International Journal of Pharmaceutics, 95 (1993) 219–228

IJP 03205

In vitro release of timolol maleate from an in situ gelling polymer system

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(Received 1 December 1992)
(Modified version received 18 January 1993)
(Accepted 20 January 1993)

Key words: Timolol maleate; Ethyl(hydroxyethyl)cellulose; In situ gelling vehicle; Phase behavior; In vitro release

Summary

A thermogelling drug delivery system composed of a cellulose ether (ethyl(hydroxyethyl)cellulose – EHEC), an ionic surfactant and water was thoroughly characterized in the presence of timolol maleate with respect to phase and rheological behaviour, as well as in vitro drug release. The phase studies reveal that gelling systems may be formed with 0.34% (w/w) timolol maleate, and that the gelling behaviour is sensitive to the surfactant concentration and ionic strength of the solution. The release of timolol maleate from the gels is retarded compared to a non-gelling EHEC system. The release rate is about equal for systems with 1 and 2% (w/w) EHEC, implying that the release is controlled by a low convection in the gels and not by any drug-polymer interaction.

Introduction

Ethyl(hydroxyethyl)cellulose (EHEC) is a water-soluble nonionic cellulose ether polymer (schematic structure segment in Fig. 1a) which under certain conditions can form thermoreversible gels in the presence of ionic surfactants (Carlsson et al., 1990; Lindman et al., 1990). Semi-dilute aqueous solutions of a rather hydrophobic type of EHEC have been shown to exhibit a drastic viscosity increase as the temperature is raised from room temperature. This property makes the system of potential interest for use as an in situ gelling drug delivery system (Carlsson et al., 1991; Lindman et al., 1992; Rydén and Edman, 1992a,b).

The drug timolol maleate is used among other things in eye-drops for the treatment of glaucoma but topically applied ophthalmic solutions often exhibit low bioavailability due to rapid tear fluid turn-over and drainage. One way to overcome this problem which recently has drawn much attention, is the use of in situ gelling polymer systems, such as Gelrite®, as vehicles for ocular drug delivery (Lee, 1990).

Gelrite® is a polysaccharide which gels at low concentrations, e.g., 0.6% (w/w), when mono- or divalent cations are present. The sodium concentration in tears is sufficient to induce the gelation

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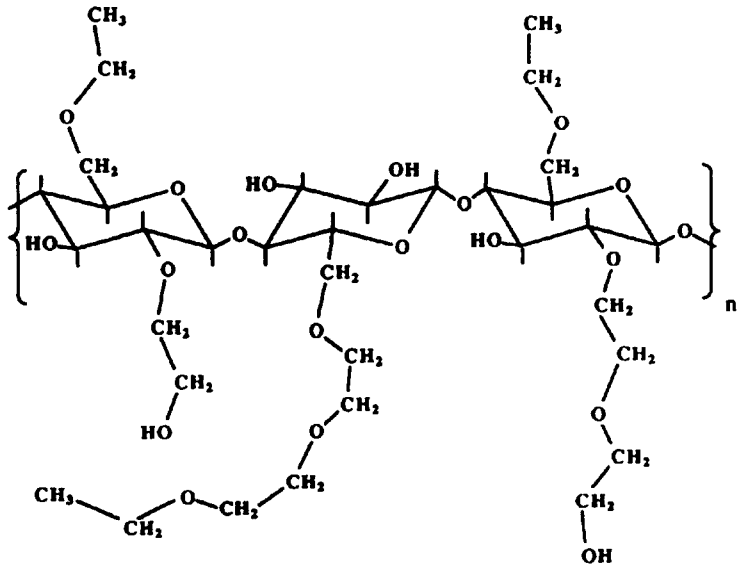
as the solution is instilled into the conjunctival sac (Moorhouse et al., 1981; Rozier et al., 1989). The EHEC system resembles Gelrite® with respect to the polymer concentration required to accomplish gelling, normally about 1% (w/w), but the gelation is temperature-induced.

The objective of this work is to investigate the gel-forming capacity of this system in the presence of timolol maleate and to monitor the in vitro release of drug from the EHEC gel. We have also chosen, for reasons of comparison, to study the in vitro release of timolol maleate from Gelrite® and a more hydrophilic non-gelling EHEC system.

Materials and Methods

Ethyl(hydroxyethyl)cellulose (EHEC) of two types was obtained as a gift from Berol Nobel AB (Stenungsund, Sweden): type A with $DS_{ethyl} = 1.7$ and $MS_{EO} = 1.0$ and average molecular weight of 100 000 g/mol, and type B with $DS_{ethyl} = 0.8$ and $MS_{EO} = 0.8$, having a similar molecular weight distribution to type A. (The degree of substitution (DS) denotes the number of hydroxyl groups on each anhydroglucose unit which have been substituted, and can vary between 0 and 3. The molar substitution (MS) term has been introduced as alkylene oxides (e.g., EO) can form



(a)

(b)

Fig. 1. A possible structure segment of the EHEC polymer (a) and molecular formula for the ionic surfactant long chain alkyl betainate (tetradecyl oxycarbonyl-*N,N,N*-trimethyl methanaminium chloride) (b).

oligomeric chains on the cellulose. This value could thus theoretically exceed 3.)

Gelrite® (Kelco Division of Merck, U.S.A.), timolol maleate, glycerol, D-mannitol and Trizmal™ buffer (Sigma, U.S.A.) were used as received, and the ionic surfactant long-chain alkyl betainate (tetradecyl oxycarbonyl-*N,N,N*-trimethyl methanaminium chloride) used in the EHEC system to cause gel formation was a gift from Berol Nobel AB and used without further purification.

Doubly distilled water was used, and remaining excipients (simple salts) were of analytical grade and purchased from Sigma.

Preparation of solutions

EHEC aqueous solutions, containing 1.0% (or 2.0%) (w/w) polymer, were prepared by the addition of hot distilled water during agitation with a magnetic stirrer. The solutions were then kept cold (in a refrigerator) for at least 2 days to simplify the dissolution. Long-chain alkylbetainate, timolol maleate (TM) and glycerol were added to the solution by weighing. The formulations were then mixed by magnetic stirring or with a Whirlimixer™ shaking machine.

A 0.6% (w/w) Gelrite® dispersion with 0.34% timolol maleate was prepared by dissolving the components in a 0.01 M Tris maleate buffer. Mannitol (4.5%) was used as an isotonizing agent to keep the ion content below the critical concentration of gelation. The dispersion was then heated and stirred at 90°C for 15 min prior to cooling.

All solutions, regardless of type, were left to equilibrate in darkness, at room temperature until the following day, when the experiments were started.

Phase studies

Phase diagrams for 1.0 and 2.0% EHEC type A aqueous solutions, and various amounts of alkyl betainate, with and without 0.34% TM, were obtained in the temperature interval of 20–50°C. Samples, prepared in glass tubes, were equilibrated for 2 h at each temperature in a block thermostat (Grant BT3), and the phase behaviour was studied by visual observation. The macroscopic fluidity was also estimated to define regions with the desired thermogelling property. Glycerol was added as isotonizing agent to the samples with the most pronounced gelling behaviour. It was ensured that the addition of glycerol did not alter the gelling behaviour.

Rheology

A Bohlin VOR rheometer system (Bohlin Rheology, Lund, Sweden) was used to monitor the steady-flow viscosity (η), at various, stepwise increasing shear rates (D). The measuring system consisted of a concentric (C25) cylinder, connected to an exchangeable torque element. The instrument was equipped with a temperature control unit and the samples were equilibrated for 10 min before the measurements.

In vitro drug release

The in vitro release of timolol maleate was investigated with an USP rotating paddle apparatus (Prolabo Dissolutest, France). Diffusion cells, originally designed for adhesive cutaneous forms (Pro 07174906, Prolabo), with a cylindrical 2.6 mm counterbore (diffusion surface 21.23 cm^2) to be put at the bottom of the flasks, were used. 4.0 g of test solution was poured into the cells and covered with a 30-mesh stainless steel net. The samples were then preheated at 37°C for 30 min and the experiment was started as each cell was immersed in a flask filled with 500 ml simulated tear fluid (Rozier et al., 1989) which had been equilibrated at 37°C. The stirring rate was 20 rpm. The release of timolol maleate was spectrophotometrically detected at selected time intervals at 295 nm (UV-lamp, Lambda 2, Perkin-Elmer, Germany) by the use of a peristaltic pump (Ismatech IPN-16, Labinett, Switzerland), continuous flow cuvettes and tubings.

Results

Phase behaviour

EHEC belongs to a class of nonionic polymers which exhibit a quite complex phase behaviour. Instead of becoming more water soluble with increasing temperature, aqueous EHEC solutions

separate into two phases above a certain critical temperature, the so-called cloud point (CP). This phenomenon is easy to detect with the eye as the turbidity of the solution increases very rapidly at the clouding temperature and gives the sample a milky appearance.

The CP is strongly influenced by the degree of substitution and balance between the substituents, hydrophilic ethylene oxide (EO) groups and hydrophobic ethyl groups, in addition to polymer concentration and degree of polymerization. This can be illustrated with the two EHEC types used in this work. The clouding temperature in 1.0% pure aqueous solution is 30°C for the more hydrophobic type A polymer, and 63°C for the more hydrophilic type B polymer.

1% EHEC without addition of drug

The phase behaviour of 1.0% (w/w) EHEC type A, and the ionic surfactant long chain alkyl betainate in various concentrations, is illustrated in Fig. 2a. The cloud point (CP) of pure 1.0%

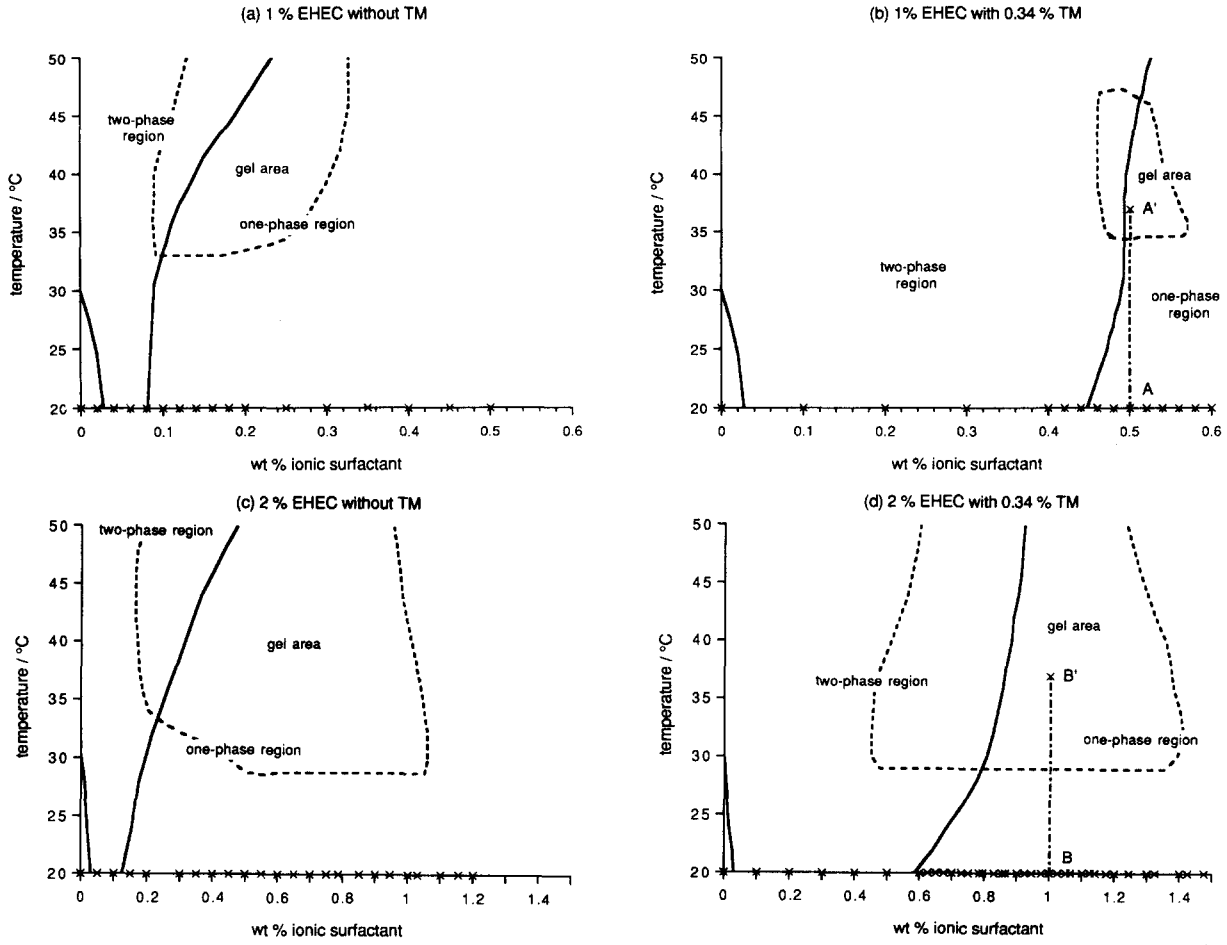


Fig. 2. Phase diagrams of the aqueous systems (a) 1% EHEC A without timolol maleate (TM), (b) 1% EHEC A with 0.34% TM, (c) 2% EHEC A without TM and (d) 2% EHEC A with 0.34% TM at various concentrations of ionic surfactant (long chain alkyl betainate). Continuous lines show the phase boundaries between the one- and two-phase regions. The areas exhibiting gelling characteristics are indicated with dashed lines. The crosses show the surfactant concentration of the samples from which the phase diagrams are estimated. The points A (B) and A' (B') indicate the composition and states at 20 and 37°C of the 1% (2%) EHEC systems with 0.34% TM chosen for further investigations in this work.

polymer solution is 30°C (taken as the last sign of haziness on cooling). The initial effect of adding small amounts of surfactant is a slight decrease in the CP, which goes through a minimum, below room temperature, at approx. 0.05 wt% surfactant (corresponding to a concentration of 1.4 mmolal). The solubility is then monotonically increased with increasing surfactant concentration.

As to the macroscopic viscosity, it is observed that in a sample with between 0.08 and 0.32% surfactant, a rise in temperature makes the system more viscous and there is an area (in temperature and concentration) exhibiting gel characteristics (indicated in Fig. 2a). This area is partly in the two-phase region, i.e., at temperatures above the CP. These gels have a more or less pronounced milky appearance, and if left to stand, they separate into two phases – one highly viscous and the other with water-like fluidity. Gels formed in the one-phase area are, on the other hand, glass clear and do not separate.

A moderate increase in viscosity on heating from 20–35°C is observed at surfactant concentrations slightly beyond the area of gelling (0.33–0.38%). At more elevated temperatures (45–50°C) there is a gradual decrease, and the samples become liquid-like, but are still clear and monophasic. The thermothickening property is totally lost at surfactant concentrations above 0.40%, as the solutions become less viscous when the temperature is raised. In addition, there is a slight tendency for increased viscosity with rising surfactant concentration at room temperature.

1% EHEC with addition of drug

Fig. 2b shows the effect on the phase diagram after adding 0.34% timolol maleate (TM). The CP of the EHEC/TM solution without ionic surfactant is unaltered at 30°C, and the decrease in clouding temperature when small amounts of surfactant are added to the system is similar to the case without TM. The range of the two-phase region, however, is much more extended with TM. The area exhibiting thermogelling characteristics is also simultaneously shifted to higher surfactant concentrations (0.46–0.56%) and restricted to a narrower range of concentration and temperature.

Based on these facts, it is possible to deduce the most appropriate surfactant concentration in a formulation to be used for drug delivery of 0.34% TM with respect to temperature-induced gelling. A system containing 1.00% (w/w) EHEC type A, 0.34% TM and 0.50% surfactant is a clear solution with high fluidity at room temperature (20°C). This is indicated as point A in Fig. 2b. It will become a clear monophasic gel (indicated as point A'), when administered to a cavity at body temperature.

2% EHEC without addition of drug

The phase diagram of a system containing twice as much polymer is presented in Fig. 2c. In comparison with the 1.0% EHEC system, the CP remains unaltered at 30°C when no surfactant is added. Here as well, the effect of adding small amounts of surfactant is an initial decrease in solubility but the subsequent increase, with increasing surfactant concentration, is less steep. The area exhibiting thermogelling characteristics is found between 0.18 and 1.05% surfactant, with a starting point at 0.25% of the one-phase domain. This area is thus much wider with respect to surfactant concentration range.

2% EHEC with addition of drug

Fig. 2d illustrates the phase diagram when 0.34% TM is present in the 2.0% EHEC system. The CP with no surfactant remains unaltered and the initial decrease in solubility at very low surfactant concentration is identical with those in the previously presented systems. The CP depression is much more pronounced with TM, which was also noted for the 1.0% EHEC system. Similarities with the 2.0% EHEC system without drug include the less steep solubility increase with surfactant concentration than in the 1.0% systems, and the more expanded area with thermogelling properties – between 0.50 and 1.40% surfactant with the lowest concentration of 0.80% for any monophasic gel. It is also evident that the addition of drug shifts the gelling area to higher surfactant concentrations.

In analogy with the reasoning about the most suitable composition of a TM-containing thermogelling system with 1.0% polymer, a system con-

TABLE 1

In vitro release system compositions

Component/system (w/w %)	Timolol maleate (aq)	EHEC B (1%)	Gelrite® [a]	EHEC A (1%)	EHEC A (2%)
Timolol maleate	0.34	0.34	0.34	0.34	0.34
NaCl	0.85	–	–	–	–
EHEC type B	–	1.00	–	–	–
EHEC type A	–	–	–	1.00	2.00
Alkyl betainate surfactant	–	0.50	–	0.50	1.00
Gelrite polysaccharide	–	–	0.60	–	–
Glycerol	–	2.50	–	2.50	2.40
D-Mannitol	–	–	4.50	–	–

[a] Also including 0.01 M Tris maleate buffer.

taining 2.00% (w/w) EHEC type A, 0.34% TM and 1.00% surfactant should roughly exhibit the same properties: a fluid solution at room temperature, indicated as point B in Fig 2d, which is converted to a stiff and clear monophasic gel upon heating to 37°C (point B').

The most evident difference between these systems from a macroscopical point of view is the higher viscosity of the system with doubled polymer and surfactant concentration, both at room temperature and at 37°C.

Viscosity

The steady-flow viscosity results of the two EHEC systems chosen from the phase studies, with compositions given in Table 1 (EHEC A, 1 and 2%), are presented in Fig. 3a and b. Both systems exhibit a slight shear-thinning tendency at 20°C, as the shear rate is increased stepwise from 0.185 to 2.31 s^{-1}. The viscosity is strongly enhanced at 37°C. This effect is especially pronounced at low shear rates, with a 100-fold increase in the 1% system. For the 2% polymer system, the viscosity is increased more than 30-fold. The systems are also more shear-thinning at this temperature.

In vitro release

In Fig. 4 the in vitro release of timolol maleate (TM) is shown for five different systems (compositions given in Table 1). The release from the reference physiological NaCl aqueous solution is

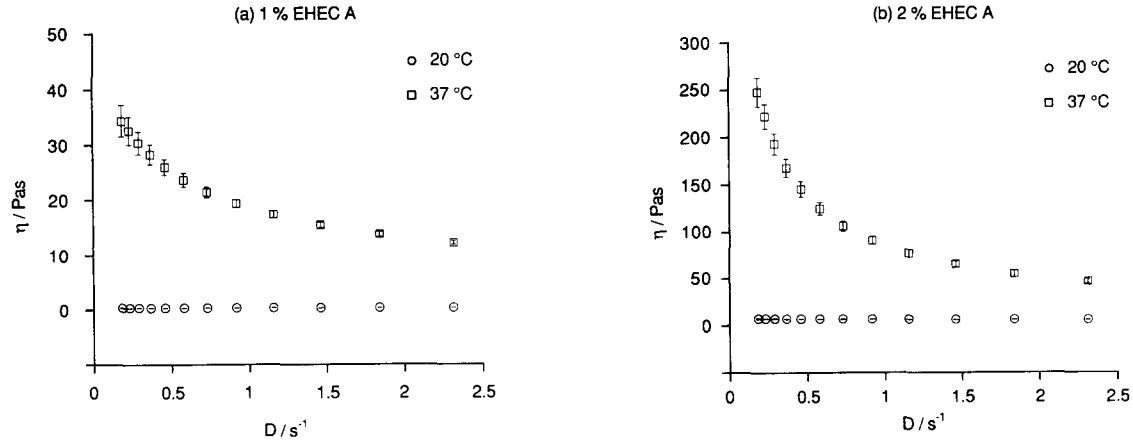


Fig. 3. Steady-flow viscosity vs shear rate at 20 and 37°C for (a) 1% EHEC A and (b) 2% EHEC A thermogelling systems. The compositions are given in Table 1.

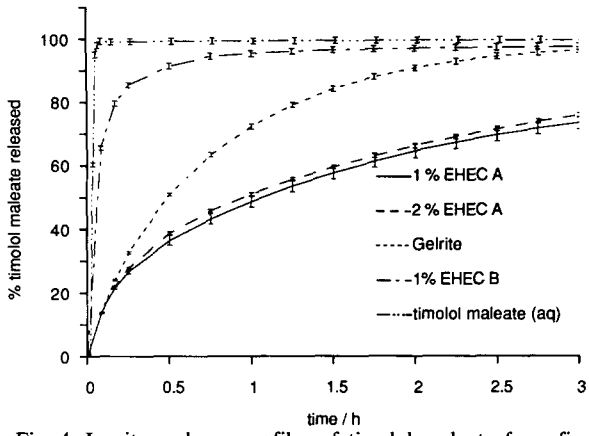


Fig. 4. In vitro release profiles of timolol maleate from five different formulations. The compositions are given in Table 1.

almost instantaneous, with 100% TM released within a few minutes. With a 1.00% non-gelling EHEC type B system (CP at 67°C), the drug release is slightly retarded compared to the reference solution, but more than 90% is released within the first 30 min. The two selected thermogelling EHEC systems exhibited the most retarded release of the drug in this study. There is no significant difference in the release rates obtained with the 1.0 and 2.0% gels. Half of the drug amount is released after 1 h and 75% after 3 h. A pronounced decrease in release rate of the drug is also attained with the in situ gelling Gelrite® system. Approx. 50% of the total TM is released after 30 min and 1 h later, this amount exceeds 80%.

Discussion

Phase behaviour

Much effort has been made to investigate and explain the complex physico-chemical behaviour of semi-dilute aqueous solutions of EHEC interacting with surfactants, inorganic electrolytes and other cosolutes (Carlsson, 1989).

A mechanism for the gel formation, based on a cooperative binding of the ionic surfactant to the polymer, leading to the formation of micelle-like clusters that could interconnect hydrophobic segments in different polymer chains, has been proposed (Carlsson et al., 1990; Lindman et al., 1990). The formation of such polymer networks would strongly increase the viscosity.

The polymer-surfactant interaction has, moreover, been found to exhibit a reverse temperature dependence with a higher degree of surfactant binding at increased temperatures, which may explain the thermo-setting property of these gels (Carlsson et al., 1989a,b; Karlström et al., 1990). The decrease in viscosity at more elevated temperatures is thought to be caused by a destruction of the networks due to thermal motions.

Small amounts of electrolytes has been shown to drastically affect the phase behaviour of systems containing non-ionic polymers and ionic surfactants (Carlsson et al., 1986; Karlström et al., 1990). This is indeed true for the EHEC-surfactant system investigated in this study. When as little as 0.34%, or 8 mmolal timolol maleate is added to the system, the phase boundaries as well as the area with gelling characteristics are shifted to considerably higher concentrations of surfactant. This effect has been denoted synergistic due to the fact that electrolytes at these low concentrations do not normally influence the CP of the pure polymer solution, and the surfactant would in itself give rise to an almost monotonic increase of the solubility if no other electrolytes were present. The resulting effect, as when both are simultaneously present, is a more or less pronounced depression of the solubility, which can be overcome at more elevated concentrations of surfactant. The extension and depth of this solubility gap is dependent on the concentration of electrolyte as well as the type of electrolyte and surfactant (Carlsson, 1989; Lindman et al., 1990). The very fact that there is an initial, narrower solubility decrease, even when no TM is present in our systems (Fig. 2a and c), can be explained by impurities in the substances used. Both the EHEC and the surfactant were used as received, without any further purification.

Investigations of the thermogelling hydrophobic EHEC/ionic surfactant system performed in aqueous solutions without additives have shown that the gelling effect is very general with respect to surfactant (Carlsson et al., 1990). It could be either anionic or cationic, as long as it exceeds a

lower limit in hydrophobicity, which is reflected in its critical micelle concentration (CMC). No effect is usually seen from surfactants with hydrocarbon chains of less than 10 carbons. The amphiphilic local anesthetic tetracaine · HCl, for example, with a CMC of approx. 70 mM, did not bring about any gel formation with the EHEC type A quality used in this work.

In the present study a number of different ionic surfactants were initially tested as the surfactant in a 0.34% TM-containing 1.0% EHEC type A system; anionics such as sodium dodecyl sulphate (SDS) and sodium oleate, cationics such as cetyltrimethylammonium bromide (CTAB) and its analogue with a 14-carbon hydrocarbon tail, beside the long chain alkyl betainate. All but one, the long chain carbonyl betainate, failed to accomplish any thermogelling in the monophasic area with respect to surfactant concentration, implying more specific demands on the structure of the ionic surfactant when a third component, TM, was added. The molecular structure of the long chain carbonyl betainate (in Fig. 1b) is strikingly similar to the CTAB structure – the only differences being an ester linkage at the second carbon from the head-group in the betainate, and different counterions (chloride instead of bromide). These findings are very interesting and should be more methodically investigated, but are beyond the scope of this work.

There is another property of the long chain carbonyl betainate that needs to be discussed. This substance has an in-built instability due to the ester linkage. The cationic charge adjacent to the ester linkage makes the bond more stable in acidic media but very labile under alkaline conditions (Thompson and Allenmark, 1989). No pH adjustments were made in our investigations and the test solutions exhibited pH values of about 4.5, implying a very low rate of hydrolysis and the phase studies were performed within 24 h.

One interesting observation during this investigation was that the surfactant seemed to become more stable when drug was added, i.e., samples from the system with 2% EHEC, 0.34% TM and surfactant, stored for 3 months at room temperature, exhibited the same phase behaviour as when first checked. In results from a recent study, the presence of counterions with a more amphiphilic character, such as tetradecanoic acid, were seen to almost completely stop the hydrolysis of this long chain alkyl betainate (Thompson and Allenmark, 1992). Inorganic salts, like NaBr, were also reported to have a negative influence on the rate of hydrolysis. It might thus be possible that the hydrogen maleate ions in our system can exhibit a similar effect on the rate of hydrolysis of the surfactant.

In vitro release

It should be noted that the conditions at the start of the in vitro release experiments were slightly different for the Gelrite® system, when compared to those containing EHEC. As the samples were preheated to 37°C, the EHEC systems could be regarded as fully transformed at the start, whereas the gelation first was initiated on contact with the simulated tear medium in the Gelrite® case. The difference is believed to be of minor importance, as this process is almost instantaneous and no burst-like effects can be seen in the release profile from the Gelrite® system.

The Gelrite® polysaccharide was dispersed according to a method regarded as producing equivalently well-dispersed solutions as does terminal autoclaving (Deasy and Quigley, 1991). The release profile of TM from the Gelrite® system in our investigation differs slightly from the results of an earlier study performed under similar conditions (Rozier et al., 1989). This could be explained by a difference in the diffusion cell design, giving nonidentical diffusion surface to sample volume ratios.

As the release experiments were completed, no remaining signs of the samples were seen in the diffusion cells, when the reference NaCl(aq) or the non-gelling EHEC type B systems were tested. In the Gelrite® case, a clear and stiff gel was found, and the remainder of the EHEC type A system was a less voluminous, clouded and more brittle gel. At similar, earlier performed experiments with the EHEC type A systems, but in a low-salt (8.15 mM NaCl) medium, the remainder was found as a clear gel (Lindell et al., 1991). No significant differences in the TM release profile were seen in comparison to the

simulated tear medium experiments. Consequently the isotonization of the EHEC system with glycerol does not counteract the long-term influx of electrolytes from the medium, and a clouded skin is seen to be formed at the sample surfaces, which grows deeper with time.

No distinctions could be made between the TM release profiles of the two EHEC type A systems, even though the 2.0% polymer system is much more viscous, implying that the primary cause of the TM release retardation could be derived from a simple convection-hampering effect, keeping the samples at place in the diffusion cells, avoiding subsequent mixing with the receptor medium.

The surfactant release was simultaneously monitored with the release of drug in a study of the in vitro release of riboflavin from a gel initially containing 1.0% EHEC, 3.0 mM SDS and 0.004% riboflavin (Lindman et al., 1992). The SDS release was here found to be considerably slower than the release of riboflavin, indicating the stronger interaction between surfactant and EHEC.

In summary, this study shows that thermoreversible gels, including 0.34 wt% timolol maleate, may be formed with a relatively hydrophobic EHEC polymer and an ionic surfactant. The systems are, however, sensitive to the choice of ionic surfactant and the amount of added electrolyte (e.g., timolol maleate). The in vitro release of the drug is retarded in comparison with a non-gelling EHEC system, and the release rate is similar to the in situ gelling system produced by the polysaccharide Gelrite® (Rozier et al., 1989). This study clearly demonstrates that, in order to use the thermogelling property of the EHEC-ionic surfactant system for drug delivery, careful studies of the phase behaviour of the system, including the drug, must be performed.

Acknowledgements

This work was supported by Kabi Invent AB. Conny Bogentoft, Anders Carlsson, Bodil Ericsson, Björn Lindman, Lars Andersson, Christian von Corswant and V.J. Rajadhyaksha are thanked for their interest and kind help in different ways.

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