation of a much larger number of micelles which could act as cross-links between polymer chains. At higher temperatures and higher concentrations of surfactant, the cross-linking ability is lost, as too many surfactant aggregates in relation to polymer chains are formed, and thus the probability that more than one chain is involved in the aggregates decreases. (The system is presently examined by means of small angle neutron scattering (SANS) in order to gain some insight into the structures connected with the thermogelling behaviour.) If all these experiences above are combined, a vision of a highly complex system emerges, that requires a very delicate balancing in order to accomplish the behaviour desired in this investigation.

4.2. 1% EHEC, increasing amount of TM with CTAB

The thermal gelation phenomenon appears to be very intimately related to the phase separation, or clouding in the system, as can be seen in all the phase maps presented. If salt is added, the phase separation tendency is always overcome by an increased amount of surfactant, whereas the

gelation seems more restricted to a finite balance of the surfactant to polymer ratio, consequently leading to a separation of the gelation from the single phase regions, at some concentration of salt, depending on the type of salt and ionic surfactant used. One interpretation of this behaviour could be to consider the number of micelle-like aggregates as too high in relationship to the number of polymer chains at the more elevated surfactant concentrations needed to overcome the phase separation tendency in the presence of salt.

We have chosen to introduce the term 'turbidity boundary' instead of cloud-point (CP) curve or phase boundary, which are often considered as synonyms when phase diagrams of aqueous EHEC systems are obtained. The reasons are mainly two; the polymer used in this study is rather polydisperse and we cannot guarantee that the thermodynamical phase boundary exactly coincides with the first sign of haziness, especially in the domain exhibiting thermogelling properties. The samples in the area called clouded gel scatter visible light, implying that they include larger objects or repetitively ordered units in the same size range as the wavelength of the light, but they seem, at least, very kinetically stable. Some samples have been equilibrated for several weeks without any signs of macroscopic phase separation, perhaps due to the high viscosity. The turbidity boundary can thus be regarded as the 'worst case' phase boundary, ensuring that no overestimations of the single phase area are made.

4.3. Effects of the surfactant alkyl chain length

The surfactant alkyl chain length is seen to strongly affect the behaviour, even with no addition of extra salt, as shown with the alkyltrimethylammonium bromide series in Fig. 1a and 2a–c. The decrease in the critical micelle concentration of the surfactant in the presence of polymer can be used as a tool to monitor the strength of the polymer-surfactant interaction, as mentioned above. The CAC values for most of the surfactants used in our work have been determined by means of titration microcalorimetry in the presence of a similar type of EHEC at a concentration of 0.25% (w/w) and temperature 25° C (Wang and Olofsson, 1994). The values for DoTAB, TTAB and CTAB were reported to be 12, 2.5 and 0.3 mmol/kg, respectively, to be compared with the CMCs listed in Table 1. A clear connection is seen with the characteristics of the phase maps. For DoTAB, with the shortest alkyl chain length and the highest CMC, the interaction with EHEC is the weakest, giving the highest CAC, also in relation to the CMC, and a phase behaviour as if there were an 'inbuilt' synergistic effect due to the high counterion and monomer concentrations at the onset of aggregation. For TTAB, with a lower CMC and CAC, the interaction is stronger, but still a small initial drop in the turbidity line is seen. For CTAB, which has an even lower CMC and where the CAC is only about one-third of this value, the interaction is stronger, which is also reflected in the monotonic increase of the turbidity line. No CAC has been reported for OTAB, with the lowest CMC in this series, and the phase map is restricted to temperatures exceeding the Krafft point. This part of the map is, however, very similar to the map with CTAB, implying that, even if the interaction with EHEC might be somewhat stronger, the overall effect of the phase behaviour is not markedly different.

4.4. Comparison with an anionic surfactant

Wang and Olofsson (1994) have also investigated the influences of the same type of EHEC on the SDS micellisation, and a corresponding CAC of 1.6 mmol/kg is reported. This value is definitely lower than for CTAB if put in relation to the CMC, indicating a stronger interaction with the polymer, which is also seen as a stronger viscosity enhancement with SDS (Carlsson et al., 1990a,b; Wang and Olofsson, 1994). The stronger interaction seems, moreover, to lead to a somewhat higher 'specificity' with respect to the surfactant concentration interval exhibiting the thermosetting property, which is seen to be narrower for SDS than for CTAB (Fig. 3a and 1a). The capacity to incorporate TM is obviously much lower for SDS and we believe this is related to the organic character of the drug. A simple com-

patibility test was performed, where TM was added to a 20 mM aqueous solution of SDS without EHEC up to a concentration of 80 mM, resulting in the formation of an oily-like precipitate, which then could be resolubilized with more SDS.

4.5. Effect of the salt type

From our perspective, a fair interpretation of the results listed in Table 2 is to consider the type of counterions (to the surfactant) introduced by the addition of salt, although it must be stressed that we are dealing with multicomponent systems, mixed counterions and possible differences in the dissociation degree of the (hydrogen)maleate ion. It is seen with the cationic surfactant CTAB that the chloride salts (small inorganic anion with low polarisability) exhibit less drastic effects than the (hydrogen)maleate salts (larger organic anion) and the same tendencies are shown with the anionic surfactant SDS – less influence with the sodium salts than with the large organic timolols. A change of the timolol counterion from (hydrogen)maleate to chloride leads to a more than 40% reduction in the CTAB concentration needed to produce the desired phase behaviour. We find this comparison very relevant, since the change of counterions of a drug, in order to reach a specific property, is a well-known tradition within the pharmaceutical field, and most drugs are very organic in their character. The polymer effects on the micellar ionisation degree for CTAC and SDS have been investigated with a similar type of EHEC (Zana et al., 1992; Kamenka et al., 1994). They reported an almost exactly doubled degree of ionisation at 20° C for both surfactants in the presence of 0.5% (w/w) EHEC. It is well known that the degree of counterion binding is influenced by the polarisability of the ions for example, and we think that this could have a substantial effect on the behaviour in this system. The effects of equimolar concentrations of NaCl, NaBr, NaI and NaSCN on the phase behaviour of a 0.9% (w/w) aqueous solution of a more hydrophilic (non-gelling) type of EHEC with CTAB have been investigated (Karlström et al., 1990). A trend towards a wider two-phase area, with respect to surfactant concentration range with the more polarisable anions was seen. The timolol cation in particular, but also the (hydrogen)maleate are rather large and might also contribute with 'steric' hindrance, at least around the oppositely charged surfactant clusters (or even be partly solubilized), and thus interfere more strongly with the polymer-micelle arrangements.

4.6. Effect of surfactant counterion

The reasoning above can also account for the positive effect of the exchange of the counterion of CTA^+ from bromide to chloride, since the micellar ionisation degree is known to be higher for the chloride salt in aqueous solutions (Fabre et al., 1980). The CAC for CTAC has also been determined in the presence of a similar EHEC by Wang and Olofsson. It is reported to be 0.3 mmol/kg, thus indicating a somewhat stronger interaction than for CTAB, as the CMC for CTAC is slightly higher than for CTAB.

An optimum alkyl chain length for alkyltrimethylammonium halides seems to be C_{16}, whereas C_{18} seems to be equivalent, if soluble*, and an ordered list of the cationic surfactants tested in this investigation, with decreasing effectiveness can be made:

OTAC and CTAC > CTAB > TTAB ≫ DoTAB and OTAB*

It might seem rather preposterous to make such effort in order to optimise the properties of the thermogelling EHEC system, which appears to be very sensitive to salt, being aware of the much higher ionic strengths in physiological fluids. Despite this fact, it has been seen that if the system is readily isotonized with a nonionic excipient, e.g., glycerol, it has the capacity to form gels if poured into excess volumes of physiological saline, diluted HCl(aq), or simulated gastric and intestinal juices at 37° C (Lindman et al., 1991). These gels, moreover, did not change their volume appreciably within 1 h, although they had a somewhat milky appearance at the interfaces to the medium, indicating an enhanced salt tolerance once the gels are formed. This type of 'skin'

formation was also seen in a simulated tear medium for the isotonized TM-containing thermogelling system (Lindell and Engström, 1993). Interestingly, no differences in the in vitro release profile of TM could be detected in comparison with that observed for gels in a low-salt medium, where the remaining gels were unchanged (clear) at the end of the experiment (Lindell et al., 1991). These findings indicate that the system might be used as an in situ gelling matrix for drug delivery. Another application, which has been proposed, is a drinkable bulk laxative, or 'liquid fibre', and in vivo effect studies in the rat and humans have shown that spontaneous gelation occurs in the gastro-enteric system and retards the gastric emptying markedly (Tomlin et al., 1993).

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