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Thermosetting microemulsions and mixed micellar solutions as drug delivery systems for periodontal anesthesia

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Abstract

In the present study, thermosetting microemulsions and mixed micellar solutions were investigated as drug delivery systems for anesthetizing the periodontal pocket. The structure of the systems, consisting of the active ingredients lidocaine and prilocaine, as well as two block copolymers (Lutrol® F127 and Lutrol® F68), was investigated by NMR spectroscopy and photon correlation spectroscopy (PCS). The results obtained for dilute (1–3% w/w) solutions show discrete micelles with a diameter of 20–30 nm and a critical micellization temperature of 25–35°C. Gel permeation chromatography (GPC) was used to study the distribution of the active ingredients, and indicates a preferential solubilization of the active components in micelles over unimers. Analogous to the Lutrol® F127 single component system these formulations display an abrupt gelation on increasing temperature. The gelation temperature was found to depend on both the drug ionization and concentration. These systems have several advantages over emulsion-based formulations including good stability, ease of preparation, increased drug release rate, and improved handling due to the transparency of the formulations.

Keywords: Microemulsion; Lidocaine; Prilocaine; PEO-PPO-PEO block copolymers; Periodontal pocket; Temperature of gelation

1. Introduction

Emulsions as drug delivery systems have been used in pharmaceutical and medical practice since the earliest days. Oil-in-water emulsions offer advantages in topical administration, including water miscibility, thus making them washable, ease of spreading onto the application site, and a low degree of irritation (Becher, 1985). In a previous study (Scherlund et al., 1998) oil-in-water emulsions for delivery of local anesthetics to the periodontal pocket were investigated. In these emulsions the active ingredients lidocaine and prilocaine (Fig. 1) were combined with a block copolymer (Lutrol® F127) to give emulsions with rheological properties suitable for the intended use. It was found that with anionic, cationic and nonionic surfactant additions an increased initial release rate compared to the benchmark (EMLA®

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cream) could be achieved. However, the storage stability of the emulsions was too short for practical use.

Microemulsions are systems well known for their excellent long-term stability and ease of preparation. They are defined as systems containing water, oil and amphiphile(s) constituting a single optically isotropic and thermodynamically stable liquid solution. Homogenized emulsions, on the contrary, are kinetically stabilized, but thermodynamically unstable, dispersions. Thus, the average droplet size of emulsions grows continuously with time until the emulsion separates into two macroscopic phases. The properties of microemulsions, on the other hand, are time independent (in the absence of chemical degradation) and also independent of the order of mixing. The microemulsion structure can be oil-in-water (o/w), water-in-oil (w/o), or bicontinuous, i.e. effectively continuous in both water and oil. Beside the obvious advantages of microemulsions, including physical stability and ease of preparation, these systems may offer additional benefits for periodontal uses. These include an increased drug release rate, due to smaller particle size compared to emulsions, and improved handling due to the transparency of the formulation, the latter making it easy to see the instruments in the working area, i.e. the periodontal pocket. The use of microemulsions in pharmaceutical formulations has been discussed in greater detail in several recent reviews (Israelachvili, 1994; Lawrence, 1994; Constantinides, 1995; Paul and Moulik, 1997; Kumar and Mittal, in press).

Particularly interesting microemulsion systems for pharmaceutical applications are those based on nonionic surfactants, since these require no additional surfactant or cosurfactant to form a microemulsion. This means that opposed to most microemulsions formed by ionic surfactants, those formed by nonionic ones are stable even when diluted, e.g. in an excess of water. Of special interest among the nonionic surfactants are the PEO-PPO-PEO block copolymers (PEO and PPO being poly(ethylene oxide) and poly(propylene oxide), respectively) which have been shown to form microemulsions of various types (Tontisakis et al., 1990; Alexandridis et al., 1997; Alexandridis and Andersson, 1997a,b; Holmqvist et al., 1997a). Considering the limited storage stability of the previously investigated o/w emulsion systems, we were interested in determining whether a microemulsion formulation would perform better than the emulsion-based formulation regarding anesthetizing the periodontal pocket. In order to be successful, these systems need to be easy to apply, stay at the application site, have a fast onset time, be non-irritant, and stable at normal storage conditions. Since Lutrol® F127 gels have the required mechanical properties this polymer together with an additional block copolymer were investigated in this study. The structure of the systems formed was investigated by NMR spectroscopy and photon correlation spectroscopy (PCS). Furthermore, gel permeation chromatography (GPC) was used to study the distribution of the active ingredients within the system. Also, the rheology, the drug release properties, and the robustness of the systems were investigated.

2. Materials and methods

2.1. Chemicals

Lutrol® F68 (EO_{79} PO_{28} EO_{79}) and F127 (EO_{99} PO_{65} EO_{99}) were obtained from BASF Svenska AB, Sweden, whereas prilocaine, lidocaine and EMLA® cream 5% w/w were from Astra AB, Södertälje, Sweden. T-butanol, D_2O, DCl and NaOD (all of 99.8% purity) were from Sigma Chemicals, USA. Distilled water, 2 M hydrochloric acid and 2 M sodium hydroxide were used as appropriate. All chemicals were used as supplied.

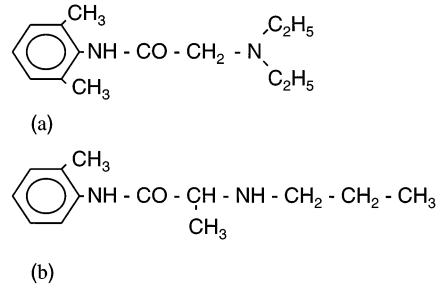

Fig. 1. Structural formula of (a) lidocaine, and (b) prilocaine.

Table 1
Compositions used in PCS and NMR experiments

Sample no.	Concentration of lidocaine (% w/w)	Concentration of prilocaine (% w/w)	Concentration of Lutrol® F68 (% w/w)	Concentration of Lutrol® F127 (% w/w)	pH
1	1.00	1.00	4.00	15.50	8
2	2.50	2.50	4.00	15.50	8
3	4.00	4.00	4.00	15.50	8
4	2.50	2.50	2.00	17.00	8
5	2.50	2.50	4.50	16.00	8
6	2.50	2.50	6.00	13.00	8

2.2. Preparation of formulations

For all formulations prilocaine and lidocaine were mixed in a ratio of 1:1 (giving a eutectic mixture) (Brodin et al., 1984), and heated to 70°C together with Lutrol® F68 and F127, until a uniform melt was formed. Distilled water at 70°C was slowly added to the melt during manual agitation. The pH was measured and adjusted to 5, 7, 7.5, 8 or 8.5 with 2 M hydrochloric acid and to pH 10 with 2 M sodium hydroxide, after which the weight was adjusted to its final value with distilled water. The pH was measured again and found to be the same as before the final water addition. For formulations used in NMR studies D_2O, DCl and NaOD were used instead of the corresponding hydrogenated compounds. The pure polymer solutions of Lutrol® F68 and Lutrol® F127 were prepared according to the so-called cold method (Schmolka, 1977a). In short the block copolymer powder was added to cold distilled water (for the NMR measurements D_2O was used) in portions during agitation, after which the samples were kept in a refrigerator at 4–8°C until turning clear.

2.3. Self-diffusion NMR studies

For the self-diffusion NMR measurements the FTPGSE technique (Stilbs, 1987) was used with a Bruker MSL 100 instrument. The technique is based on a 90°-τ-180°-τ- echo pulse sequence with two additional rectangular magnetic field gradients pulses of magnitude G, separation Δ, and duration δ. At 2τ, the echo amplitude (A) is given by:

$$A\ (2\tau) = A\ (0)\ \exp\ [-2\tau/T_2 - \gamma^2 G^2 D\delta^2\ (\Delta - \delta/3)] \quad (1)$$

where T_2 is the transverse relaxation time and γ is the magnetogyric ratio (Holmqvist et al., 1997b). The hydrodynamic radius, R_h, of the aggregates were calculated from the diffusion coefficients (D) thus measured according to the Stokes–Einstein equation:

$$R_h = kT/6\pi\eta D \quad (2)$$

where k is Boltzman constant, T is the temperature, and η the viscosity of the continuous phase. The formulations investigated are shown in Table 1. Samples were run at 25 and 35°C. Prior to running the experiments the formulations were diluted to 1 or 3% w/w with D_2O.

2.4. Photon correlation spectroscopy

A frequency-stabilized Coherent Innova Ar ion laser operating at 488 nm was used for the photon correlation spectroscopy (PCS) measurements. The detector optics were coupled to an ITT FW130 photomultiplier by a 4 μm-diameter monomodal fiber. The signal analyzer, producing the intensity autocorrelation function, $g^{(2)}(t)$, was an ALV-5000 digital multiple-τ correlator with 288 exponentially spaced channels (Langen GmbH). Inverse Laplace transformation of the intensity autocorrelation functions was performed using a constrained regularization calculation algorithm (REPES) as incorporated in the analysis package GENDIST (Schillén et al., 1994). In this analysis procedure the fitting is performed directly

to the measured intensity autocorrelation function. The result is presented as a relaxation time distribution from which the diffusion coefficients, D, may be obtained. The hydrodynamic radii, R_h, of the aggregates were calculated from the measured diffusion coefficients according to Eq. (2). The concentrations of ingredients in the different formulations are given in Table 1. Samples were run at 20 and 35°C and before running the experiments the formulations were diluted to 1 or 3% w/w with distilled water.

2.5. Gel permeation chromatography

A Superose 6 HR 10/30 column from Amersham Pharmacia Biotech, Sweden, was connected to an HPLC and detector system, consisting of a Gynkotek M 480 G pump, a Gynkotek UVD 170 S UV detector and an ERC 7517 A RI detector. The system was operated by Gynkosoft software. Samples were also run with the column connected to an HPLC system consisting of an LKB 2150 HPLC pump, a Spectroflow 757 absorbance detector from ABI Analytical Kratos Division and a Chrom Jet SP 4400 integrator from Thermo Separation Products, which gave comparable results. The eluent was degassed distilled water. The flow was set at 0.3 ml min^{-1}, the wavelength at 220 nm, and the absorbance at 0.05. The sample volume was 200 μl for the first and 80 μl for the second HPLC system. The first system, containing both UV and RI detection, was used to check which peaks corresponded to the micelles and unimers in the formulations. Thereafter, the second system was used. The concentrations of the ingredients in the different formulations investigated are given in Table 2. All formulations containing active ingredients were diluted 100 times with water whereas the polymer solutions were diluted 10 times with water. All experiments were run at 45°C and selected experiments at 20°C. All measurements were performed in duplicates.

2.6. Rheology measurements

A StressTech Rheometer from Reologica AB, Sweden, was used to measure the rheological behavior of the formulations. All measurements were performed using a cone/plate system with a cone diameter of 40 mm and an angle of 4° (C40 4PC). The temperature stability and range of the temperature unit were ± 0.1°C and 5–90°C, respectively.

The formulations were placed on the plate and the excess material was removed after lowering the cone. A solvent trap was used in order to prevent sample evaporation. For each sample the following measurements were performed:

1. Oscillation stress sweep obtaining the linear viscoelastic region (LVER).
2. Oscillation temperature sweep from 10–40°C with constant stress in the LVER, measuring the temperature of gelation and the elastic and loss moduli (G' and G'') of the formulations.

The measurements were performed in duplicates for all samples.

Table 2
Compositions used in GPC experiments

Sample no.	Concentration of lidocaine (% w/w)	Concentration of prilocaine (% w/w)	Concentration of Lutrol® F68 (% w/w)	Concentration of Lutrol® F127 (% w/w)	pH
1	1.25	1.25	5.50	15.50	7, 7.5, 8, 8.5
2	2.50	2.50	5.50	15.50	5, 7, 7.5, 8, 8.5, 10
3	3.75	3.75	5.50	15.50	7, 7.5, 8, 8.5
4	5.00	5.00	5.50	15.50	7, 7.5, 8, 8.5
5	–	–	5.50	15.50	5, 7, 7.5, 8, 8.5, 10

2.7. Drug release studies — in vitro

Diffusion cells consisting of two glass compartments, a sampling port, a poly(tetrafluoroethylene) magnetic stirrer and a synthetic membrane were used for the drug release studies (Scherlund et al., 1998). A synthetic cellulose membrane with a specified pore size (Spectra/Por® 4, MWCO 12 000–14 000) was used since the availability and reproducibility of mucosa derived from a suitable animal are limited. The formulations were compared against a reference product, EMLA®, which has been reported to be effective in anesthetizing the oral mucosa (Holst and Evers, 1985). Degassed distilled water was used as sink solution. The diffusion cells were placed in a water bath maintained at 35°C, with magnetic stirrers set at 500 rpm. After equilibrating at room temperature for 60 min, 1 g of sample was placed in the donor part of the cell onto the membrane using a syringe. The timer was started just before the sample was applied. Samples of 500 μl were removed from the receptor compartment every 15 min for the first hour and then every 30 min for up to 4 h. Every sample removed was replaced with the same amount of degassed distilled water. An HPLC system consisting of an LKB 2150 HPLC pump, a Spectroflow 757 absorbance detector from ABI Analytical Kratos Division, a Chrom Jet SP 4400 integrator from Thermo Separation Products, and a μ-Bondapak™ C18 reversed phase column from Waters were used to analyze the samples. As eluent a mixture of 65% methanol and 35% phosphate buffer solution (pH 8) was used. The flow was set at 0.8 ml min^{-1}, the absorbance at 0.05, and the wavelength at 220 nm. The amounts of prilocaine and lidocaine released in μmol/cm^2 h were calculated from standard curves of prilocaine and lidocaine being prepared for each drug diffusion experiment. The measurements were performed in at least duplicates for each formulation and for each experiment two samples of EMLA® cream 5% w/w (containing 25 mg/g of lidocaine and prilocaine respectively) were included as controls. The release rate of lidocaine and prilocaine measured for samples stored for up to 12 months was measured on-line with a Lambda 20 UV spectrometer from Perkin Elmer. Apart from that the same technique as described above was used.

3. Results and discussion

3.1. General considerations

All formulations investigated were based on the active ingredients lidocaine and prilocaine (Fig. 1) and the block copolymers Lutrol® F127 and F68. The concentrations of the components were varied between 2 and 10% w/w for the active ingredients, between 13 and 17% w/w for Lutrol® F127, and between 2 and 6% w/w for Lutrol® F68. The pH of the formulations was varied between 5 and 10. The aim was to achieve a stable formulation with a gelation temperature between room and body temperature and with a high initial release rate according to the requirements stated in the introduction. Most of the combinations were found to result in clear solutions presumably being o/w microemulsions or mixed micellar solutions depending on the pH of the system. Note that the term 'microemulsion' should be used with some care, since this denotes a rather well-defined type of systems, and since literature in the past has described numerous systems as 'microemulsions' when in fact they were other types of structures, e.g. emulsions of a small droplet size, etc. The properties of the active ingredients used in this study change dramatically with pH. Thus, at sufficiently low pH, lidocaine and prilocaine are positively charged, and they could be expected to behave largely as water-soluble cationic surfactants, hence possibly forming mixed micelles. At high pH, on the other hand, the substances are poorly soluble (0.52% w/v in 1 mM NaOH at 32°C) (Nyqvist-Mayer et al., 1986), and could be expected to act largely as hydrophobic solutes. Clearly lidocaine/prilocaine are also highly surface active under these conditions, but this is due to their poor water solubility, which again is analogous to the behaviour of sparingly soluble solutes in general, and not restricted to amphiphiles. Thus, the formulations are presumably o/w microemulsions at high pH and mixed micellar solutions at low pH. In order to

Table 3
Micellar size obtained by PCS

Sample no.[a]	Sample concentration (% w/w)	Temperature (°C)	Radius, R_h (nm)
1	1	35	10.3
1	3	35	12.1
2	1	35	10.6
2	3	35	11.9
3	1	35	12.2
3	3	35	11.9
4	1	35	11.0
4	3	35	11.9
5	1	35	11.6
5	3	35	11.6
6	1	35	11.4
6	3	35	12.2

[a] Compositions shown in Table 1.

investigate this in more detail experiments were performed using various techniques.

3.2. Self-diffusion NMR and PCS

To determine the size of the swollen micellar aggregates presumably present in the diluted formulation, PCS and self-diffusion NMR experiments were performed. Particularly NMR offers interesting opportunities for probing the structure of surfactant systems in general, and microemulsions in particular, as discussed extensively before (Stilbs, 1987; Delpuech, 1995). An aspect of NMR self-diffusion measurement making it especially interesting for these types of systems is that the diffusion of all components may be monitored simultaneously. In order to obtain more information regarding the structure of the formulations, we investigated the diffusion coefficients of water, the polymers, and the active ingredients. The self-diffusion coefficient observed for water ($D_w \approx 1.8 \times 10^{-9} m^2/s$ at 25°C) should be compared to that of neat water ($D_0 = 1.90 \times 10^{-9} m^2/s$ at this temperature (Mills, 1973)) which clearly shows that the system is water continuous. Considering that the formulation has been extensively diluted, this is not surprising. However, also more concentrated PEO-PPO-PEO copolymer systems display water coefficients close to that of neat water, as discussed in detail previously (Malmsten and Lindman, 1992b). Furthermore, the diffusion coefficients for the polymer component in the formulation agrees quite well with that for Lutrol® F127 in itself, and indicates a temperature dependent micellization, with a critical micellization temperature (cmt) between 25°C and 35°C. (Note, however, that we can not exclude very small oligomer micelles even at temperatures lower than 25°C) Analogous findings for the Lutrol® F127 single component system have been discussed in more detail previously (Malmsten and Lindman, 1992a). The diffusion coefficient of the active components ($\approx 4 \times 10^{-10} m^2/s$), finally, falls in between that of the polymers and water, suggesting that a substantial fraction of the active components diffuse freely in the aqueous solution.

At 20°C the low light scattering in the PCS measurements indicates that no or only very small micelles are present at this temperature, although quantification is difficult. At 35°C, on the other hand, there are micelles present in the systems as indicated in Table 3, which is also supported by results from the NMR experiments (Table 4). As found previously (Malmsten and Lindman, 1992a), the tendency for micelle formation by Lutrol® F127 increases with temperature in this temperature range and therefore the present NMR diffusion finding of larger hydrodynamic radii at 35°C than at 25°C is not unexpected. Also quantitatively, the present results are quite similar to those found previously for the size of micelles formed by Lutrol® F127 alone (Wanka et al.,

Table 4
Micellar size obtained by self-diffusion NMR[a]

Sample no.[b]	Temperature (°C)	Micelle size (nm)
1	25	4.54
2	25	4.65
3	25	5.04
4	25	4.75
5	25	4.87
6	25	4.61
1	35	14.2
2	35	14.7
3	35	15.2
4	35	14.6
5	35	15.1
6	35	14.4

[a] For all measurements the sample concentration was 3% w/w.
[b] Compositions shown in Table 1.

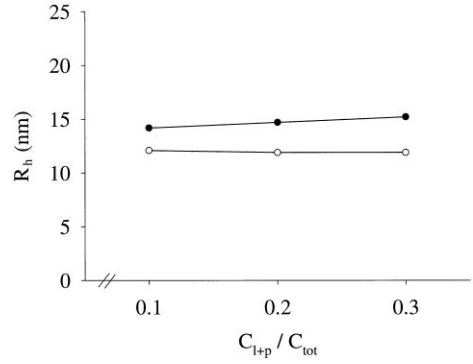

Fig. 2. Influence of different concentrations of active ingredients on the hydrodynamic radius of the micelles at 35°C. NMR results (3% w/w solutions) (filled circles) and PCS results (3% w/w solutions) (open circles) are shown. C_{tot} corresponds to the total concentration in weight basis of polymers and active components.

1990; Malmsten and Lindman, 1992a). PCS and NMR results indicate a cmt between 25°C and 35°C for a range of formulation compositions. As indicated by PCS and NMR measurements, neither the polymer concentration nor the sample composition have any major effect on the micellar size, for the ranges investigated. Furthermore, the amount solubilized drug molecules or the ratio of poloxamers has essentially no influence on R_h in the range investigated (Fig. 2). (At 1% a slightly increasing R_h with increasing concentration of lidocaine and prilocaine was observed, but again the dependence is quite limited) This minor change is as can be expected, considering the relatively low concentration of the active ingredients in comparison to that of the polymers taken together.

3.3. GPC experiments

The PEO-PPO-PEO block copolymers are known for their ability to self-assemble, thus forming various associated structures, e.g. micelles, liquid crystalline phases, reversed liquid crystalline phases and microemulsions (Linse, 1993a,b; Mortensen and Pedersen, 1993; Wanka et al., 1994; Wu et al., 1994; Alexandridis et al., 1995; Alexandridis and Hatton, 1995; Zhang and Khan, 1995; Alexandridis et al., 1996). The PPO being quite hydrophobic results in micellar solutions capable of solubilizing hydrophobic solutes (Alexandridis and Hatton, 1995), although due to the slight polarity of the PPO core the solubilization capacity is higher for aromatic than aliphatic substances (Nagarajan et al., 1986). Furthermore, the solubilization capacity increases with temperature and relative PO content (Hurter and Hatton, 1992; Saito et al., 1994).

There have been numerous reports on the use of PEO-PPO-PEO block copolymers as drug delivery systems for hydrophobic substances. Their fairly low toxicity makes them interesting for various administration routes such as dermal, oral, buccal, nasal, ocular, rectal, vaginal and parenteral (Schmolka, 1977b; Miller and Donovan, 1982; Miyazaki et al., 1986; Carlfors et al., 1991; Wang and Johnston, 1995). Apart from increasing the often low solubility of hydrophobic drugs through solubilization the block copolymers work as stabilizers against hydrolysis since the micellar core protects the drug from the surrounding aqueous environment. Just to give one example, the hydrolysis rate of indomethacin was reported to be reduced after solubilization into PEO-PPO-PEO micelles (Lin and Kawashima, 1985).

An interesting feature of block copolymer micelles, with or without solubilized drug, is their

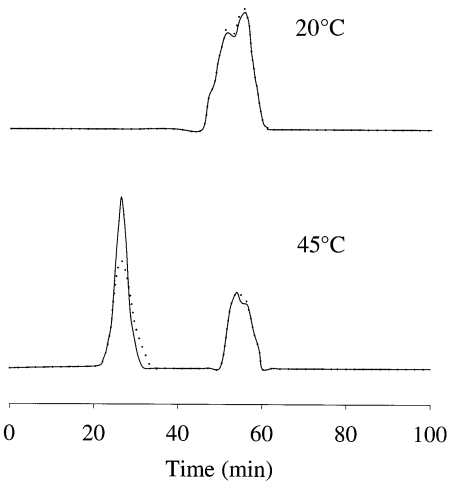

Fig. 3. GPC chromatograms for the mixed polymer solution (solid line) and a typical formulation at pH 8 (dotted line) below (20°C) and above (45°C) the critical micellization temperature.

extremely slow disintegration kinetics. For example, Lutrol® F127 micelles have been found to be stable for more than an hour after dilution below cmc, which facilitates studies of the micellization with, e.g. GPC (Malmsten and Lindman, 1992a). Given this, we were interested in investigating the micellization and solubilization with this technique. Typical results from the GPC experiments performed on the mixed polymer solution (Lutrol® F127 15.5% w/w and Lutrol® F68 5.5% w/w) at 20 and 45°C can be seen in Fig. 3. These results confirm previous findings for Lutrol® F127 by Malmsten and Lindman, where the GPC chromatograms at low temperature are characterized by one peak corresponding to unimers. Above a critical micellization temperature, on the other hand, the chromatograms contain also another peak, corresponding to micelles. With increasing temperature the peak corresponding to micelles increases at the same time as the unimer peak decreases, i.e. the cmc decreases with increasing temperature. This has been discussed in detail previously (Linse and Malmsten, 1992; Linse, 1993a; Wanka et al., 1994; Alexandridis and Hatton, 1995).

Some of the formulations (i.e. including the active components) were initially run at 20 and 45°C and were found to show a pattern matching that seen for the mixed polymer solution in absence of lidocaine and prilocaine (see Fig. 3). The rest of the formulations were only run at 45°C where micellization is ensured. Since the polymer solutions were found to display UV absorbance at the same wavelength as lidocaine and prilocaine, i.e. 220 nm, the peak areas for the polymers were subtracted from those of the formulations. In Fig. 4 the areas under the curve (AUC) of the peaks corresponding to the micelles and unimers at increasing concentration of active ingredients are shown for a formulation at pH 7.5. As can be seen an increasing amount of the active ingredients results in an increasing amount solubilized in the micelles, whereas almost no solubilization is found for the unimers. Formulations at lower and higher pH values were also investigated and contrary to our expectations, considering the pK_a values of lidocaine and prilocaine being 7.86 and 7.89, respectively (Nyqvist-Mayer et al., 1986), the lower the pH the more of the active ingredients were found in micelles. The origin of this effect is unclear at present, but due to the amphiphilic nature of the active components (see Fig. 1) they could be expected to bear some resemblance to short-chain cationic surfactants in its ionized form. Since PEO has been found to interact with and bind such surfactants (although weakly), this may possibly affect the 'solubilization'. However, artifacts such as absorption of active ingredients

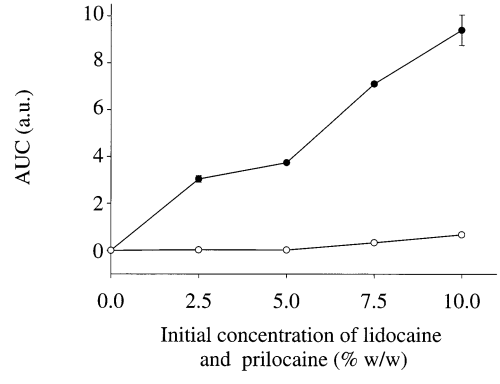

Fig. 4. The area under the curve (AUC) of the micellar peak (filled circles) and unimer peak (open circles) at varying concentrations of active ingredients at pH 7.5. Normalized AUC.

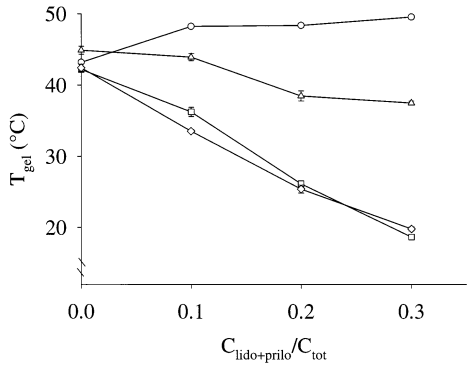

Fig. 5. The effect of concentration of active ingredients on the gelation temperature at pH 5 (open circles), 7 (open triangles), 8 (open squares) and 10 (open diamonds). The concentration of Lutrol® F127 and Lutrol® F68 was 15.5 and 4% w/w, respectively, for all formulations.

to tubing, filters etc, at higher pH values, can not be excluded and further investigations are therefore required. Nevertheless, a preferential solubilization of active ingredients in micelles over unimers with increasing concentration was found for all pH values investigated.

3.4. Rheology measurements

The PEO-PPO-PEO block copolymers are mainly known for their ability to form gels on heating. At low temperatures (e.g. at refrigerator or room temperatures) a 20% w/w aqueous solution of Lutrol® F127 shows a viscosity of about 0.02 Pa s, (Malmsten and Lindman, 1993). When the temperature is increased, a rigid 'gel' is formed at a well-defined temperature which depends on the polymer concentration, composition and molecular weight of the polymer, and addition of different cosolutes such as surfactants, polymers, salt, and hydrophobic compounds (Malmsten and Lindman, 1992a, 1993; Alexandridis and Hatton, 1995). It has been shown previously that the gelation of Lutrol® F127 is influenced by the active ingredients in the presently investigated formulations, i.e. lidocaine and prilocaine, and that considerations have to be taken to this fact when choosing a suitable concentration of the polymer (Scherlund et al., 1998). In Fig. 5 the temperature of gelation is plotted for different concentrations of active ingredients (ratio 1:1) at fixed polymer concentrations and pH. It can be seen that at high pH, i.e. when the active components are uncharged and thus sparingly soluble in water, the gelation point decreases with increasing amount of lidocaine and prilocaine. This is in agreement with previous findings on the effects of hydrophobic compounds on the gel formation of PEO-PPO-PEO block copolymer systems. For example, Malmsten and Lindman (1992a) investigated the gelation of Lutrol® F127 and found that t-butylbenzene lowers the gelation temperature for this polymer. In analogy, Gilbert et al. (1987) found that benzoic acid and *p*-hydroxybenzoate esters caused a decrease in the gelation temperature of the block copolymer and that the more hydrophobic the solute the greater the decrease in gelation temperature. The origin of this effect is that the presence of a hydrophobic component induces micellization (lowers cmc) and causes a micellar growth, in agreement with the behavior of low molecular weight surfactants (Lindman and Wennerström, 1980) (This notion is supported by the findings of solubilization of active components in the block copolymer micelles discussed above). Since 'gelation' in the presently investigated system is strongly related to the micellization (see previous discussions on structural aspects, e.g. Malmsten and Lindman, 1992a; Mortensen et al., 1992; Alexandridis and Hatton, 1995) the induced micellization and the micellar growth are expected to result in a decreased gelation temperature. At pH 5, on the other hand, the opposite behavior is found, i.e. the gelation temperature increases somewhat with an increasing concentration of the active components. This is quite interesting and may indicate that the active ingredients, which are almost completely (99.9%) in their hydrophilic ionized form at this pH, interact with the hydrophilic PEO part of the polymer in a similar way as found for short-chain cationic surfactants. By introducing charges on the polymer backbone the solvency of the polymer effectively improves (Goddard and Ananthapadmanabhan, 1993) resulting in an increase in gelation temperature.

In Fig. 6 the gelation curves for compositions containing the same amount of active ingredients

(i.e. 5% w/w) with varying amounts of Lutrol® F127 and Lutrol® F68 are shown. It can be seen that the more Lutrol® F127 present the lower the gelation point, whereas, the opposite holds for Lutrol® F68. From NMR and PCS data, we infer that the copoymer mixture composition has no significant effect in this range regarding the micellar size. However, we can not exclude small variations in the micellar size, which might have an accumulative comparably large effect on gelation at present. In conclusion, therefore, the mechanism for the effects of the copolymer composition on micellization and gelation is currently somewhat unclear and further studies of mixed micelle formation are needed. Nevertheless, for our purpose it is sufficient to know that by altering the concentrations of Lutrol® F127 and F68, the temperature of gelation can be set to the desired value. This can be seen in Fig. 6 where a minor change in the F127/F68 ratio (at a constant total polymer concentration) causes a clear change in the gelation temperature.

3.5. Drug release studies — in vitro

The in vitro drug release of lidocaine and prilocaine from formulations with varying pH values, compared to the reference EMLA® cream 5% w/w, is presented in Fig. 7a. It can be seen that the release rates for the formulations are higher than that for the reference. In order to highlight the effect of pH, on the release rate of active ingredients, the results are summarized in Fig. 7b where it can be seen that the release rate increases with decreasing pH. The reason for this behavior is that the lower the pH the more of the active ingredients are present in ionized form and hence more is present in the aqueous part of the system. This is in agreement with findings by La et al. (1996) who studied the release of indomethacin (a weak acid) from PEO-poly(β-benzyl L-aspartate) block copolymer micelles in aqueous solution, and found an increased release rate above pKa of indomethacin. It should also be noted that at pH 5 and 7.5 the formulations are in a liquid state at 35°C, while the formulations of pH 7.8 and 10 are in a gel state at this temperature. It can also be seen that after 4 h 50% w/w of the active ingredients have already been released at pH 5. By choosing a pH in the area of 7.8 it is possible to maintain a high release rate at the same time as having a system forming a gel in the desired temperature range.

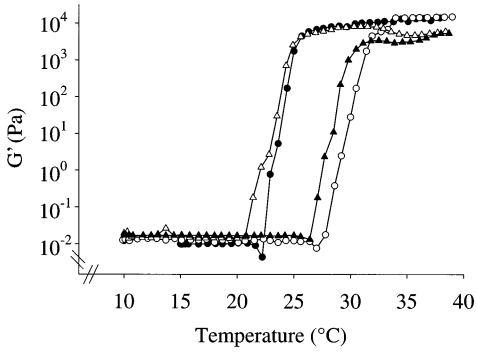

Fig. 6. Elastic modulus (G') of formulations containing 5% w/w Lutrol® F68 and 14.5% w/w of Lutrol® F127 (open circles), 4% w/w Lutrol® F68 and 15.5% w/w of Lutrol® F127 (filled circles), 5% w/w Lutrol® F68 and 16% w/w of Lutrol® F127 (open triangles), and 5.5% w/w Lutrol® F68 and 15.5% w/w of Lutrol® F127 (filled triangles). All formulations contained 5% w/w of active ingredients (1:1) and had a pH of 8.

In Fig. 7c the relative release rates of the formulations are compared to the emulsion-based formulations previously investigated (Scherlund et al., 1998). It can be seen that the presently investigated formulations have a much higher release rate (except at the highest pH) compared to the emulsion-based formulations. Naturally, this is due to the smaller aggregates solubilizing the active components (Scherlund et al., 1998). Considering the indication for the formulation a fast onset time is required, and therefore a high initial release rate is important. These results are encouraging since EMLA® cream has been tested on oral mucosa previously and found to be effective (Holst and Evers, 1985). Furthermore, it is noteworthy that there seem to be a difference in release rate between lidocaine and prilocaine at different pH values. As seen in Fig. 7d the same amount of the two active ingredients is released at pH 5. With increasing pH, however, there is an increase in the release of prilocaine over lidocaine. Considering that lidocaine is more hydrophobic than prilocaine this behavior is expected (Nyqvist-Mayer et al., 1986).

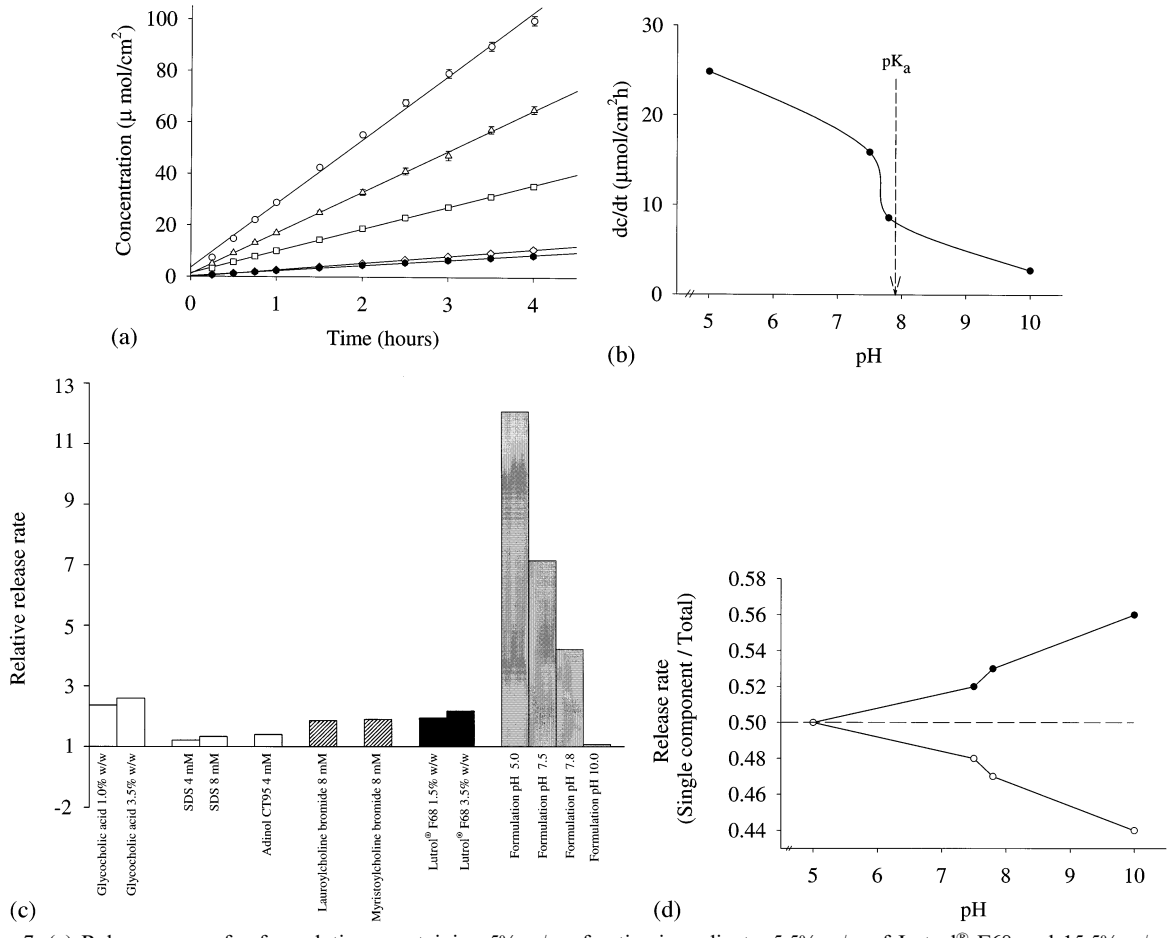

Fig. 7. (a) Release curves for formulations containing 5% w/w of active ingredients, 5.5% w/w of Lutrol® F68 and 15.5% w/w of Lutrol® F127 at pH 5 (open circles), 7.5 (open triangles), 7.8 (open squares) and 10 (open diamonds) compared to EMLA® cream 5% w/w (filled hexagons). (b) Initial release rate, taken over the first 4 h, as a function of pH. The arrow indicates the pK_a value of lidocaine and prilocaine being 7.9, respectively. (c) The relative release rate of lidocaine and prilocaine from emulsion-based formulations containing active ingredients 4.5% w/w, Lutrol® F127 14% w/w and anionic surfactants (unfilled bars), cationic surfactants (striped bars) and nonionic surfactants (filled bars), all divided by the release rate of the reference formulation EMLA® cream 5% w/w (2.58 μmol/cm²h), compared to formulations containing 5% w/w of active ingredients, 15.5% w/w of Lutrol® F127 and 5.5% w/w of Lutrol® F68 at various pH values (grey bars), all divided by the release rate of the reference formulation EMLA® cream 5% w/w (2.39 μmol/cm²h). (d) The release rate ratio of the individual components divided by the total release rate of lidocaine (open circles) and prilocaine (filled circles) at different pH values. All formulations contained 15.5% w/w of Lutrol® F127 and 5.5% w/w of Lutrol® F68. The lines are merely guides to the eye.

3.6. Robustness of the system

The system seems to be quite robust since clear solutions have been found in the region of 2–5% w/w of active ingredients, 13–17% w/w of Lutrol® F127 and 2–6% w/w of Lutrol® F68. In order to find the maximum and minimum amounts for each component giving a microemulsion, a complete phase diagram has to be mapped. This, however, is out of the scope of the current investigation. As expected the storage stability of the formulations is good, showing no significant variance with respect to macroscopic appearance, temperature of gelation, amount of active ingredients, pH, and drug release rate when stored for 12 months at 25°C (Table 5).

Table 5
Storage stability of a typical formulation at 25°C[a]

Time (months)	Active ingredients (% w/w)	pH	Gelation temperature (°C)	Release rate[b] (μmol/cm²h)	Macroscopic appearance
0	5.04	7.7	28.1 ± 0.42	8.14 ± 0.08	At 25°C: a clear solution. Above the gelation point: a clear gel.
6	5.04	7.8	28.7 ± 0.14	–	Same as at 0 months
12	4.96	7.8	26.5 ± 0.07	7.34 ± 0.28	Same as at 0 months

[a] The composition is shown in Table 2, sample no 2.
[b] The release rate of reference EMLA® cream 5% w/w was 2.49 μmol/cm²h.

4. Conclusions

When mixing the active ingredients lidocaine and prilocaine, in a ratio of 1:1, together with the nonionic block copolymers Lutrol® F127 and Lutrol® F68 clear stable micellar solutions are obtained. Self-diffusion NMR and PCS measurements of the diluted formulations show discrete micelles with a diameter of 20–30 nm and a critical micellization temperature of 25–35°C. The size of the micelles is largely independent of concentration and composition in the range investigated. The GPC experiments indicate a preferential solubilization of the active components in micelles over unimers.

In order to achieve a formulation with rheological properties suitable for the intended application, care has to be taken in choosing both pH and appropriate concentrations of the components included, since both the polymers and the active ingredients have an impact on the temperature of gelation. The release rate was shown to increase with decreasing pH of the formulations and to be higher or the same as the reference EMLA® cream for all the formulations tested. By choosing a pH in the area of 7.8 it is possible to have a system with a gelation point between room and body temperature displaying a high initial release rate, thus making it suitable for the intended use.

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