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Micellization and gelation in block copolymer systems containing local anesthetics

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Abstract

A formulation consisting of a eutectic mixture of lidocaine and prilocaine, Lutrol® F68 and Lutrol® F127, suitable for anesthetizing the periodontal pocket has previously been developed. This consists of discrete micelles with a diameter of 20–30 nm and has a suitable gelation temperature, a good release profile and excellent long-term stability. In this study, the unimer/micelle transition and gel formation of the formulation, in its concentrated state, are investigated using differential scanning calorimetry (DSC), dye solubilization, rheology, and nuclear magnetic resonance (NMR) self-diffusion. The critical micellization temperature (cmt) and gelation temperature are found to be interconnected and influenced by cosolutes, such as electrolytes and hydrophobic substances, the latter as found particularly for the eutectic mixture of the local anesthetic agents lidocaine and prilocaine. Both cmt and the gelation temperature decrease with increasing pH of the system, i.e. at reduced solubility of the active ingredients. Moreover, both cmt and the gelation temperature increase upon diluting the system with water. The ratio between the two block copolymers present in the system also has an impact on both cmt and the gelation temperature, resulting in a decrease in onset temperature of both processes with an increase of Lutrol® F127. The amount of the active ingredients present in the micelle phase depends on the pH of the system being approximately 0% w/w at pH 5, 50–60% w/w at pH 7.8 and 80% w/w at pH 9.

Keywords: Micellization; Gelation; Microemulsion; Lidocaine; Prilocaine; PEO–PPO–PEO block copolymers

1. Introduction

Poly(ethylene oxide)–poly(propylene oxide)–poly(ethylene oxide) block copolymers have been used in pharmaceutical formulations owing to their ability to self-aggregate, thereby displaying a rich phase behavior, forming, e.g. micelles and liquid crystalline phases (Alexandridis et al., 1994; Alexandridis and Hatton, 1995). The micelles formed consist of a hydrophobic core of propylene oxide and a hydrophilic corona of ethylene oxide (Zhou and Chu, 1988; Wanka et al., 1990;

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Brown et al., 1991; Linse and Malmsten, 1992; Malmsten and Lindman, 1992; Mortensen, 1992; Yu et al., 1992; Mortensen and Pedersen, 1993; Alexandridis et al., 1994; Wanka et al., 1994; Hecht et al., 1995) thus being able to solubilize hydrophobic substances (Alexandridis and Hatton, 1995). A notable feature of these systems is that the self-assembly is highly temperature dependent. More specifically, an increasing temperature facilitates self-association, resulting in a strong decrease in the critical micellization concentration (cmc) (Linse and Malmsten, 1992) and at a given polymer concentration a critical micellization temperature (cmt) occurs (Alexandridis and Hatton, 1995).

Several techniques have been described in the literature for measuring the cmc/cmt of block copolymers, e.g. light scattering and fluorescence spectroscopy, surface tension, nuclear magnetic resonance (NMR) spectroscopy and differential scanning calorimetry (DSC) (Alexandridis and Hatton, 1995). Of special interest to the present investigation are the possibilities to study block copolymer self-assembly by means of the latter technique. The micellization, detected with DSC, has been shown to be endothermic and the self-assembly therefore inferred to be driven by the entropy gain when the micelles form, presumably an effect of both hydrophobically driven self-assembly, and the increase in the non-polar conformational state of PEO with increasing temperature (Wanka et al., 1990; Yu et al., 1992; Linse, 1993a,b; Alexandridis et al., 1994; Hecht and Hoffman, 1994; Wanka et al., 1994; Alexandridis and Hatton, 1995; Patterson et al., 1996).

Another characteristic property of PEO–PPO–PEO block copolymers is the thermoreversible gelation displayed by some concentrated block copolymers at temperatures close to room temperature (Wanka et al., 1990; Brown et al., 1991; Wang and Johnston, 1991; Yu et al., 1992). The gel formation occurs when the micelle concentration reaches the critical volume fraction of 0.53, i.e. where the micelles are locked into a hard sphere crystalline structure due to their high volume density (Mortensen et al., 1992; Mortensen, 1992; Mortensen and Pedersen, 1993; Alexandridis et al., 1994; Hecht and Hoffman 1994; Alexandridis and Hatton, 1995; Mortensen, 1996). The onset of gelation can easily be detected by, e.g. oscillating rheological measurements (Wanka et al., 1990; Brown et al., 1991; Scherlund et al., 2000).

Both the micellization and gelation are affected by a range of factors, e.g. temperature, copolymer composition, molecular weight, concentration and presence of cosolutes, such as surfactants, electrolytes, and hydrophobic substances (Malmsten and Lindman, 1992; Linse, 1993a,b; Mortensen and Pedersen, 1993; Wanka et al., 1994; Alexandridis et al., 1995; Alexandridis and Hatton, 1995; Alexandridis et al., 1996; Alexandridis and Holzwarth, 1997).

Of particular interest to the present investigation are the effects of the concentration and solubilization of the local anesthetic agents lidocaine and prilocaine. In previous studies, a microemulsion formulation based on these active ingredients and the block copolymers Lutrol® F127 and F68, suitable for anesthetizing the periodontal pocket, was presented. The system has a gelation temperature between room and body temperature depending on the pH and concentration of the formulation components. The active ingredients in the form of a eutectic mixture of lidocaine and prilocaine, are mainly solubilized in the micelles of the system, albeit depending on the pH (see below). Using NMR and photon correlation spectroscopy (PCS), on diluted systems, the cmt was found to be 25–35°C, above which micelles of the diameter 20–30 nm were formed. However, very small oligomer micelles could not be excluded even at temperatures lower than 25°C (Scherlund et al., 1998, 2000). The formulation developed also displayed a pH dependent release of the active ingredients, but at pH 7.8, a good release profile could be combined with a suitable gelation behavior, excellent long-term stability, etc. (Scherlund et al., 2000).

Considering the many promising features of the previously developed formulation, we were interested in gaining a deeper knowledge of the unimer/micelle transition as well as the gel formation of the system in its concentrated state, and how these processes are influenced by the factors described above. Thus, the aim of the present study was to investigate the influence of active

ingredients, block copolymer ratio, pH, and addition of cosolutes such as salts on these processes using DSC, dye solubilization, rheology, and NMR self-diffusion.

2. Materials and methods

2.1. Chemicals

Lutrol® F68 (EO_{79} PO_{28} EO_{79}), F127 (EO_{99} PO_{65} EO_{99}) and Pluronic® P85 ($EO_{26}PO_{40}EO_{26}$) were obtained from BASF Svenska AB, Sweden, whereas prilocaine and lidocaine, were from Astra AB, Södertälje, Sweden. 1,6-diphenyl-1,3,5-hexatriene (DPH), NaCl, NaSCN, *t*-butanol, D_2O, DCl and NaOD (all of 99.8% purity) were from Sigma Chemicals, USA. Distilled water, 2M HCl and 2M NaOH were used as appropriate. All chemicals were used as supplied.

2.2. Preparation of formulations

For formulations containing active ingredients, prilocaine and lidocaine were either mixed in a ratio of 1:1 (giving a eutectic mixture) (Brodin et al., 1984), or used as single components. Lutrol® F68 and F127 were heated to 70°C together with the active ingredient(s), until a uniform melt was formed. Thereafter distilled water of 70°C was slowly added to the melt during manual agitation. The pH was adjusted to 5, 6, 7 or 7.8 with 2 M HCl and to pH 9 or 10 with 2 M NaOH, and the weight adjusted to its final value with distilled water. After dilution, pH was checked once more, and found to be the same as before dilution. In the NMR studies, D_2O, DCl and NaOD were used instead of the corresponding hydrogenated compounds. For the spectroscopic measurements DPH was added to the formulation. The pure polymer solutions and placebo formulations of Lutrol® F68, Lutrol® F127 and Pluronic® P85 were prepared according to the so-called cold method (Schmolka, 1977a). In short, the block copolymer powder was added to cold distilled water with or without NaCl or NaSCN (for the NMR measurements D_2O was used) in portions during agitation, after which the samples were kept in a refrigerator until turning clear. Distilled water was used as dilution medium when needed (for the NMR measurements D_2O was used). Also here, pH was checked once more after dilution, and found to be the same as before dilution.

2.3. Differential scanning calorimetry

For the DSC measurements an Exstar 6000 DSC6200 SII (Seiko Instruments, Japan) was used. The samples were weighed into 70 μl aluminum containers that were hermetically sealed and thereafter the heat transfer was measured in the temperature range of -10–50°C, with a scanning rate of 5°C/min. In order to ensure the reversibility of the micellization process, scans were run going both up and down in temperature for each sample and the hysteresis was found to be quite small (the difference in position of the peak on going up and down was found to be less than ± 1°C). The measurements gave rise to an endothermic peak upon increasing the temperature and an exothermic peak upon decreasing the temperature. The cmt was taken as the onset of the endothermic peak (Fig. 1a).

All samples were run in duplicates with a variation of less than ± 1°C.

2.4. Rheology measurements

All measurements were performed on a StressTech Rheometer (Reologica AB, Sweden) with a cone/plate and solvent trap system. The polycarbonate cone diameter and angle was 40 mm and 4°, respectively. For the viscosity measurement of the most diluted formulation used in the NMR study, a stainless steel bob/cup system was used and the results were compared to the results obtained using the cone/plate system. The difference between the two systems was found to be less than 10%. Thereafter, the cone/plate system was used for all measurements. The temperature unit had a stability of ± 0.1°C and a temperature range of 5–90°C. Oscillation temperature sweep measurements were performed with a constant stress in the linear viscoelastic region (LVER) from 5–90°C giving the temperature of gelation and the elastic and loss moduli (G' and

G'') of the formulations (Scherlund et al., 1998). The viscosities of the formulations were measured at constant shear rates, i.e. 20 shear rates between 30 and 100 s^{-1}, and at temperatures corresponding to the ones used in the NMR study. The variation in the results were less than $\pm 2\%$.

The measurements were performed in duplicates for all samples.

2.5. Dye solubilization

The non-polar DPH probe was used to study the micelle formation in a system containing 5% w/w active ingredients, 15.5% w/w Lutrol® F127 and 5.5% w/w Lutrol® F68 at pH 7.8, diluted to 70% w/w with distilled water. The probe has a maximum absorbance at 356 nm, which was detected using a UV/VIS Lambda 12 spectrometer (Perkin Elmer, USA) and has been used in several previous studies to investigate the micelle formation of block copolymers (Chattopadhyay and London, 1984; Alexandridis et al., 1994; Svensson et al., 1995). 0.1% w/w of the probe was added to the solution which was shaken and kept in a water bath at different temperatures, within the investigated temperature range, i.e. 5–50°C, for 4 or 12 h before conducting the measurements. Water together with DPH was kept at the same temperatures, as a control, giving negligible absorbencies. Corresponding measurements were also performed for a pure block copolymer system containing 5% w/w of Lutrol® F127.

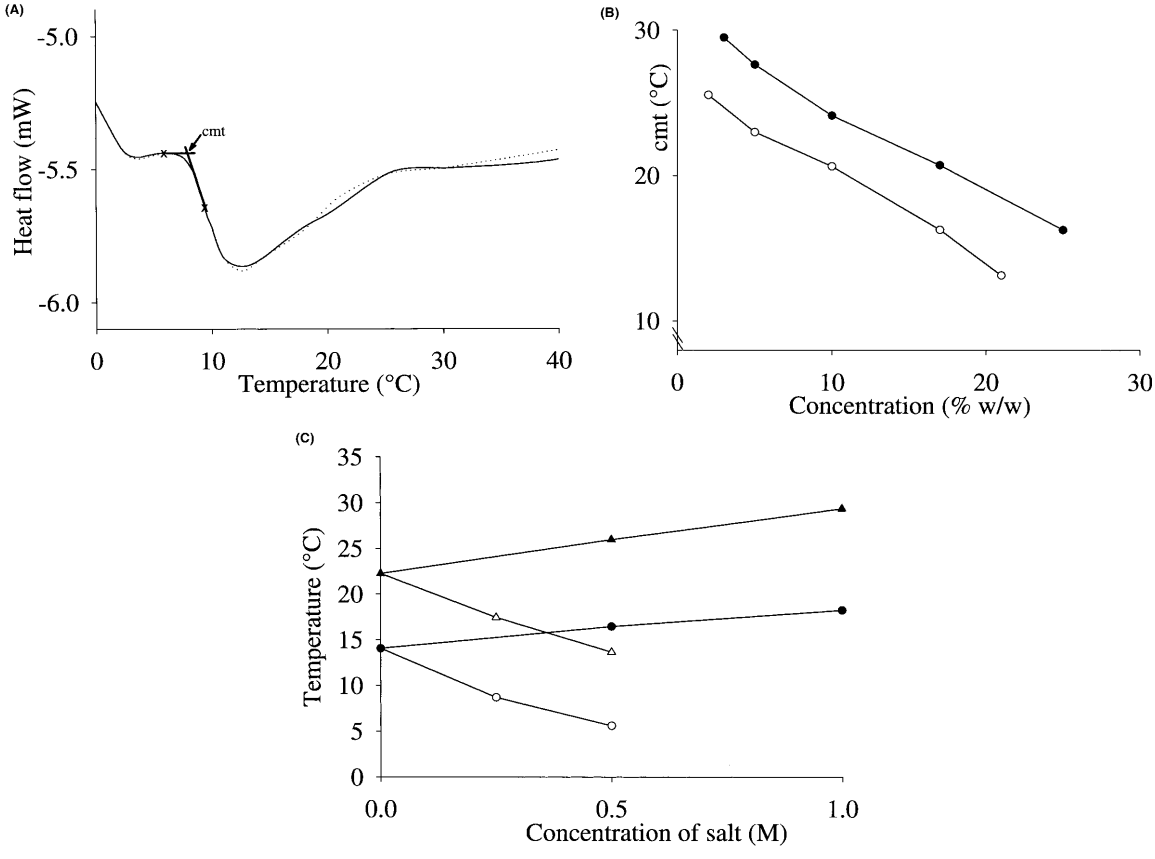

Fig. 1. (a) Endothermic DSC peak of a formulation containing 5% w/w of active ingredients, 15.5% w/w of Lutrol® F127 and 5.5% w/w of Lutrol® F68 at pH 7, first run (filled line) and second run (dotted line). (b) cmt as a function of concentration of Pluronic® P85 (filled circles) and Lutrol F127 (open circles). (c) cmt (circles) and gelation temperature (T_{gel}) (triangles) as a function of salt concentration for a formulation containing 20% w/w of Lutrol® F127 and either NaCl (open symbols) or NaSCN (filled symbols).

Three measurements were performed for each sample.

2.6. Gel filtration

A Superdex 200 HR 10/30 column (Amersham Pharmacia Biotech, Sweden), was connected to a 2150 HPLC pump (LKB, Sweden) and saturated with 25% w/w aqueous solutions of the formulations investigated by adding at least a column volume, i.e. 24 ml, to the column according to the tail-end analysis method (Suzuki and Sasaki, 1971; Ayako and Fumio, 1978; Nyqvist-Mayer et al., 1985). Distilled water of the same pH as the formulation being eluted was used as eluent at 0.5 ml/min and 0.5 ml samples of the filtrate was collected for 2 h using a fraction collector. The amount of active ingredients in each fraction was calculated from a standard curve after measuring the absorbance at 220 nm with a UV/VIS Lambda 12 spectrometer (Perkin Elmer, USA). All experiments were run at 40°C.

2.7. Photon correlation spectroscopy

A System 4700 sub-μm particle analyzer (Malvern Instruments, Sweden), connected to a helium–neon laser operating at 632.8 nm (Spectra-Physics Stabilite, Sweden), was used to determine the particle size at an angle of 90° in selected samples collected during the tail-end experiment. The particle diameter and polydispersity of the measured samples were calculated according to the cumulant method (Koppel, 1972). Samples taken from the first and second plateaus, for each formulation in the gel filtration experiments, were examined and found to have a high polydispersity that was not found for the samples measured before application to the column. The samples were thereafter measured after filtration through a 0.22 μm filter in order to eliminate dust particles interfering with the measurement. This resulted in a polydispersity below 0.3 indicating a uniform distribution in particle size. All measurements were performed at 40°C on diluted solutions, having a total polymer concentration of 1–10% w/w.

2.8. NMR self-diffusion measurements

A Unity Inova 500 MHz spectrometer (Varian, USA), equipped with a DOTY probe (DOTY Scientific, USA) was used for the NMR self-diffusion measurements. The standard Stejskal–Tanner pulse sequence with 40 equally spaced values was used for the sineshaped pulsed field gradients (Price and Kuchel, 1991). In order to achieve a good signal-to-noise value 16 scans were accumulated for each value. A bimodal distribution of diffusion coefficients was fitted to the data by using a Levenberg–Marquardt routine in Matlab. In addition, a Monte Carlo method (Alper and Gelb, 1990) was utilized in order to obtain a good estimate of the error for both diffusion coefficients and their fractions.

The experiments were run at 20°C for all samples except the most diluted formulation, which was measured at 25°C.

3. Results and discussion

3.1. Differential scanning calorimetry and rheology measurements

In order to probe the onset of micellization of concentrated block copolymer formulations, and to investigate how cmt depends on parameters, such as the polymer concentration, the composition of copolymer mixtures, the concentration of the active ingredients lidocaine and prilocaine, and the pH dependent ionization of the latter, DSC investigations were performed. As can be seen in Fig. 1a, such measurements typically result in an endothermic peak on formation at the onset of micellization. This peak has previously been attributed to a hydrophobic entropy gain on micellization (Wanka et al., 1990; Yu et al., 1992; Alexandridis et al., 1994; Hecht and Hoffman, 1994; Wanka et al., 1994; Alexandridis and Hatton, 1995; Patterson et al., 1996).

However, given the complex nature of the formulations to be investigated, initial experiments were first performed with two single copolymer systems, i.e., Lutrol® F127 and Pluronic® P 85. The results of these studies are shown in Fig. 1b.

As can be seen, the cmt decreases with increasing polymer concentration. The decrease in the cmt with increasing polymer concentration for both copolymers is expected, and analogous to concentration-induced self-assembly in both block copolymer and surfactant systems (Alexandridis et al., 1994; Jönsson et al., 1998). Considering that the cmt decreases both with an increase in the copolymer PPO content and molecular weight (Alexandridis et al., 1995) the lower cmt for F127 than for P85 at the same concentration is not surprising. The results were found to correlate reasonably well, given the broadness in the thermal transition, with results previously reported in the literature for these systems obtained both with DSC and with other techniques (Wanka et al., 1990; Mortensen, 1992; Yu et al., 1992; Mortensen and Pedersen, 1993; Alexandridis et al., 1994; Wanka et al., 1994; Alexandridis and Hatton, 1995; Hecht and Hoffman, 1995).

The phase behavior of block copolymers is greatly affected by cosolutes, e.g., electrolytes. Thus, both the entire gel region and the cloud point are shifted to lower temperatures upon the addition of NaCl (a typical 'salting out' cosolute) to an aqueous solution of Lutrol® F127, whereas the opposite is found for NaSCN (a typical 'salting in' cosolute) (Malmsten and Lindman, 1992). As can be seen in Fig. 1c, NaCl and NaSCN have the same effect on the cmt of a Lutrol® F127 solution, demonstrating the strong connection between the micellization and gelation processes. In the context of the present study, this serves as a second sample control system. Qualitatively, the results obtained agree well with previous findings (Malmsten and Lindman, 1992; Hecht and Hoffman, 1995; Pandit et al., 2000).

The effect of the ratio between Lutrol® F127 and F68 on self-assembly was investigated by measuring cmt and the gelation temperature for formulations containing a total polymer concentration of 21% w/w. As can be seen in Fig. 2, both the cmt and gelation temperature decrease with increasing fraction of Lutrol® F127, although in the former case, the decrease is quite minor. Since the relative PPO content and molecular weight is higher for F127 than for F68, self-assembly is expected to be more pronounced in the former

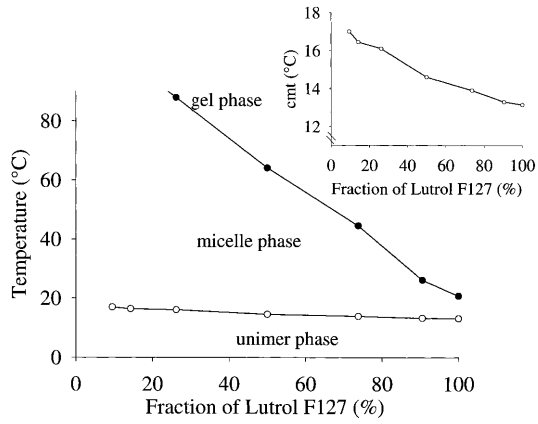

Fig. 2. cmt (open circles) and T_{gel} (filled circles) as a function of the ratio of F127/F68 in a placebo formulation containing a total polymer concentration of 21% w/w. Enlargement of cmt as a function of the ratio of F127/F68 in a placebo formulation containing a total polymer concentration of 21% w/w (inserted figure).

system. Indeed, lower cmc/cmt and gel formation temperatures for F127 compared to F68 have been found in several previous investigations (Schmolka, 1977b; Alexandridis et al., 1994; Wanka et al., 1994; Alexandridis and Hatton, 1995). With an increasing fraction of Lutrol® F127 in the system, the average molecular weight and PPO content in the system increases, which is therefore expected to result in a facilitated self-assembly, as also found. Note, however, that for a multi-component system, there is not a prior reason why this should associate ideally. Instead, the composition of the micelles is expected to differ from that of the average composition, and also to depend on the total polymer concentration (Linse, 1994a,b). Nevertheless, with a decreasing concentration of the more self-associating component, self-assembly should eventually be precluded. However, the magnitude of the effect on the gelation temperature of the composition of the copolymer mixture is rather surprising considering the minor effect this has been found to have on the size of the micelles (Scherlund et al., 2000) as well as on the cmt. A possible explanation might be the difference in aggregation number between the two polymers. Judging from literature values, the aggregation number for Lutrol® F68 seem to be lower than for Lutrol® F127

although the spread in the results reported is quite substantial (Rassing and Attwood, 1983; Linse 1993a; Nigarajan, 1999). However, more work needs to be done in order to verify this hypothesis.

Both cmt and the gelation temperature are affected by the addition of hydrophobic substances. As can be seen in Fig. 3, cmt decreases with increasing amount of the active ingredients. The hydrophobicity of lidocaine and prilocaine (pK_a value of 7.86 and 7.89, respectively (Nyqvist-Mayer et al., 1986)) depends on the pH of the system. At pH 5, where 99.9% w/w of the active ingredients are in their ionized form, the effect on cmt is minor. At pH 7.8, on the other hand, a sizable fraction of these substances are uncharged and therfore poorly soluble. Thus, addition of lidocaine and prilocaine at this pH causes the cmt to decrease substantially, in analogy, e.g., to the effects caused by medium chain alcohols (Carlsson et al., 1986; Malmsten and Lindman, 1990). The effect of pH on the system is also shown in Fig. 4, where it can be seen that the cmt decreases drastically with increasing pH until 50% w/w of the active ingredients are in their hydrophobic state, i.e., at pH 7.8. Thereafter, a further increase in pH seems to be of minor importance for the onset of micellization.

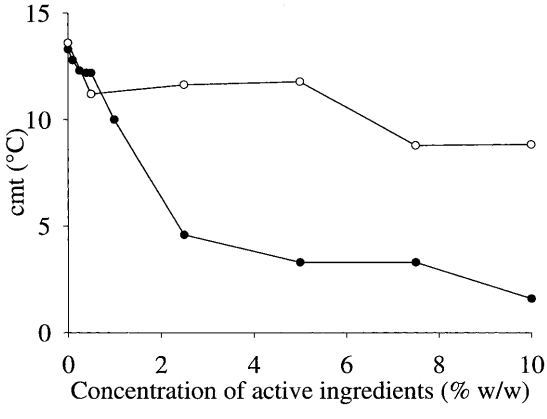

Fig. 3. cmt as a function of concentration of active ingredients in a formulation containing 15.5% w/w of Lutrol® F127 and 5.5% w/w of Lutrol® F68 at pH 5 (open circles) and 7.8 (filled circles).

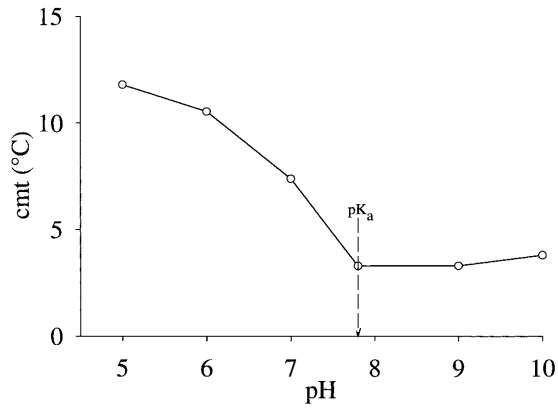

Fig. 4. cmt as a function of pH for a formulation containing 5% w/w of active ingredients, 15.5% w/w of Lutrol® F127 and 5.5% w/w of Lutrol® F68.

No significant difference in effect on cmt or gelation temperature can be distinguished between the eutectic mixture, on one hand, and the lidocaine and prilocaine single components, on the other hand (Fig. 5). Considering that lidocaine is more hydrophobic than prilocaine (the water solubility of lidocaine and prilocaine being 4–6 and 6–8.4 mg/ml, respectively) one could perhaps have expected a stronger concentration dependent decrease in both cmt and gelation temperature for lidocaine than for prilocaine (Nyqvist-Mayer et

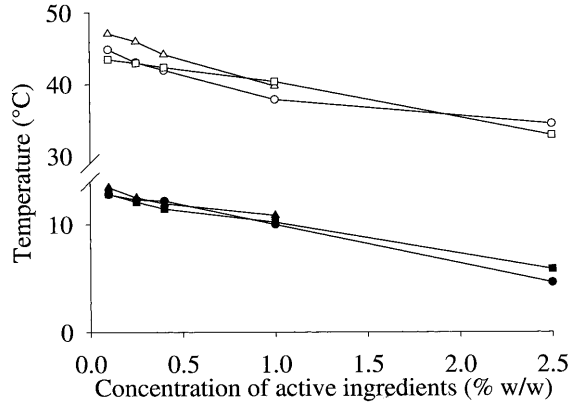

Fig. 5. cmt (filled symbols) and T_{gel} (open symbols) as a function of concentration of active ingredients for formulations containing lidocaine and prilocaine (1:1) (circles), lidocaine (triangles) and prilocaine (squares). All formulations contained 5.5% w/w Lutrol® F68 and 15.5% w/w Lutrol® F127 and had a pH of 7.8.

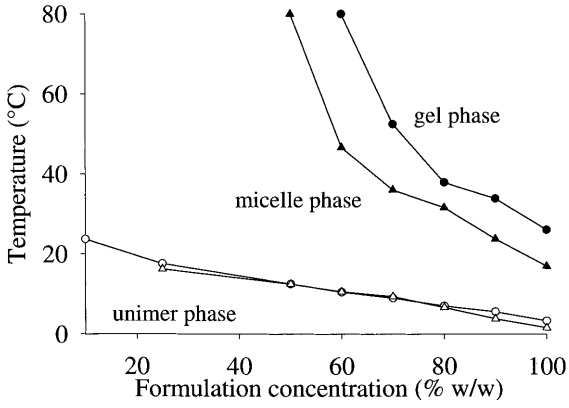

Fig. 6. cmt (open symbols) and T_{gel} (filled symbols) as a function of formulation concentration. A concentration of 100% w/w corresponds to 5% w/w active ingredients (circles) or 10% w/w (triangles), 15.5% w/w Lutrol® F127 and 5.5% w/w Lutrol® F68 at a pH of 7.8.

al., 1986). Apparently, this difference in hydrophobicity is insufficient to affect the self-association. In parenthesis it could be said that the lidocaine formulation resulted in a phase separation, i.e., crystallization of lidocaine, at a concentration of 2.5% w/w upon storage in a refrigerator, i.e., 2–8°C, whereas the same amount of prilocaine could be solubilized in the system without causing phase separation. Furthermore, since the solubility capacity of the system for the eutectic mixture by far exceeds that of the single components, no such effect were observed for the mixture of the active ingredients.

The effect of diluting the system with water is shown in Fig. 6 and as can be seen, increasing the water content causes an increase in both cmt and gelation temperature. The same concentrations of active ingredients were also tested at pH 10 giving analogous results (not shown). As is clearly seen, the effect on the gelation temperature is much more dramatic than the effect on cmt. This might be explained by the gelation temperature being determined by both the self-assembly and the close-packing of micelles (Mortensen, 1992; Mortensen et al., 1992; Mortensen and Pedersen, 1993; Mortensen, 1996), the latter naturally being rather sensitive to total copolymer concentration.

3.2. Dye solubilization

The cmt has also been measured for an aqueous solution of Lutrol® F127 and a formulation containing 5% w/w of active ingredients using a dye solubilization approach with the hydrophobic probe, DPH. The results of these measurements are shown in Fig. 7. The cmt values obtained with the DPH solubilization were somewhat higher than those found by DSC (indicated in Fig. 7). This is the case also for the simpler Lutrol® F127 system (results not shown). The origin of this effect is not entirely clear at present, but may be related to the dye solubilization being precluded for small micelles. If this is the case, solubilization is expected to be facilitated by an increased temperature, due to the well-known micellar growth with increasing temperature for this type of systems (Zhou and Chu, 1988; Tontisakis et al., 1990; Wanka et al., 1990; Brown et al., 1991; Linse and Malmsten, 1992; Alexandridis et al., 1995). Interestingly, the process is kinetically controlled, as indicated by a lower absorption intensity of the different samples after an incubation time of 4 h compared to 12 h. Nevertheless, the cmt determined after an incubation time of 4 and 12 h were virtually identical, showing that this is of minor importance for the present discussion.

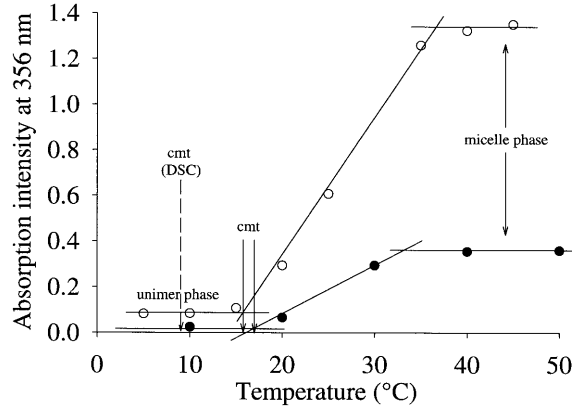

Fig. 7. The absorption intensity at 356 nm for a formulation containing 5% w/w of active ingredients, 15.5% w/w Lutrol® F127 and 5.5% w/w Lutrol® F68 at pH 7.8 diluted to 70% w/w with distilled water incubated with DPH probe at different temperatures for 4 h (filled circles) and 12 h (open circles). The cmt and the phases of the micellization process are indicated together with cmt values determined by DSC.

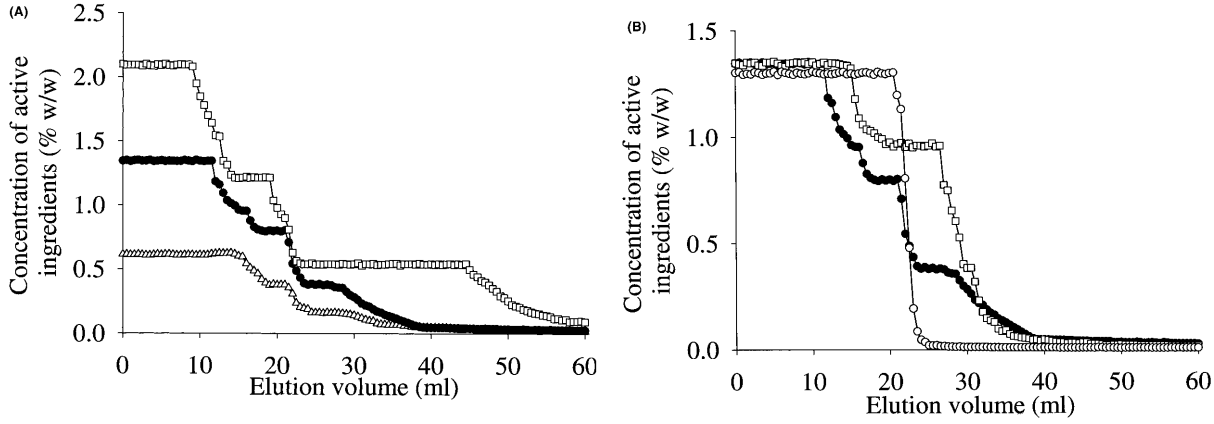

Fig. 8. (a) The concentration of active ingredients as a function of elution volume after gel filtration for formulations containing 15.5% w/w Lutrol® F127, 5.5% w/w Lutrol® F68 and 2.5% w/w (open triangles), 5% w/w (filled circles) or 10% w/w (open squares) of active ingredients at pH 7.8. (b) The concentration of active ingredients as a function of elution volume after gel filtration for formulations containing 15.5% w/w Lutrol® F127, 5.5% w/w Lutrol® F68 and 5% w/w of active ingredients at pH 5 (open circles), 7.8 (filled circles) and 9 (open squares). All formulations were diluted to 25% w/w with distilled water prior to the gel filtration.

3.3. Gel filtration

Block copolymer micelles have been shown to display extremely slow disintegration kinetics, i.e., being stable for more than 1 h after dilution below cmc, making it possible to study the micellization with, e.g., gel filtration (Tuzar et al., 1974; Booth et al., 1978; Malmsten and Lindman, 1992). Considering this, the tail-end analysis was used to study the amount of active ingredients present in the different phases of the system (Suzuki and Sasaki, 1971; Ayako and Fumio, 1978; Nyqvist-Mayer et al., 1985). The results are shown in Fig. 8a and b and as can be seen three plateaus are found during the elution. A reasonable assumption is that these correspond to the concentration of the active components in the added formulation, micelle phase, and unimer and/or freely soluble phase, respectively. In an attempt to clarify whether or not this is the case, the particle size of each of the three plateaus were investigated by PCS after filtration through a 0.22 μm filter in order to eliminate dust particles. Such measurements showed that samples from the first and middle fraction contained aggregates with a diameter (Z average) in the range of 20–30 nm, respectively (the polydispersity being below 0.3 indicating a rather uniform distribution in particle size). This was found for all systems investigated, and correlates well with previous findings for these systems using PCS and NMR techniques (Scherlund et al., 2000). For the expected unimer and freely soluble phase, very low scattering intensities were found, once more in analogy to previous findings (Scherlund et al., 2000). From Fig. 8a and b, it can be seen that the concentration of active ingredients in the first plateau corresponds very well with the initial concentration of the formulations diluted to 25% w/w (0.625, 1.25 and 2.5% w/w, respectively). The amount of active ingredients present in the micelle and unimer and freely soluble phases are 50–60 and 40–50% w/w respectively for all formulations at pH 7.8.

The solubilization of lidocaine and prilocaine was found to be affected by the pH dependent ionization of these active ingredients. Thus, with increasing pH (and thereby decreasing degree of ionization and reduced solubility) the extent of micelle solubilization increases. More specifically, at pH 5 and 9 (Fig. 8b), the relative amount of lidocaine and prilocaine present in the micelle phase is approximately 0% and 80%, respectively, as compared to 50–60% at pH 7.8, while the amount present in the unimer and freely soluble phase is 100% at pH 5, 40–50% at pH 7.8 and 20% at pH 9. Considering the pK_a values of the formulations the amount of active ingredients present in their uncharged state is 0.2% w/w at

pH 5, 50% w/w at pH 7.8, and 94% w/w at pH 9, which seems to correlate quite well with our findings of the amount of active ingredients solubilized at these pH values.

The pH dependent micelle solubilization of the active ingredients was previously investigated for dilute systems by the authors (Scherlund et al., 2000) and contrary to our expectations, the apparent amount solubilized in these previous investigation was found to increase with decreasing pH. As pointed out in this previous study, artifacts related to the adsorption of the active components at high pH, could not be excluded, and were identified as a source of these unexpected findings. That similar effects were not observed in the present studies may have different reasons, but most likely, this is an effect of the much higher concentration of active ingredient in the processed solution which results in a much larger amount active ingredient to surface area ratio, thereby making the present approach much less sensitive to material loss due to adsorption at tubing walls, etc.

3.4. NMR self-diffusion measurements

In order to learn more about the dynamics of the systems investigated, polymer self-diffusion

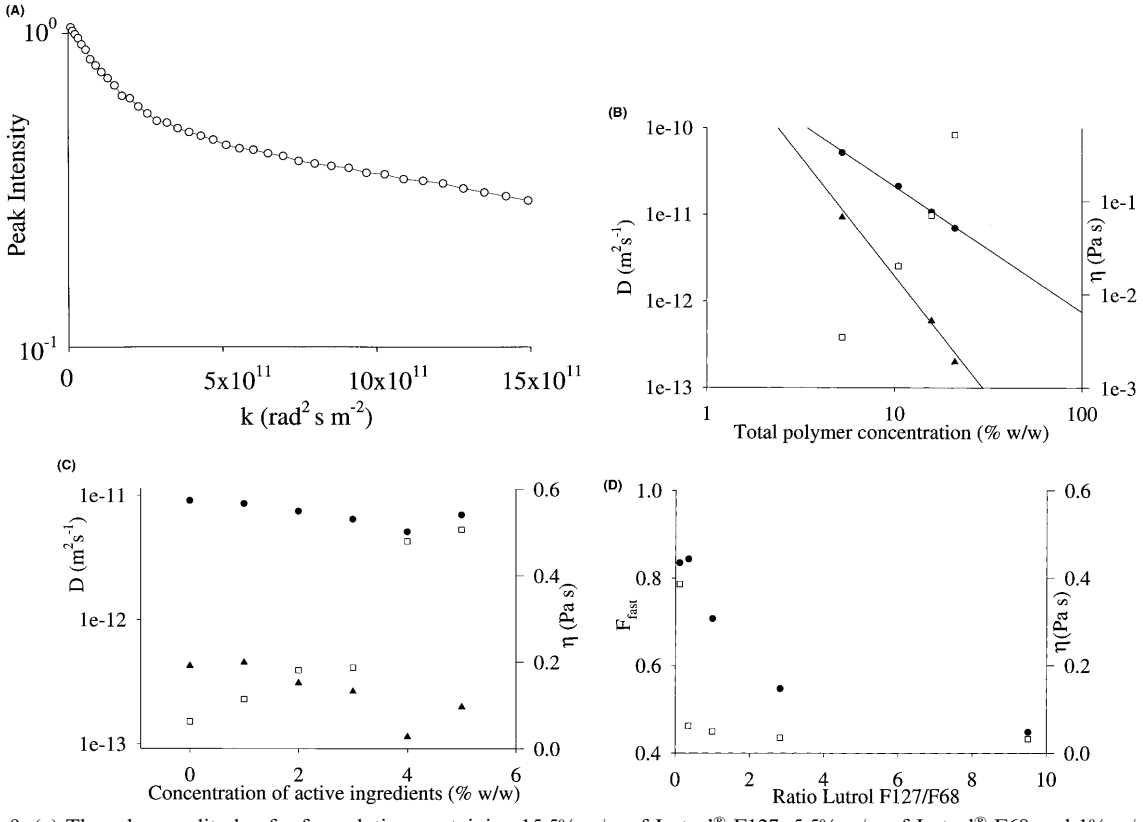

Fig. 9. (a) The echo amplitude of a formulation containing 15.5% w/w of Lutrol® F127, 5.5% w/w of Lutrol® F68 and 1% w/w of active ingredients at pH 7.8. (b) The dependence of the diffusion coefficients for the fast (filled circles) and the slow (filled triangles) and the zero-shear viscosity (open squares) as a function of total polymer concentration. The original formulation contained 15.5% w/w Lutrol® F127, 5.5% Lutrol® F68 and 5% w/w active ingredients at pH 7.8. (c) The dependence of the diffusion coefficients for the fast (filled circles) and the slow (filled triangles) and the zero-shear viscosity (open squares) as a function of concentration of active ingredients. All formulations contained 15.5% w/w Lutrol® F127, 5.5% Lutrol® F68 and had a pH of 7.8. (d) The fraction of the fast diffusion mode (filled circles) and zero-shear viscosity (open squares) as a function of the Lutrol® F127/F68 ratio. All formulations had a pH of 7.8.

measurements were performed. As can be seen in Fig. 9a, the echo amplitude decay shows existence of two rather distinct populations, diffusing quite differently. With increasing concentration, the two corresponding diffusion coefficients decrease in a power-law-dependent way, with exponents of 1.4 and 2.8 for the fast and slow mode, respectively (Fig. 9b). Simultaneously, the zero-shear viscosity increases, although not with a well-defined component (Fig. 9b). Clearly, neither the self-diffusion behavior nor the viscosity is compatible with reptation in semi-dilute polymer solutions (de Gennes, 1979; Callaghan and Pinder, 1983; von Meerwall et al., 1985; Goddard and Ananthapadmanabhan, 1993; Johansson and Löfroth, 1993). However, given the self-assembly and the presence of micellar aggregates in the presently investigated systems, this is hardly surprising.

With increasing concentration of the active ingredients at pH 7.8, where approximately 50% of the molecules are in their uncharged form, both diffusion coefficients decrease, whereas the viscosity increases (Fig. 9c), both effects presumably as a result of solubilization-induced micellization and micellar growth (seen above). At the highest concentration of active ingredients, the diffusion coefficients increase once more. The reason for this behavior is not known at present and further investigations are required. However, this is considered to be out of the scope of the current investigation. Interestingly, the relative fraction of the two diffusion modes remains unaffected of both the total polymer concentration and the concentration of active ingredients (approximately 50% of both in all cases). Finally, with increasing ratio between Lutrol® F127 and Lutrol® F68, no clear trend is observed in the diffusion coefficients (results not shown), but a clear decrease in the fraction of the fast diffusion mode and an increase in the viscosity (Fig. 9d) are found.

Although the effects of the total copolymer concentration, the concentration of active ingredients, and the copolymer ratio on self-diffusion and viscosity yields interesting information, the interpretation of the results is not entirely straightforward. From the echo amplitude decay it is clear that there are two modes of self-diffusion. At present, it is not certain what these correspond to. As a first alternative, one could perhaps have thought these to correspond to micelles and unimers, respectively. However, since the relative fraction of the two populations remains unchanged with the total polymer concentration, this is clearly not the case, Alternatively, one could think that the two populations correspond to Lutrol® F127 and Lutrol® F68, respectively. However, since the presently studied mixture of the two polymers has previously been found to form micelles with a rather narrow size distribution in dilute solution, it is uncertain if this happens in the more concentrated solutions. Furthermore, since the echo amplitude decay of the methylene groups was followed, the decay contains information about the diffusion of both the polymer and the active ingredients. However, the concentration of the latter is much smaller than the former, and their diffusion much faster ($D \approx 4 \times 10^{-10}$) (Scherlund et al., 2000), which means that their contribution to the echo amplitude decay is quite small. Furthermore, the fraction of populations is essentially unchanged on increasing the concentration of active ingredients in the range of 0–5% w/w (results not shown), indicating that this is not due to polymers and active ingredient diffusion. Clearly, further work on this is required, which, however is out of the scope of the present investigation.

4. Conclusions

The cmt and gelation temperature of the local anesthetic microemulsion formulation are interconnected and influenced by cosolutes, such as electrolytes and hydrophobic substances, the latter as found particularly for the eutectic mixture of lidocaine and prilocaine. Both the cmt and the gelation temperature decrease with increasing pH of the system, at reduced solubility of the active ingredients. They also increase upon diluting the system with water. The ratio between the two block copolymers present in the system has an impact on both cmt and the gelation temperature, resulting in a decrease in onset temperature of both processes with an increase of Lutrol® F127.

Finally, the amount of the active ingredients present in the micelle phase are dependent on the pH of the system being approximately 0% w/w at pH 5, 50–60% at pH 7.8 and 80% at pH 9. These findings are in line with expectations from the pH dependent solubility, and previously found release data, showing that the release rate for lidocaine and prilocaine increases with decreasing pH. Finally, neither the self-diffusion behavior nor the viscosity is compatible with reptation in semi-dilute polymer solutions.

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References

Alexandridis, P., Holzwarth, J.F., Hatton, T.A., 1994. Micellization of poly(ethylene oxide)–poly(propylene oxide)–poly(ethylene oxide) triblock copolymers in aqueous solutions: thermodynamics of copolymer association. Macromolecules 27, 2414–2425.

Alexandridis, P., Hatton, T.A., 1995. Poly(ethylene oxide)–poly(propylene oxide)–poly(ethylene oxide) block copolymer surfactants in aqueous solutions and at interfaces: thermodynamics, structure, dynamics, and modeling. Colloids Surf. A 96, 1–46.

Alexandridis, P., Olsson, U., Lindman, B., 1995. Self-assembly of amphiphilic block copolymers: the $(EO)_{13}(PO)_{30}(EO)_{13}$-water-*p*-xylene system. Macromolecules 28, 7700–7710.

Alexandridis, P., Olsson, U., Lindman, B., 1996. Phase behavior of amphiphilic block copolymers in water–oil mixtures: the pluronic 25R4-water-*p*-xylene system. J. Phys. Chem. 100, 280–288.

Alexandridis, P., Holzwarth, J.F., 1997. Differential scanning calorimetry investigation of the effects of salts on aqueous solution properties of an amphiphilic block copolymer (poloxamer). Langmuir 13, 6074–6082.

Alper, J.S., Gelb, R.I., 1990. Standard errors and confidence intervals in nonlinear regression: comparison of Monte Carlo and parametric statistics. J. Phys. Chem. 94, 4747–4751.

Ayako, G., Fumio, E., 1978. The distribution of alkyl paraben in aqueous sodium lauryl sulfate micellar solutions. J. Colloid Interf. Sci. 66, 26–32.

Booth, C., Naylor, T.D., Price, C., Rajab, N.S., Stubbersfield, R.B., 1978. Investigation of the size distribution of non-ionic micelles formed from a polystyrene–polyisoprene block copolymer in *N*,*N*-dimethylacetamide. J. Chem. Soc. Faraday Trans. 1 (74), 2352–2362.

Brodin, A., Nyqvist-Mayer, A., Wadsten, T., Forslund, B., Broberg, F., 1984. Phase diagram and aqueous solubility of the lidocaine–prilocaine binary system. J. Pharm. Sci. 73, 481–484.

Brown, W., Schillen, K., Almgren, M., Hvidt, S., Bahadur, P., 1991. Micelle and gel formation in a poly(ethylene oxide)/poly(propylene oxide)/poly(ethylene oxide) triblock copolymer in water solution: dynamic and static light scattering and oscillatory shear measurements. J. Phys. Chem. 95, 1850–1858.

Callaghan, P.T., Pinder, D.N., 1983. A pulsed field gradient NMR study of self-diffusion in a polydisperse polymer system: dextran in water. Macromolecules 16, 968–973.

Carlsson, A., Karlström, G., Lindman, B., 1986. Synergistic surfactant-electrolyte effect in polymer solutions. Langmuir 2, 536–537.

Chattopadhyay, A., London, E., 1984. Fluorimetric determination of critical micelle concentration avoiding interference from detergent charge. Anal. Biochem. 139, 408–412.

de Gennes, P.-G., 1979. Scaling Concepts in Polymer Physics. Cornell University Press, Ithaca, NY.

Goddard, E.D., Ananthapadmanabhan, K.P., 1993. Interactions of Surfactants with Polymers and Proteins. CRC Press, Boca Raton.

Hecht, E., Hoffman, H., 1994. Interaction of ABA block copolymers with ionic surfactants in aqueous solution. Langmuir 10, 86–91.

Hecht, E., Hoffman, H., 1995. Kinetic and calorimetric investigations on micelle formation of block copolymers of the poloxamer type. Colloids Surf. A 96, 181–197.

Hecht, E., Mortensen, K., Hoffman, H., 1995. L3 phase in a binary block copolymer/water system. Macromolecules 28, 5465–5476.

Johansson, L., Löfroth, J-E., 1993. Diffusion and interactionin gels and solutions. IV: hard sphere Brownian dynamics simulations. J. Chem. Phys. 98, 7471–7479.

Jönsson, B., Lindman, B., Holmberg, K., Kronberg, B., 1998. Surfactats and Polymers in Aqueous Solution. Wiley, Chichester, England.

Koppel, D., 1972. Analysis of macromolecular polydispersity in intensity correlation spectroscopy. J. Chem. Phys. 57, 4814–4820.

Linse, P., 1993a. Micellization of poly(ethylene oxide)–poly(propylene oxide) block copolymers in aqueous solution. Macromolecules 26, 4437–4449.

Linse, P., 1993b. Phase-behavior of poly(ethylene oxide)–poly(propylene oxide) block copolymers in aqueous solution. J. Phys. Chem. 97, 13896–13902.

Linse, P., 1994a. Micellization of poly(ethylene oxide)–poly(propylene oxide) block copolymers in aqueous solution – effect of polymer impurities. Macromolecules 27, 2685–2693.

Linse, P., 1994b. Micellization of poly(ethylene oxide)–poly(propylene oxide) block copolymers in aqueous solution – effect of polymer polydispersity. Macromolecules 27, 6404–6417.

Linse, P., Malmsten, M., 1992. Temperature-dependent micellization in aqueous block copolymer solutions. Macromolecules 25, 5434–5439.

Malmsten, M., Lindman, B., 1990. Ellipsometry studies of the adsorption of cellulose ethers. Langmuir 6, 357–364.

Malmsten, M., Lindman, B., 1992. Self-assembly in aqueous block copolymer solutions. Macromolecules 25, 5440–5445.

von Meerwall, E.D., Amis, E.J., Ferry, D., 1985. Self-diffusion in solutions of polystyrene in tetrahydrofuran: comparison of concentration dependences of the diffusion coefficients of polymer, solvent, and ternary probe component. Macromolecules 18, 260–266.

Mortensen, K., 1992. Phase behaviour of poly(ethylene oxide)–poly(propylene oxide)–poly(ethylene oxide) triblock-copolymer dissolved in water. Europhys. Letts. 19, 599–604.

Mortensen, K., Brown, W., Nordén, B., 1992. Inverse melting transition and evidence of three-dimensional cubatic structure in a block-copolymer micellar system. Phys. Rev. Lett. 68, 2340–2343.

Mortensen, K., Pedersen, J.S., 1993. Structural study on the micelle formation of poly(ethylene oxide)–poly(propylene oxide)–poly(ethylene oxide) triblock copolymer in aqueous solution. Macromolecules 26, 805–812.

Mortensen, K., 1996. Structural study of aqueous solutions of PEO–PPO–PEO triblock copolymers, their micellar aggregates and mesophases; a small-angle neutron scattering study. J. Phys. Condens. Matter 8, 103–124.

Nagarajan, R., 1999. Solubilization of hydrocarbons and resulting aggregate shape transitions in aqueous solutions of Pluronic® (PEO–PPO–PEO) block copolymers. Colloids Surf. B 16, 55–72.

Nyqvist-Mayer, A.A., Brodin, A.F., Frank, S.G., 1985. Phase distribution studies on an oil–water emulsion based on a eutectic mixture of lidocaine and prilocaine as the dispersed phase. J. Pharm. Sci. 74, 1192–1195.

Nyqvist-Mayer, A.A., Brodin, A.F., Frank, S.G., 1986. Drug release studies on an oil–water emulsion based on a eutectic mixture of lidocaine and prilocaine as the dispersed phase. J. Pharm. Sci. 75, 365–373.

Pandit, N., Trygstad, T., Croy, S., Bohorquez, M., Kock, C., 2000. Effect of salts on the micellization, clouding, and solubilization behavior of pluronic F127 solutions. J. Colloid Interf. Sci. 222, 213–220.

Patterson, I., Chowdhry, B.Z., Leharne, S., 1996. Evaluation of surfactant monomer concentrations in equilibrium with micelles and their temperature dependence for oxyethylene/oxypropylene/oxyethylene triblock copolymers using DSC. Colloids Surf. A Physicochem. Eng. Aspects 111, 213–222.

Price, W.S., Kuchel, P.W., 1991. Effect of nonrectangular field gradient pulses in the Stejskal and Tanner (diffusion) pulse sequence. J. Magn. Reson. 94, 133–139.

Rassing, J., Attwood, D., 1983. Ultrasonic velocity and light-scattering studies on the polyoxyethylene–polyoxypropylene copolymer pluronic F127 in aqueous solution. Int. J. Pharm. 13, 47–55.

Scherlund, M., Malmsten, M., Brodin, A., 1998. Stabilization of a thermosetting emulsion system using ionic and non-ionic surfactants. Int. J. Pharm. 173, 103–116.

Scherlund, M., Malmsten, M., Holmqvist, P., Brodin, A., 2000. Thermosetting microemulsions and mixed micellar solutions as drug delivery systems for periodontal anesthesia. Int. J. Pharm. 194, 103–116.

Schmolka, I.R., 1977a. BWC surfactants in gel cosmetics. Cosmet. Toiletries 92, 77–79.

Schmolka, I.R., 1977b. A review of block polymer surfactants. J. Am. Oil Chem. Soc. 54, 110–116.

Suzuki, H., Sasaki, T., 1971. Gel filtration of aqueous sodium dodecyl sulfate. Bull. Chem. Soc. Jpn. 44, 2630–2643.

Svensson, M., Linse, P., Tjerneld, F., 1995. Phase behaviour in aqueous two-phase systems containing micelle-forming block copolymers. Macromolecules 28, 3597–3603.

Tontisakis, A., Hilfiker, R., Chu, B., 1990. Effect of xylene on micellar solutions of block-copoly(oxyethylene/oxypropylene/oxyethylene) in water. J. Colloid Interface Sci. 135, 427–434.

Tuzar, Z., Petrus, V., Kratochvil, P., 1974. Sedimentation and light scattering study of block copolymer association. Makromol. Chem. 175, 3181–3192.

Wang, P.L., Johnston, T.P., 1991. Kinetics of sol-to-gel transition for poloxamer polyols. J. Appl. Polym. Sci. 43, 283–292.

Wanka, G., Hoffman, H., Ulbricht, W., 1990. The aggregation of poly(oxyethylene)–poly(oxypropylene)-block-copolymers in aqueous solution. Colloid Polym. Sci. 268, 101–117.

Wanka, G., Hoffman, H., Ulbricht, W., 1994. Phase-diagrams and aggregation behavior of poly(oxyethylene)–poly(-oxypropylene)–poly(oxyethylene) triblock copolymers in aqueous solutions. Macromolecules 27, 4145–4159.

Yu, G., Deng, Y., Dalton, S., Wang, Q-G., Attwood, D., Price, C., Booth, C., 1992. Micellisation and gelation in triblock copoly(oxyethylene)/oxypropylene/oxyethylene), F127. J. Chem. Soc. Faraday Trans. 88, 2537–2544.

Zhou, Z., Chu, B., 1988. Light-scattering study on the association behavior of triblock polymers of ethylene oxide and propylene oxide in aqueous solution. J. Colloid Interface Sci. 126, 171–180.