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Stabilization of a thermosetting emulsion system using ionic and nonionic surfactants

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Abstract

Ways of achieving a suitable local anesthetic formulation for use in the periodontal cavity were investigated in this study. By choosing poly(ethylene oxide)–poly(propylene oxide)–poly(ethylene oxide) block copolymers as excipients, formulations which are low viscosity fluids at room temperature and rigid elastic gels at body temperature are obtained. Despite the solubilizing capacity of these polymers, formulations containing Lutrol® F127 ($EO_{99}PO_{65}EO_{99}$) and the active ingredients lidocaine and prilocaine at the desired concentrations, i.e. approximately 25 mg g^{-1} of each component, are unstable. In order to achieve a more stable formulation a second surfactant can be added to the system since it could help both to solubilize the hydrophobic active ingredients and to stabilize the droplets of lidocaine and prilocaine from flocculation and coalescence. Thus, formulations containing local anesthetic compounds comprising the oil phase, a block copolymer giving the system unique rheological properties, and a suitable second surfactant were evaluated with regard to rheological behavior, drug release properties and stability. The system needs to be balanced regarding the concentration of polymer, active ingredients and surfactant in order to achieve a formulation with suitable properties. Stable formulations with appropriate characteristics for the application in focus here were obtained with anionic, cationic and nonionic surfactants.

Keywords: PEO-PPO-PEO block copolymers; Thermoreversible gelation; Surfactants; Periodontal pocket; Lidocaine; Prilocaine

1. Introduction

Many people in need of periodontal scaling procedures suffer from fear of visiting dental clinics due to the pain associated with needle injections. A topical formulation containing local

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anesthetic compounds would enable the patients to have painless treatment without the distress of an injection. Such a formulation needs to be easy to apply, stay at the application site, have a fast onset time, be a non-irritant, and be stable under normal storage conditions. By choosing an excipient from a group of low-toxicity block copolymers, showing reversed temperature dependent gelation, a formulation which is a low viscosity fluid at room temperature and a rigid elastic gel at body temperature, can be achieved. Therefore, a formulation which efficiently fills a cavity, e.g. the periodontal cavity, may be easily applied, due to its low viscosity, after which the rigid gel forms ensuring an effective fixation of the formulation at the application site. After treatment, the gel may be removed by simple rinsing with cold water.

Of special interest to the present study are the local anesthetic compounds lidocaine and prilocaine, which, e.g. are the active ingredients in EMLA® cream. These substances are quite hydrophobic and practically insoluble in water (Brodin et al., 1984) consequently an aqueous system with the concentration required to obtain the maximum release rate from the system, i.e. 50 mg g^{-1} (Nyqvist-Mayer et al., 1986) of the active substances will typically be an oil-in-water (o/w) emulsion.

In the present investigation, we have focused on poly(ethylene oxide)–poly(propylene oxide)–poly(ethylene oxide) (EO_m-PO_n-EO_m) block copolymers, since these are commercially available in a range of molecular weights and compositions, they have fairly low toxicity, and, in particular, they display a pronounced reversed temperature gelation. Furthermore, these polymers form micelles in aqueous solution consisting of a PPO core and a PEO palisade. Since the PPO core is quite hydrophobic, the micellar solutions are capable of solubilizing hydrophobic solutes (Alexandridis and Hatton, 1995) such as lidocaine and prilocaine. The solubility of lidocaine in Lutrol® F127 gels has been described in the literature and determined to be 2.4, 2.7 and 3.2% w/w for Lutrol® F127 gels of 20, 25 and 30% w/w, respectively (Chen-Chow and Frank, 1981). Despite all the promising features of these systems, however, the formulations prepared in this way are not necessarily stable, but rather can undergo phase separation on storage, as a result of emulsion droplet flocculation and coalescence. Naturally, the latter processes are particularly important when the lidocaine and prilocaine concentrations required for a functional formulation (50 mg g^{-1}) (Nyqvist-Mayer et al., 1986) greatly exceed the solubility limit of the micellar solution. Given the necessity to reach a therapeutically effective dose and that the polymer concentration required for complete micellar solubilization would be very high (rendering practical handling difficult), the emulsion formed needs to be stabilized in another way.

A well known approach for improving the stability of disperse systems (including emulsions) is to add surfactants which, following their adsorption at the 'oil–water' interface, result in repulsive interdroplet interactions due to either electrostatic or steric mechanisms (Israelachvili, 1991). Also for the present systems, it was found that upon the addition of nonionic, anionic or cationic surfactants an increased formulation stability can be obtained.

The aim of the present study was, therefore, to investigate the effects of various surfactants on the performance of the systems, particularly with regard to their stability, rheology, and drug release properties, as well as to evaluate the possibilities of preparing formulations fulfilling the requirements stated above.

2. Materials and methods

2.1. Chemicals

The block copolymers used, i.e. Lutrol® F68 ($EO_{79}PO_{28}EO_{79}$) and F127 ($EO_{99}PO_{65}EO_{99}$), were commercial products from BASF Svenska, Sweden. The bile salt was a 99% purified grade of glycocholic acid sodium salt obtained from Sigma Chemicals, USA. Sodium dodecyl sulfate (C_{12}), (SDS), cmc value of 8.1 mM (Israelachvili, 1991) came from Biochemical, UK, whereas the sodium lauryl oligooxyethylene sulfates (C_{12}), (Texapon® N70 EO_2, Texapon® N70LS EO_3 and Chemsalan®

RLM70 EO_2, cmc values of 1.54, < 1.5 and 0.44 mM, respectively (Linfeld, 1976 and supplier Mataki Kemi)) were commercial products from Mataki Kemi, Sweden. Sodium *N*-methyl *N*-cocoyl taurate (C_{12}), (Adinol® CT95, cmc value of 1 mM given by the supplier) and sodium *N*-lauroyl sarcosinate (C_{12}), (Crodasinic® LS30, cmc value of 7–9 mM given by the supplier) were obtained from Croda Nordica, Sweden. Lauroylcholine bromide (C_{12}), cmc value of 6.8 mM (Chelminska-Bertilsson et al., 1993) and myristoylcholine bromide (C_{14}), cmc value of 0.56 mM (Chelminska-Bertilsson) were a gift from the department of Clinical Bacteriology, University of Göteborg, Sweden. The active ingredients prilocaine and lidocaine came from Astra, Södertälje, Sweden, as did the PEG-54 (54 mol ethylene oxide) hydrogenated castor oil (98% C_{18}), (Arlatone® 289). Distilled water and 2 M hydrochloric acid were used as appropriate. For the drug release studies EMLA® cream 5% w/w, obtained from Astra, Södertälje, Sweden, was used.

All chemicals were used without further purification.

2.2. Preparation of formulations

Concentrated solutions of Lutrol® F127 were prepared according to the so-called cold method (Schmolka, 1977). The block copolymer powder was added to cold distilled water in portions during agitation after which the samples were kept in a refrigerator at 4–8°C until clear systems were obtained. For all formulations prilocaine and lidocaine were mixed in a ratio of 1:1 (giving a eutectic mixture) (Brodin et al., 1984), with or without Arlatone® 289, and heated to 70°C until a homogeneous melt was obtained. Then the temperature was lowered to 60°C. Distilled water at 60°C was slowly added to the melt during agitation with a Ystral® X10/25 (Bergius Trading, Sweden) homogenizer at 2000 rpm for 1 min during cooling resulting in a concentrated emulsion. In the cases where another emulsifying agent was added to the formulation it was dissolved in the 60°C water portion. For the polymer-free emulsions of lidocaine and prilocaine (1:1) used for MasterSizer® measurements and phase separation studies, distilled water was added to the concentrated emulsion during continued homogenization at 2000 rpm for 3 min. The pH was measured and adjusted to 8 or 9 with 2 M hydrochloric acid 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.

To prepare the gel formulations of lidocaine and prilocaine (1:1) used for rheology, phase separation, and drug release studies, concentrated Lutrol® F127 solutions, of 22% w/w, were added in small portions during agitation. The pH was adjusted to 8 or 9 with 2 M hydrochloric acid and the weight was adjusted to its final value with distilled water.

2.3. Rheology measurements

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

After the formulations had been placed on the plate and the cone lowered, the excess material was removed and then a solvent trap was applied in order to prevent sample evaporation. Before the first measurement, each sample was preshearred for 5 min at a shear rate of 0.5 s^{-1}, followed by an equilibration time of 60 min. Preshearing of the samples was tested at longer and shorter preshearing times (2.5–10 min) with higher and lower shear rates (0.1–1.0 s^{-1}), and with longer and shorter equilibrium times (30–120 min) all giving the same results. The following measurements were performed for each sample:

1. Oscillation stress sweep to obtain the linear viscoelastic region.
2. Oscillating temperature sweep from 10–40°C with constant stress in the linear viscoelastic region, to determine the temperature of gelation and the elastic and loss moduli (G' and G'') of the gels.

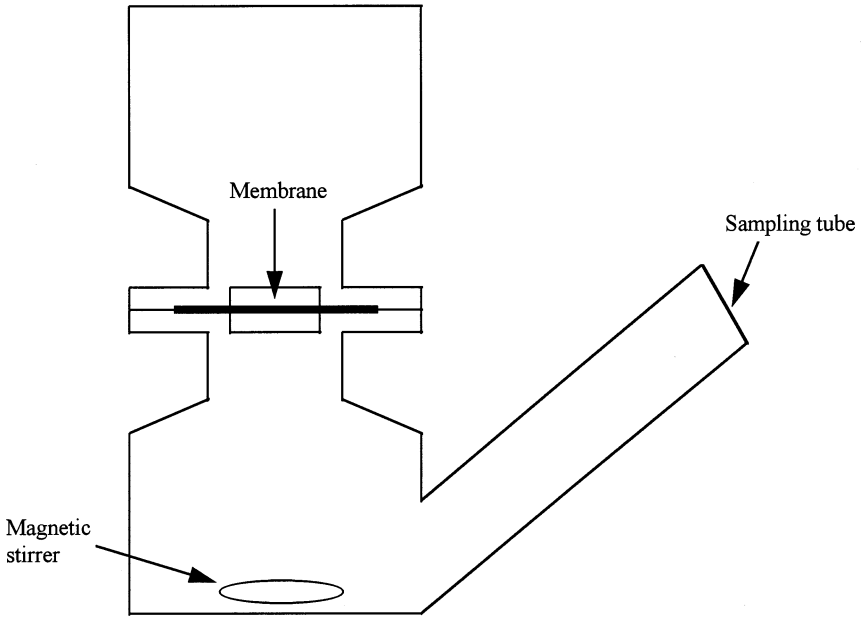

Fig. 1. A schematic illustration of the apparatus used for the drug diffusion studies.

Duplicate measurements, at least, were performed for all samples with a variation of less than ± 1°C.

2.4. Phase separation studies

The stability of the formulations was studied by storing samples (approximately 200 ml) at a low temperature (4–8°C). The samples were inspected visually at different storage times and their appearance was noted according to the following scale:

1. No phase separation.
2. Slight phase separation—a very thin creaming layer at the top.
3. Moderate phase separation—a thin creaming layer at the top.
4. Severe phase separation—a distinct separation into two phases.
5. Precipitation/crystallization.

2.5. Particle size measurements

Measurements of emulsion droplet size were made with a Malvern MasterSizer, Malvern Instruments, UK. A diluted dispersion/emulsion is pumped through a measuring device where it is illuminated by a laser beam (He–Ne: 633 nm). The scattered light is caught by a lens and focused upon a detector. The measured scattering pattern will depend upon the particle size distribution which is determined by these measurements.

The droplet size distribution of different emulsions were evaluated in the 0.1–80 μm range with a 2NHD polydisperse model. The solvent used as suspension medium was a phosphate buffer solution at pH 8 ($Na_2HPO_4*2\ H_2O$ 16.25 mM and $NaH_2PO_4*H_2O$ 12.5 mM). A sample was added to the suspension medium until the obscurity (related to the particle concentration) was between 10–30%, after which the measurement was conducted. The droplet sizes given are values of d (0.5), i.e. the volume median diameter.

The measurements were performed in triplicate at room temperature.

2.6. Drug release studies in vitro

The amount of prilocaine and lidocaine diffusing through a synthetic membrane was studied using diffusion cells (Fig. 1). The diffusion cell consisted of two glass compartments, a poly(tetrafluoroethylene) magnetic stirrer, a sampling port, and a membrane. Because of the limited availability and reproducibility of mucosa derived from a suitable animal a synthetic cellulose membrane with a specified pore size (Spectra/Por® 4, MWCO 12000–14000) was used. Due to the difficulty in finding a membrane that can actually predict what will happen in vivo the formulations were compared against a reference product, EMLA®, which has been tested on oral mucosa and found to be effective (Holst and Evers, 1985). The sink solution was degassed distilled water. The diffusion cells were placed in a water bath maintained at 35°C, and the magnetic stirrer was set at 500 rpm. The sample (1 g) was allowed to equilibrate at room temperature for 60 min and was then placed in the donor part of the cell and onto the membrane using a syringe. The timer was started just before applying the sample. Samples of 500 μl were withdrawn from the receptor compartment every 15 min for the first hour and then every 30 min for up to 4 h. Each sample withdrawn was replaced with the same amount of degassed distilled water. The samples were analyzed by an HPLC system consisting of a 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. The eluent was a mixture of 65% methanol and 35% phosphate buffer solution (pH 8). The flow was set at 0.8 ml min^{-1}, the wavelength at 220 nm, and the absorbance at 0.05. A standard curve for prilocaine and lidocaine was prepared for each drug diffusion experiment and from these the amounts of prilocaine and lidocaine released in μmol cm^{-2} h^{-1} could be calculated. Two or three measurements were performed for each formulation and for each experiment two samples of EMLA® cream 5% w/w (containing 25 mg g^{-1} of lidocaine and prilocaine, respectively) were included as controls.

3. Results and discussion

3.1. General considerations

The formulations investigated in the present study are based on Lutrol® F127, also known as poloxamer 407, with the chemical formula $EO_{99}PO_{65}EO_{99}$. PEO-PPO-PEO block copolymers are useful in a host of pharmaceutical applications, which, apart from their low toxicity and commercial availability, is due to interesting physico-chemical behavior. This includes their micellization and subsequent ability to solubilize (moderately) hydrophobic substances (e.g. for reducing the hydrolysis rate or to increase drug solubility (Alexandridis and Hatton, 1995)), their ability to adsorb at various surfaces and evoke protein rejection (of major importance for preventing opsonization in parenteral drug delivery using colloidal drug carriers) (Napper, 1983; Fleer et al., 1993), and their ability to form gels on heating. Due to their versatile effects in various formulations, the Lutrols are referred to as thermosetting block copolymers and/or nonionic surfactants. Since both of these effects occur in the presently investigated systems, they are referred to as both in the text. Part of the phase behavior of aqueous solutions of Lutrol® F127 is shown in Fig. 2 where it can be seen that the temperature of gelation decreases with increasing polymer concentration. (In fact, at high temperatures, the gels 'melt' and low viscosity solutions are formed.

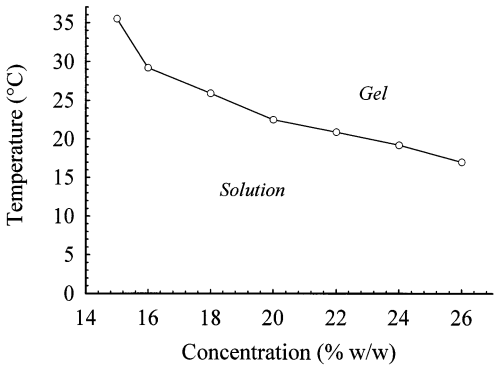

Fig. 2. The phase behavior of aqueous solutions of Lutrol® F127. The line is merely a guide to the eye.

However, although depending on a range of factors, this transition generally occurs at temperatures significantly higher than those relevant for pharmaceutical applications (Malmsten and Lindman, 1992; Alexandridis and Hatton, 1995)). A 20% w/w aqueous solution of Lutrol® F127 typically shows a quite low viscosity (about 0.02 Pa s) (Malmsten and Lindman, 1993) at low temperatures (e.g. at refrigerator or room temperatures). However upon increasing the temperature, a rigid gel is formed at a well defined temperature which may be controlled, e.g. by the molecular weight and composition of the polymer, or by addition of various cosolutes such as salt, polymers, or surfactants (Malmsten and Lindman, 1992, 1993; Alexandridis and Hatton, 1995). The gel formation typically occurs over a narrow temperature range and the elastic modulus of the gel formed is quite high (Brown et al., 1991; Malmsten and Lindman, 1993; Wanka et al., 1994; Alexandridis and Hatton, 1995). The gels display a largely elastic mechanical behavior over a wide frequency range, contrary for example, to concentrated polymer solutions which, at least at low frequencies, are largely viscous in nature (Ferry, 1980).

Despite the solubilization capacity of PEO-PPO-PEO block copolymers, formulations containing Lutrol® F127 and the active ingredients lidocaine and prilocaine at the desired concentrations; i.e. approximately 25 mg g^{-1} of each component, are unstable with regard to flocculation and coalescence, as seen from the occurrence of macroscopic phase separation (Table 1). From this, it may be concluded that the solubilizing capacity at longer storage times of Lutrol® F127 is too low for the application focused on here. The addition of a surfactant to a system is a way of achieving a more stable formulation since it could help both to solubilize the hydrophobic lidocaine and prilocaine and to stabilize droplets of lidocaine and prilocaine from flocculation and coalescence. However, introduction of an additional surfactant can affect the system not only through its effects on the hydrophobic substances, but also due to an interaction between the block copolymer and the surfactant. Thus, in a number of studies, it has been found that a range of polymers, and PEO containing polymers in particular, interact with a host of surfactants, e.g. forming mixed micelles or 'bead-necklace'-type associated structures (Goddard and Ananthapadmanabhan, 1993). Of particular interest to the present investigation is the effect of anionic surfactants self-assembly on PEO-PPO-PEO triblock copolymers which has been reported in the literature. For example, Pluronic® L64 and Pluronic® F68 do not form micelles on their own but will form mixed micelles with SDS at SDS concentrations well below its normal cmc (Almgren et al., 1991). In contrast, nonionic surfactants do not generally interact with nonionic polymers, although mixed micelle formation is still possible.

Finally, the effect of cationic surfactants self-assembly on PEO-PPO-PEO triblock copolymers has not been extensively described. This is the case particularly for the C_{12} and C_{14} choline bromides used in the present investigation. These surfactants are particularly interesting since they are biodegradable as well as antibacterial (Ahlström et al., 1995). Nevertheless, cationic surfactants are generally found to interact more weakly with nonionic polymers than anionic surfactants (Goddard and Ananthapadmanabhan, 1993).

3.2. Gelation

Depending upon the surfactant chosen the rheological properties of the polymer system will be more or less affected. In Fig. 3a the gelation curve for the pure polymer system is compared to the polymer system with the addition of the anionic surfactant Chemsalan® RLM70 or the addition of the nonionic surfactant Arlatone® 289. It can be seen that the temperature of gelation is nearly unaffected by these additives. In Fig. 3b, the effect of the concentration of the bile salt sodium glycocholate on gelation temperature is described. As can be seen the point of gelation changes dramatically with increasing amounts of bile salt. The same tendency was found for Lutrol® F68 (Table 1). This observation is very interesting and is possibly due to the size of the mixed micelles formed. To investigate this is outside the scope of the current investigation but steps have been taken to study this phenomenon further and the

Table 1
Summary of the appearance and rheology of formulations containing nonionic and cationic surfactants

Lutrol® F68 (% w/w)	Arlatone® 289 (% w/w)	C_{12} (mM)	C_{14} (mM)	Temperature of gelation (°C)	Gel strength (kPa)	Appearance after 3 weeks of storage
—	—	—	—	—	—	4
—	1.9	—	—	14.4	13.0	4
1.0	—	—	—	18.3	8.5	4
1.5	—	—	—	21.0	6.5	4
2.5	—	—	—	32.0	5.6	4
3.5	—	—	—	29.4	8.3	1[a]
4.0	—	—	—	33.2	3.4	1[a]
—	1.9	2	—	15.5	10.8	4
—	1.9	4	—	16.2	10.2	1
—	1.9	8	—	16.2	10.6	1[a]
—	1.9	—	2	17.1	9.9	5
—	1.9	—	4	16.3	9.8	5
—	1.9	—	8	17.5	9.7	1[a]

All formulations contained 4.5% w/w lidocaine and prilocaine (1:1) and 14% w/w Lutrol® F127.
[a] Microemulsions.

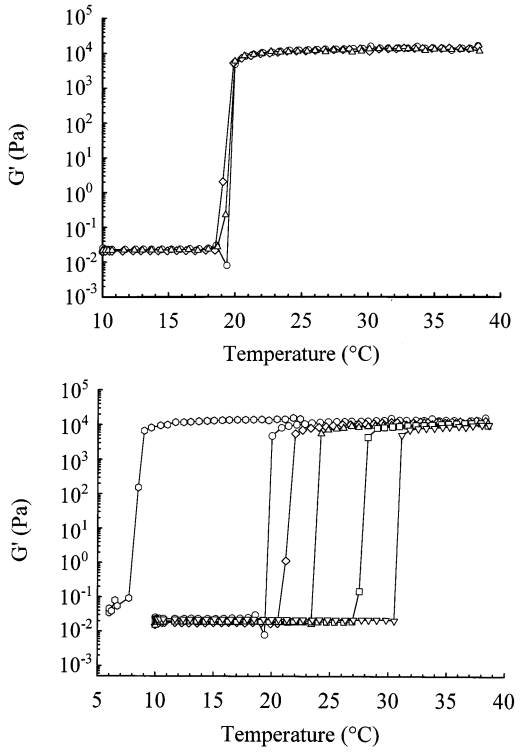

Fig. 3. (a) The elastic modulus of a 20% w/w aqueous solution of Lutrol® F127 in the absence of surfactant (○), as well as in the presence of an anionic (Chemsalan® RLM 70, 8 mM) (◇), or a nonionic (Arlatone® 289, 1.9% w/w) (△) surfactant. (b) The effect of bile salt on the elastic modulus behavior of a 20% w/w aqueous solution of Lutrol® F127. Bile salt concentrations of 0 (○), 1 (◇), 2 (△), 3 (□) and 4% w/w (▽) and bile salt 1% w/w together with lidocaine and prilocaine (1:1) 5% w/w (⬡).

results will be reported at a later stage. The effects of the surfactant on the gelation behavior is an important aspect to consider when choosing a suitable surfactant for making the formulations. However, as described above, and also shown in Tables 1 and 2, the other surfactants studied only had a marginal and controllable effect on the rheological behavior of the systems (Fig. 3a).

It is, furthermore, important to realize that the active ingredients also have a major effect on the gelation temperature. This can be seen in Fig. 3b, where the pure polymer system can be compared to that obtained on the addition of 1% w/w bile salt and that obtained on the addition of bile salt together with the active ingredients lidocaine and prilocaine. In the latter case a significant lowering of the gelation temperature can be seen. Thus, in order to achieve a formulation with a suitable gelation temperature, the system needs to be balanced with regard to the concentrations of polymer, active ingredients and surfactant.

3.3. Formulation stability

3.3.1. Anionic and nonionic surfactants

The storage stability of the formulations at refrigerator temperature are indicated in Tables 1 and 2. As can be seen from Table 1, phase separation was generally observed in the absence of charged surfactants. The reason for this could be that the storage temperature is lower than the upper consolute temperature in the presence of the hydrophobic substances (Flory, 1953). If this is the mechanism operating, the fact that the addition of the nonionic surfactant Arlatone® 289 had no effect on the formulation stability is hardly surprising. However, on the addition of Lutrol® F68 ($EO_{79}PO_{28}EO_{79}$) at high concentrations, microemulsions were formed. These quite interesting formulations will be investigated more extensively, and the results reported separately. Irrespective of the mechanism of phase separation on storage under refrigeration, increased stability may be obtained through the addition of charged surfactants by inclusion of charges in both the polymer micelles (mixed micelle formation) and at the drug droplet surface (surfactant adsorption). The choice of surfactant will depend upon both its properties and toxicity. One group of interesting substances are the anionic surfactants since these have previously been found to form mixed micelles with PEO-PPO-PEO block copolymers (Almgren et al., 1991). Therefore, these surfactants should increase the formulation stability. However, as can be seen from Table 2, using SDS as surfactant gives unstable formulations. Furthermore, it can be seen that the presence of SDS in the formulations causes problems with SDS precipitation on storage at low temperatures. This behavior can at least partly be explained by the Krafft temperature of SDS being higher (20°C) than the storage temperature, which means that

Table 2
Summary of the appearance and rheology of formulations containing anionic surfactants at various concentrations

Bile salt (% w/w)	SDS (mM)	Texapon® N70 (mM)	Texapon® N70LS (mM)	Chemsalan® RLM 70 (mM)	Adinol® CT95 (mM)	Crodasinic® LS30 (mM)	Arlatone® 289 (% w/w)	Temperature of gelation (°C)	Gel strength (kPa)	Appearance after 3 weeks of storage
1.0	—	—	—	—	—	—	—	20.2	7.0	1
2.0	—	—	—	—	—	—	—	29.4	2.0	—
2.5	—	—	—	—	—	—	—	36.3	[a]	1
3.0	—	—	—	—	—	—	—	>40	[a]	1
3.5	—	—	—	—	—	—	—	>40	[a]	1
—	2*	—	—	—	—	—	—	—	—	4
—	2*	—	—	—	—	—	1.9	—	—	1
—	2	—	—	—	—	—	1.9	—	—	5
—	4*	—	—	—	—	—	—	—	—	5
—	4*	—	—	—	—	—	1.9	—	—	1
—	4	—	—	—	—	—	1.9	16.4	8.2	1
—	8*	—	—	—	—	—	—	—	—	4
—	8*	—	—	—	—	—	1.9	—	—	1
—	8	—	—	—	—	—	1.9	15.6	10.0	1
—	—	2	—	—	—	—	1.9	16.3	9.5	3
—	—	4	—	—	—	—	1.9	17.2	9.4	3
—	—	8	—	—	—	—	1.9	15.0	10.6	3
—	—	—	2	—	—	—	1.9	15.6	10.3	5
—	—	—	4	—	—	—	1.9	15.6	11.1	2
—	—	—	8	—	—	—	1.9	14.6	10.7	3
—	—	—	—	2	—	—	1.9	16.6	9.5	5
—	—	—	—	4	—	—	1.9	14.9	11.5	2
—	—	—	—	8	—	—	1.9	15.0	10.8	2
—	—	—	—	—	2	—	1.9	15.7	10.0	1[b]
—	—	—	—	—	4	—	1.9	14.8	10.9	1[b]
—	—	—	—	—	8	—	1.9	13.8	11.5	1[b]
—	—	—	—	—	—	2	1.9	15.6	10.4	1
—	—	—	—	—	—	4	1.9	15.2	10.4	2
—	—	—	—	—	—	8	1.9	13.6	12.1	3

All formulations contained 4.5% w/w lidocaine and prilocaine (1:1) and 14% w/w Lutrol® F127, except formulations marked with an * which contain 4.5% w/w lidocaine and prilocaine (1:1) but no Lutrol® F127.
[a] No gelation; [b] microemulsions.

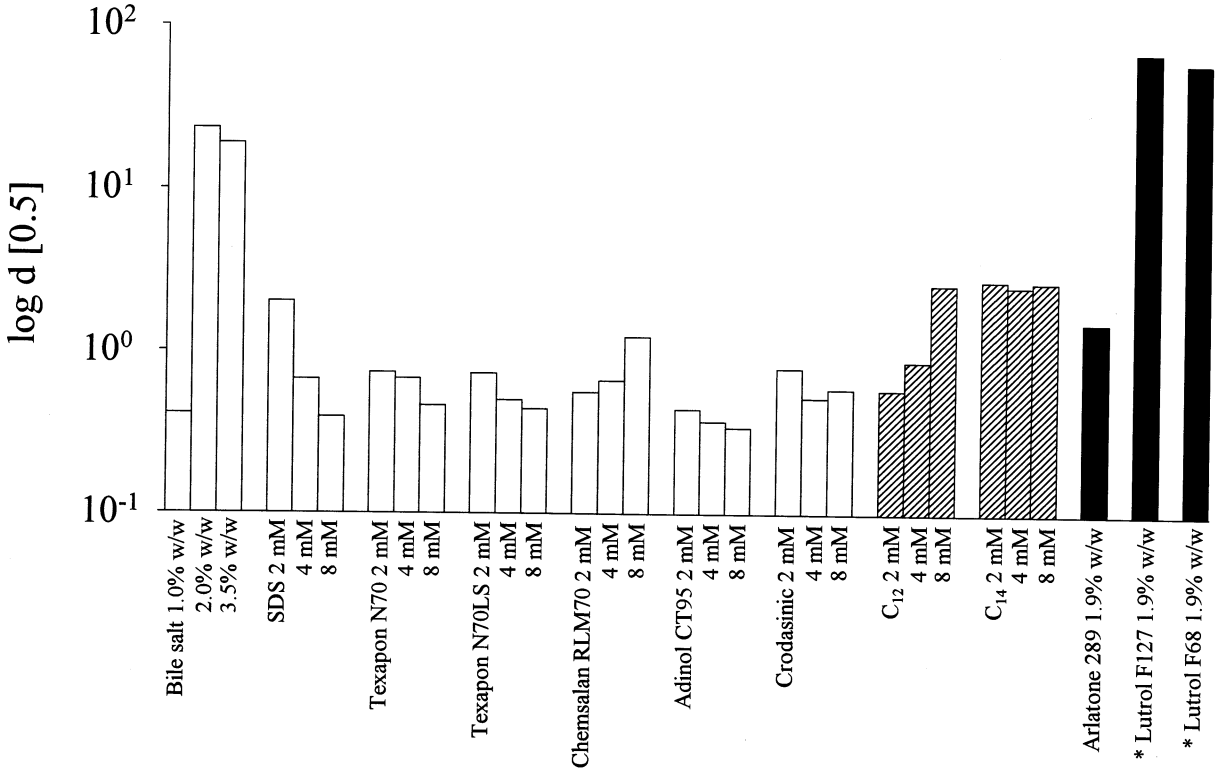

Fig. 4. The droplet size of formulations containing anionic (open bars), cationic (shaded bars) and nonionic (filled bars) surfactants. All formulations except the ones marked with an * contain Arlatone® 289, but no Lutrol® F127.

SDS crystallization occurs on storage in a refrigerator, with consecutive detrimental effects on emulsion stability.

One possibility for avoiding problems associated with the high Krafft temperature of SDS is to also include in the formulation a nonionic surfactant since this may lead to mixed micelle formation at a lower SDS concentration than the monomer solubility, thereby effectively eliminating SDS crystallization. Not unexpectedly, therefore, it was observed that when both SDS and a nonionic surfactant (Arlatone® 289) were added, stable formulations were obtained at higher concentrations of SDS. For lower concentrations of SDS, the adsorption at the oil droplet surface is limited (Wahlgren and Arnebrant, 1992), yielding an insufficient surface charge to induce emulsion stability. The droplet size of the formulations was also measured and confirmed the findings stated above (Fig. 4) although it should be noted that the stability of the final formulation is dependent not only on the surfactant added but on all the surface active components present in the system. An additional advantage of having a charged surfactant together with a nonionic surfactant in the formulations is that the amount of the charged surfactant can be kept quite low, thus minimizing the toxic effects (Siegel and Gordon, 1985).

In order to further reduce the potential Krafft point induced stability problems at longer storage times other anionic surfactants with lower Krafft points were investigated. One such class of anionic surfactants is the alkyl ether sulfates, e.g. lauryl oligooxyethylene sulfates. Since the Krafft point of these surfactants is lower than the storage temperature ($< 4°C$) no crystallization occurs, which could also be expected to increase the

formulation stability. An additional advantage of these surfactants is that they are less toxic (irritating) than SDS (Chiucta and Dodd, 1978). However, contrary to our expectations, the lauryl oligooxyethylene sulfate formulations give more or less unstable formulations for all the lauryl oligooxyethylene sulfates used (Texapon® N70, Texapon® N70LS and Chemsalan® RLM70). The origin of these effects are uncertain at present and deserves further investigations with more well defined substances, which, however, are outside the scope of the present investigation. Nevertheless, two other anionic surfactants with a higher Krafft temperature (< 8°C) were found to give stable formulations with good rheological properties. This is the case for Adinol® CT95, at all concentrations tested and for Crodasinic® LS30 formulations, particularly at low concentrations.

3.3.2. Cationic surfactants

Cationic surfactants offer a potentially interesting alternative to anionic and nonionic surfactants, mainly due to their antibacterial effect against gram-negative and gram-positive bacteria, as well as yeast. However, their high degree of affinity for biological membranes in general results in low selectivity, which can cause cell damage if the time of exposure is sufficiently long (Ahlström et al., 1995). The concentration of the cationic surfactant must, therefore, be kept as low as possible just as for the anionic surfactants. Consequently, only mixtures of cationic and nonionic surfactants were investigated. The cationic surfactants used in this study were alkanoylcholines with either C_{12} or C_{14} hydrocarbon chains (laurylcholine bromide and myristoylcholine bromide, respectively). As can be seen in Table 1, good results were obtained for the 8 mM concentration of both alkyl choline bromides, since this concentration resulted in stable formulations with good rheological properties. For the C_{14} surfactant, but not for the C_{12} surfactant, crystallization seems to be somewhat of a problem for the lower concentrations, i.e. 2 and 4 mM. However, for the C_{12} surfactant the lowest concentration investigated seems to give incomplete stabilization, presumably through insufficient adsorption at the oil droplets. Nevertheless, as can be seen from Table 1 conditions giving stable formulations may be obtained for both surfactants, allowing also for other concerns such as the drug release rate to be met, e.g. through the choice of the surfactant and its concentration.

3.4. Drug release

The amount of active ingredients released over time through a synthetic membrane, used instead of oral mucosa, was studied for the most promising formulations within each category. A typical drug release curve is shown in Fig. 5. The release rate is highest during the first hour and thereafter levels off. Considering the therapeutic indication of the formulation, the amount released over the first hour is the most interesting. In Fig. 6, the initial drug release rate for the anionic, cationic and nonionic formulations divided by that of the reference formulation, EMLA® cream (5% w/w), a product on the market containing the same active ingredients, is plotted. From the group of anionic surfactants, it can be concluded that bile salt has a positive effect on the release rate of lidocaine and prilocaine. The mechanism behind this phenomenon is currently unknown and further studies are needed. However, a possible explanation may be that bile salts could bind to the PPO chains of the block copolymers (Goddard and Ananthapadmanabhan, 1993), thereby reducing the energy cost for 'PPO'–water contacts. This, in turn, may result in more dynamic micelles

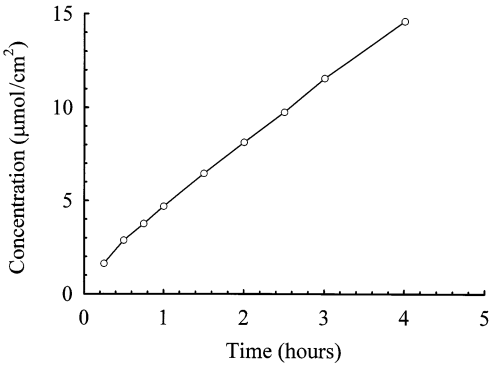

Fig. 5. A typical release curve for lidocaine and prilocaine from a formulation containing 8 mM of a C_{12} cationic surfactant (lauryl choline bromide).

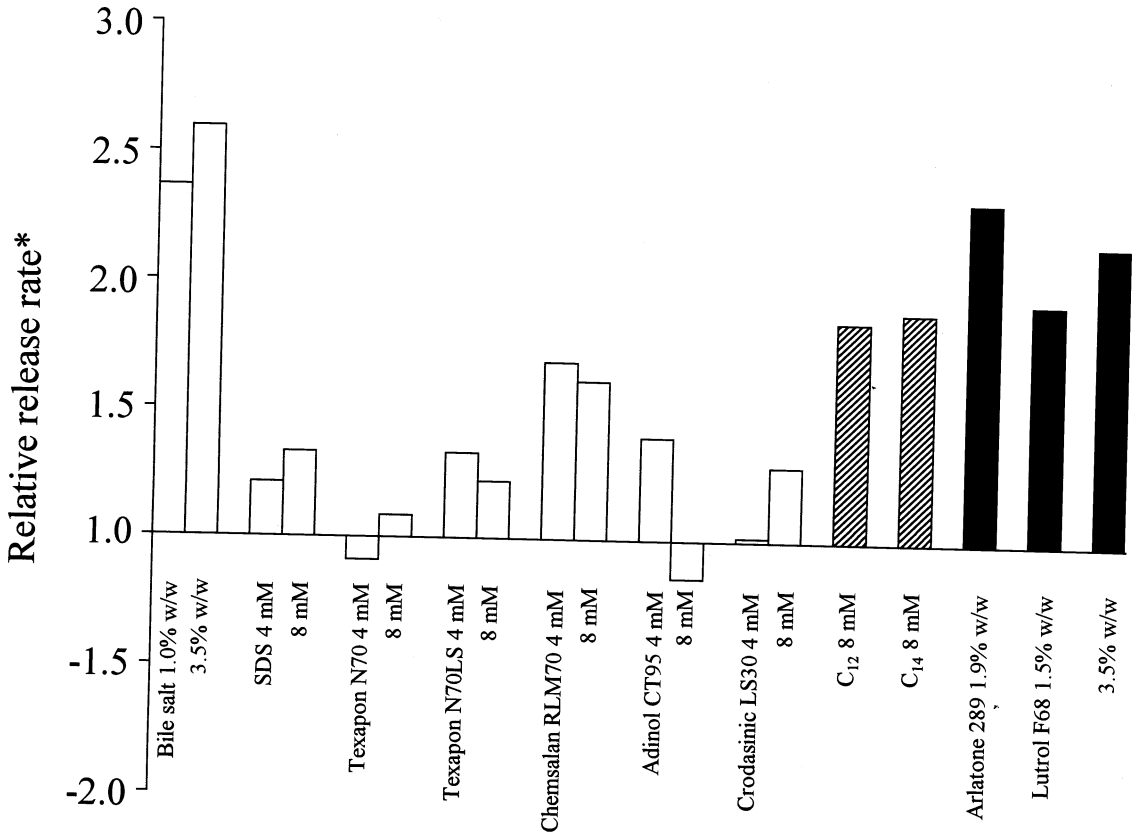

Fig. 6. Relative release rates for anionic (open bars), cationic (shaded bars) and nonionic (filled bars) surfactant formulations. * Data referes to the initial release rate for the formulations divided by that for the reference formulation, EMLA® cream 5% w/w (2.58 μmol cm^{-2} h^{-1}).

since the micellar structure fluctuations could be expected to increase and the unimer life-time in a micelle to be reduced at a lower energy penalty for PPO–water contacts (see chain-length dependence of micellar residence time for low molecular weight surfactants (Lindman and Wennerström, 1980)). Since such binding would also result in a decreased hydrophobic driving force for micellization the gelation temperature is also expected to increase. Furthermore it can be seen that both the formulation containing either SDS or 4 mM Adinol® CT95, together with the Arlatone, show promising results with regard to formulation stability and rheological properties, and also give an improved initial rate of release of the active ingredients as compared to the control EMLA® cream. The formulations containing the nonionic surfactants gave high release rates as did the cationic surfactants. Overall, there seems to be rather poor agreement between the emulsion droplet size and the drug release rate, which seems to indicate also that the Lutrol® F127 (present in the formulation but not in the droplet size measurements) is important for the resulting emulsion droplet size in the formulation, and/or that the initial drug release occurs from mixed micelles rather than from the emulsion droplets. In conclusion almost all the formulations showed a higher initial rate of release of the active ingredients compared to the reference EMLA® cream.

It should be noted that PEO-PPO-PEO block copolymers have been studied frequently in connection with pharmaceutical applications and the use of these polymers as vehicles for oral, parenteral, rectal, ocular and topical drug delivery has been described in the literature (Schmolka, 1991). The use of poloxamer 407 as a vehicle for delivery of tetracycline to the periodontal pocket was reported by Esposito et al. (1996). Although the formulations described in our paper differs in many aspects from the system described above, regarding therapeutic action and type of formulation, the poloxamer 407 was found to be a suitable vehicle, for this particular application site, in both studies.

4. Conclusions

Fulfillment of the requirements stated in the introduction can be achieved by systems containing local anesthetic compounds comprising the oil phase, a block copolymer which gives the system unique rheological properties, and by choosing a suitable second surfactant. Stable formulations were obtained with anionic, cationic and nonionic surfactants. For the anionic surfactants the best results were obtained with SDS, and Adinol® CT95 in combination with the nonionic surfactant Arlatone® 289 and bile salt. The C_{12} and C_{14} cationic surfactants both gave stable formulations at the highest concentration studied. These surfactants are also very interesting for use in the periodontal pocket considering their antibacterial effect which will be studied further. Among the nonionic surfactants, Lutrol® F68 showed very promising results in high concentrations which will be discussed in more depth in a separate study.

Finally, since it is possible to obtain formulations with suitable properties for the intended use with any of the above mentioned second surfactants, other aspects such as toxicity, effect on the environment, commercial availability and price can be taken into consideration for the most optimal choice.

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