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# A study of polar lipid drug carrier systems undergoing a thermoreversible lamellar-to-cubic phase transition

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## Summary

The polar insoluble, but swelling, lipid monoolein (glyceryl monooleate) forms a highly ordered cubic phase in excess water, which can be used to sustain the release of different types of drugs. One problem with the cubic phase, however, is its stiffness, and in order to make the administration of the cubic phase easier, precursors in the form of a lamellar phase were investigated. This phase undergoes a phase transition to the cubic phase on temperature increase; thus the lipid system formed in this way acts similar to in situ activated gel-forming polymer systems, e.g., Pluronic F-127, Gelrite and EHEC. Lipid drug carrier systems can be made from a wide variety of lipids. They can also be used in the application of a wide variety of drugs, and are very versatile as regards the site of administration. They can, therefore, be an interesting alternative to polymer systems.

## Introduction

There are a number of polymer systems discussed in the literature which possess an in situ thermal gelling behaviour. One is the amphiphilic block-co-polymer Pluronic F-127 (or Poloxamer 407) which at a concentration in the range of 20–30 wt% in water forms a thermoreversible gel with increasing temperature (Miller and Donovan, 1982). A second is the cellulose derivative EHEC (ethylhydroxyethylcellulose) which at a 1 wt% concentration in water forms a gel in the presence of low amounts (< 1%) of ionic amphiphiles such as SDS (sodium dodecyl sulphate) and CTAB (cetyltrimethylammonium bromide) (Carlsson et al., 1990; Lindell et al., 1991). A third system is the polysaccharide Gelrite which differs from the other two in that the gel forms in the presence of simple salts present in, for example, tear fluid (Rozier et al., 1989).

In this work we examine systems based on polar lipids in water having the property of undergoing a reversible phase transition upon temperature increase, from a relatively low-viscous lamellar phase ($L_\alpha$) at room temperature to a stiffer cubic phase (Q) at body temperature. Thus, these lipid systems resemble the thermoreversible gelling polymer systems, which may have clear advantages as drug delivery systems for prolonged action, e.g., as gelling eyedrops (Lee, 1990). How-

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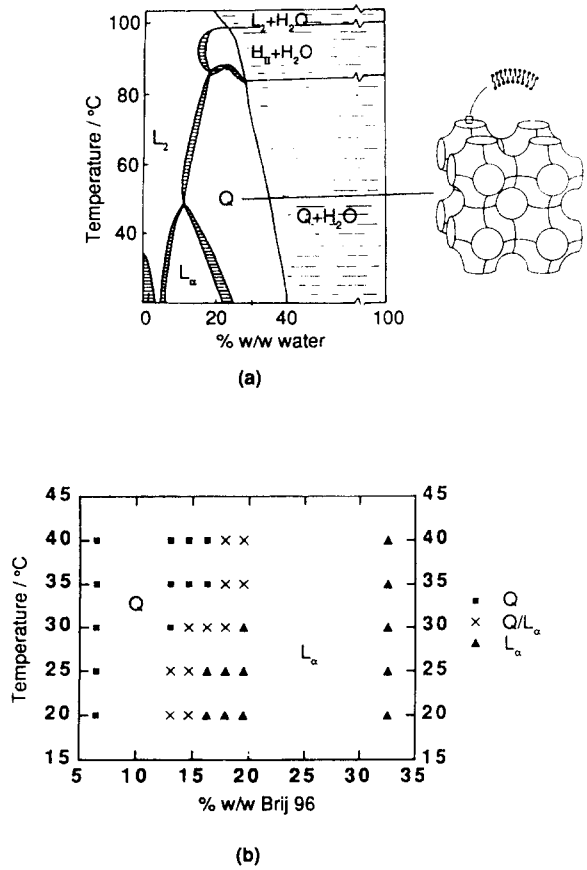


Fig. 1. Phase diagrams of (a) the monoolein-water system (adapted from Hyde et al., 1984), and (b) the Brij 96-monoolein-water (35% w/w) system.

ever, the lipid and polymer systems have very different structures, the former being liquid crystals and the latter polymer networks.

The basis of this work is a practically water-insoluble ($\approx 10^{-6}$ M) polar lipid, glyceryl monooleate (GMO) or monoolein, which swells in water giving rise to several phases with different rheological properties. Fig. 1a shows the phase diagram of the monoolein-water system with the structure of a bicontinuous cubic phase indicated as well (Hyde et al., 1984). (In fact there are two cubic phases in the cubic phase region, belonging to different space-groups.) Two properties are particularly interesting in this context: the decrease in fluidity as more water is added to monoolein, and the ability of the cubic phase to co-exist with excess water. Thus, if a sample of monoolein is put in water it will swell to a water content of approx. 35 wt%, forming the stiff cubic phase with a highly ordered structure.

The cubic phase consists of appreciable amounts of both lipid and water, as well as a large interfacial area, and is therefore able to solubilize both polar, amphiphilic and nonpolar drugs (Engström, 1990). If a drug is incorporated into the system it will diffuse out from the phase at a reduced rate (Ericsson et al., 1991). A problem with the cubic phase, from the user's point of view, is that its stiffness makes it difficult to handle and almost impossible to inject. The objective of the research reported here was therefore to create a precursor to the cubic phase, which is easy to handle at room temperature, and is converted to the cubic phase at body temperature. It turns out that the lamellar phase is well-suited for this purpose.

In order to formulate systems with this property we used a monoolein-water system with 16% (w/w) water, which according to the phase diagram in. Fig. 1a, undergoes the lamellar-to-cubic transition upon temperature increase. The drug budesonide and the bone mineral hydroxyapatite were incorporated into this kind of system. We also developed a system which contains 35% (w/w) water, i.e., near the water swelling limit of the monoolein-water cubic phase, which shows the same phase transition behaviour. This system was made through replacement of some of the monoolein by a more polar amphiphilic substance, Brij 96 ($C_{18:1}$-$(OCH_2CH_2)_{\sim 10}OH$). Timolol maleate and ethoxylated vitamin E were solubilized in this water-rich system. Finally, the local anesthetic lidocaine, a weak base with $pK_a$ slightly below 8, gives rise to a temperature induced lamellar-to-cubic phase transition in the monoolein-water (35% w/w) system within a certain pH range (Engström and Engström, 1992).

The phase transitions occurring in the systems studied have been characterized by means of visual inspection using crossed polarizers, X-ray diffraction, differential scanning calorimetry (DSC) and rheology. The release of timolol maleate from a cubic phase was measured as well. The lipid-water-systems were compared to

TABLE 1

*Fatty acid composition (% w/w) of the monoglycerides (denoted monoolein in the text) as determined by gas chromatography*

| Fatty acid | Grindsted | Eastman |
|---|---|---|
| Palmitic $C_{16:0}$ | 0.5 | 3.2 |
| Stearic $C_{18:0}$ | 2.2 | 5.0 |
| Oleic $C_{18:1}$ | 92.5 | 77.1 |
| Linoleic $C_{18:2}$ | 4.6 | 10.6 |
| Linolenic $C_{18:3}$ | trace | trace |
| Arachidonic $C_{20:4}$ | 0.2 | 0.6 |

two polymer-water systems, i.e., Pluronic F-127 and EHEC (Lindell et al., 1991).

## Materials and Methods

### *Materials*

The monoglycerides, denoted as monoolein, were manufactured by Grindsted A/S (Braband, Denmark) and Eastman Chemicals (Kingsport, U.S.A.) with fatty acid compositions according to Table 1. The products were isomerized equilibrium mixtures of the 1- and 2-isomers (about 9:1). The greater amounts of saturated long-chain fatty aicds (i.e., $C_{16:0}$ and $C_{18:0}$) in the Eastman monoglyceride caused crystallization of these in the liquid crystalline phases at room temperature.

The other substances, Brij 96 (Atlas Chemie, Germany), timolol maleate (Sigma, U.S.A.), lidocaine base and HCl (Astra Pain Control AB, Sweden), budesonide (Astra Draco AB, Sweden), ethyoxylated vitamin E (Eastman Chemicals, U.S.A.), hydroxyapatite (Pentax, Japan) and Pluronic F-127 (BASF, U.S.A.), were used as received. The water used was of doubly distilled quality.

### *Methods*

*Sample preparation* The samples were prepared in glass ampoules, which after sealing were left standing until equilibrium was reached (days to weeks). The various lipid phases were detected by using crossed polarizers in order to detect any anisotropy and polarizing microscopy in order to study the texture of the anisotropic phases.

*X-ray diffraction* The low angle X-ray diffraction studies were undertaken at 20 °C and 37 °C with a DPT camera with copper $K_\alpha$ radiation ($\lambda = 0.1542$ nm) and point focus. The resulting X-ray films were examined with an image analysing system (JAVA, Germany) equipped with a Philips CDD video camera.

*DSC* The DSC measurements were performed with a Perkin-Elmer DSC-2C instrument. The heating rate was 5 °C/min and the range was 0.84 mJ/s (0.2 mcal/s). Each temperature scan was started at 13 °C and ended at 87 °C. Varnished aluminium pans were used as sample containers, and an empty aluminium pan was used as the reference. Because of the relatively small enthalpy changes measured for the phase transitions of the liquid crystalline systems studied, the amount of sample in each pan was maximized to 20–30 mg. The prepared pans were sealed and stored overnight at 20 °C before analysis.

*Rheology* The rheological properties of the lipid and polymer systems were measured during oscillation tests in the Bohlin Rheometer (Bohlin Reologi, Sweden). The complex modulus, $G^*$, was measured and is defined as the sum of the storage (elastic) modulus, $G'$, and the loss (viscous) modulus, $G''$, according to

$$G^* = G' + i \cdot G'' \qquad (1)$$

where the phase angle $\delta$ is defined as the angle between $G^*$ and $G'$ in the complex plane. For an elastic body, $\delta = 0°$, and for a viscous liquid, $\delta = 90°$. For visco-elastic systems, $\delta$ has an intermediate value (Whorlow, 1980). A liquid-to-gel transition is characterized by a decreasing $\delta$ with time, i.e., the system changes from a viscous to an elastic system.

The oscillation was performed at a frequency of 1.0 Hz with a strain of 0.05. All samples were analyzed during a temperature increase from 25 to 40 °C at a rate of 1 °C/min. Concentric cylinders with an inner vs outer cylinder ratio of 0.91 were used for the Pluronic F-127 system. The sample volume was 2.0 ml. The lipid systems, except for the hydroxyapatite system, were analyzed with cone-and-plate equipment, using ap-

prox. 1.0 g of material. The angle of the cone was 5.4°, the diameter of the plate and the gap between the cone-and-plate were 30 mm and 150 μm, respectively. For the hydroxyapatite system, plate-plate equipment with a diameter of 30 mm was used with a gap setting of 1 mm.

*Release studies* The release of timolol maleate from the cubic phase was carried out with a USP paddle apparatus (Prolabo, France) at 37 °C. The cubic phase was placed in a cylindrical hole (diameter 29 mm, depth 3 mm) in a Teflon disk, and the latter was then positioned in the release chamber containing distilled water. The paddle speed was 20 rpm. Since the cubic phase exists in equilibrium with excess water, there was no need for a supporting membrane to keep the sample in place. Released amounts of timolol maleate were determined spectrophotometrically at 295 nm.

## Results

*Compositions and phase behaviour*

In order to be able to use the cubic phase of the monoolein-water system near its water swelling limit, i.e., 35%, some of the monoolein was replaced by a more polar lipid so that the cubic phase was converted to a lamellar phase at, as well as slightly above, room temperature. The substance chosen was Brij 96, and the monoolein-Brij 96 phase diagram at 35% water is given in Fig. 1b. From the phase diagram it is evident that one has to choose a monoolein/Brij 96 ratio around 3:1 by weight in order to have a system that undergoes the desired $L_\alpha$-to-Q transition. It should be noted that an incorporated drug may, of course, influence the phase diagram.

The compositions of the lipid systems studied in this work are given in Table 2, together with upper and lower temperatures at which the lamellar and cubic phases exist, respectively, determined by visual inspection utilizing crossed polarizers. It is evident from Table 2 that it is possible to find compositions fulfilling the requirement that the system be a relatively low-viscous lamellar phase at room temperature, and the much stiffer cubic phase at body temperature.

Some of the samples were investigated by means of X-ray diffraction in order to make a final validation of their structures. The X-ray diffraction patterns were consistent with lamellar phases at room temperature, and cubic phases at

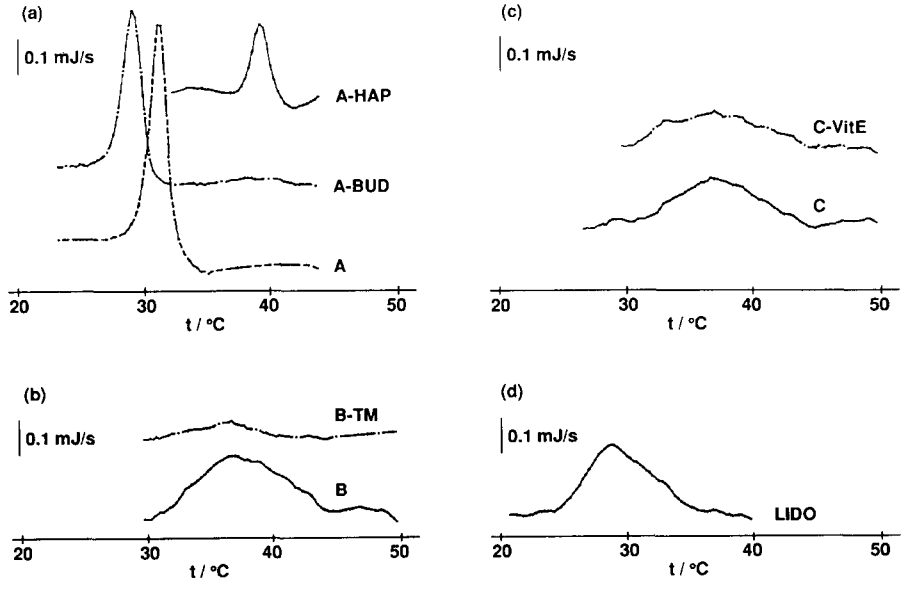


Fig. 2. Representative thermograms for the phase transition $L_\alpha$-to-Q for the systems in Table 2: (a) A, A-HAP and A-BUD, (b) B and B-TM, (c) C and C-VitE, and (d) LIDO. The heating rate was 5 °C/min.

TABLE 2

*Compositions, temperature range of lamellar-cubic two-phase region from visual inspection, and enthalpy changes ($\Delta H$) from DSC (each enthalpy is based on at least four measurements)*

| System | Composition [a] (% w/w) | $L_\alpha$ (°C) | Q (°C) | $\Delta H$ (kJ/mol lipid) |
|---|---|---|---|---|
| A | 84% GMO-G<br>16% water | < 26 | > 30 | 0.60 ± 0.06 |
| A-HAP [b] | 50% HAP<br>50% A | n.d. | n.d. | 0.11 ± 0.03 |
| A-BUD | 0.1% BUD<br>99.9% A | < 24 | > 29 | 0.28 ± 0.02 |
| B | 16.25% Brij 96<br>48.75% GMO-G<br>35.00% water | < 26 | > 33 | 0.21 ± 0.01 |
| B-TM | 0.34% TM<br>99.66% B | < 26 | > 33 | 0.14 ± 0.11 |
| C | 16.25% Brij 96<br>48.75% GMO-E<br>35.00% water | < 26 | > 34 | 0.23 ± 0.02 |
| C-VitE | 5% VitE<br>95% C | < 26 | > 34 | 0.19 ± 0.03 |
| LIDO | 2.4% L<br>2.6% LHCl<br>60% GMO-G<br>35% water | < 26 | > 33 | 0.33 ± 0.07 |
| Pluronic F-127 | 20% Pluronic F-127<br>80% water | (gels at 27 °C) | | |

[a] GMO-G, monoolein from Grindsted; GMO-E, monoolein from Eastman Chemicals; HAP, hydroxyapatite; BUD, budesonide; TM, timolol maleate; VitE, ethoxylated vitamin E; L, lidocaine base; LHCl, lidocaine HCl; n.d. not detectable.
[b] The rheological measurement was performed with 25% HAP and 75% A.

body temperature. However, there were too few lines in the diffraction patterns in order to make a precise space-group determination of the cubic phases.

*DSC*

The energy involved in the lamellar-to-cubic phase transition was measured by means of DSC. Fig. 2 shows the thermograms for the systems given in Table 2. The enthalpy change ($\Delta H$) associated with the phase transition for each system is given in Table 2, from which it is readily seen that the enthalpy changes for all systems are small and below 1 kJ/mol lipid. Our values agree well with other determinations (E.Z. Radlinska, personal communication). These $\Delta H$ values are roughly two and one orders of magnitude lower than the enthalpy changes involved in the gel-to-fluid transition and the lamellar-to-reversed hexagonal transition, respectively, for diacylphospholipids ($C_{18}$ chains) (Seddon et al., 1983). The small enthalpy values can be explained by the relatively small rearrangements of the lipids needed for converting the lamellar structure to the cubic structure.

The small enthalpy changes involved in the phase transitions are also reflected by the thermograms in Fig. 2. The water-rich systems were particularly difficult to determine with any high degree of accuracy. The rather poor agreement between the temperature intervals of the phase transition given by calorimetric measurements on the one hand, and visual inspection (see Table 2) on the other, is most probably a consequence of measuring conditions, e.g., heating rate. Another choice of heating rate may lead to better agreement, but at the expense of sensitivity in the enthalpy determination. In summary, the $\Delta H$-values in Table 2, with associated standard deviations, should be regarded as order-of-magnitude estimates of the heat involved in the $L_\alpha$-to-Q transition due to the limits set by the DSC equipment.

*Rheology*

From a user's point of view, the most striking feature of the lipid systems studied is the dramatic change in visco-elastic properties as the temperature changes from room to body temperature. This behaviour is illustrated in Fig. 3, which shows the complex modulus, $G^*$, as a function of temperature, the latter increasing at 1 °C/min. Fig. 3 shows that the complex modulus was much larger for the lipid systems than for the polymer system (which only had a $G^*$ value of a few Pascal at room temperature), a finding which agrees well with those of Bohlin et al. (1985). In Fig. 3 the difference in $\delta$ value from the beginning to the end of the temperature scan is given as a positive or negative sign. The polymer systems showed a decreasing phase angle with in-

creasing temperature, while the tendency was less pronounced in the lipid systems.

It is obvious from the slopes of the curves in Fig. 3 that at least two different polar systems can be recognized. The budesonide and hydroxyapatite systems had a similar behaviour, expressed as a very distinct transition to higher $G^*$ values. The other systems, all with a high water content (35% w/w) showed a less pronounced increase in $G^*$. The curves in Fig. 3 are shown at the upper limit of accuracy of the actual measuring system. This means that no $G^*$ values of the cubic phase at equilibrium are given, but the kinetics of the transition is implied by the curves.

Fig. 4 shows rheograms for the Pluronic F-127 (panel a), the vitamin E (b), and the lidocaine (c) systems which illustrate the situation in more detail. The change and the absolute value of the phase angle is dependent on the system under study. A thermal input normally causes a greater mobility of the molecules, which would give rise to higher values of the phase angle. However, in the Pluronic system an increase in temperature causes aggregation which in turn leads to a more ordered system, and hence a lower phase angle. The situation is less clear in the lipid systems, where the phase angle varies less than for the Pluronic system. In summary, the rheological behaviour of the lipid systems is very complex, but rheometry may nevertheless provide valuable information about the structure of the systems (Bohlin and Fontell, 1978; Bohlin et al., 1985).

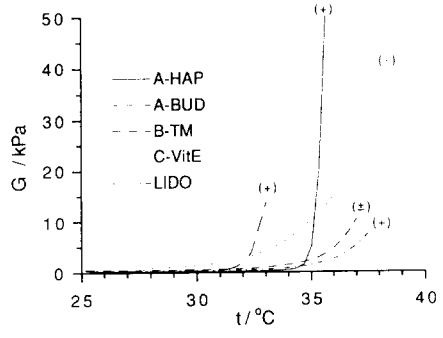


Fig. 3. The complex modulus, $G^*$, as a function of temperature for the lipid and polymer systems. The heating rate was 1 °C/min. The difference in phase angle $\Delta\delta = \delta_{37\,°C} - \delta_{25\,°C}$ is represented as either (+) or (−).

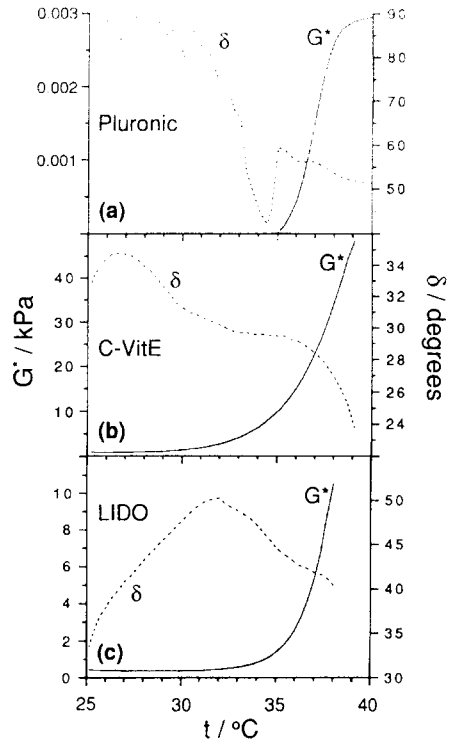


Fig. 4. Rheograms showing the complex modulus, $G^*$, and the phase angle, $\delta$, as a function of temperature for the (a) Pluronic F-127, (b) C-VitE, and (c) LIDO systems. The heating rate was 1 °C/min. Note the different scales on the ordinates.

*Release of timolol maleate from a lipid and polymer system*

The release of timolol maleate from the lipid composition B-TM (see Table 2) and an EHEC-ionic amphiphile system (Lindell et al., 1991) is given in Fig. 5. It should be noted that the comparison made in Fig. 5 is not straightforward, since the experimental conditions are not identical. In the case of the polymer system, a polyethylene net was required to keep the gel in place during the release experiment.

It is probably safe to state that the release of timolol maleate is much slower from the lipid system than from the EHEC system, as revealed by the diffusion coefficients ($D_{\text{cubic}} \approx 4.3 \times 10^{-11}$ m$^2$ s$^{-1}$ and $D_{\text{EHEC}} \approx 2.7 \times 10^{-10}$ m$^2$ s$^{-1}$) deter-

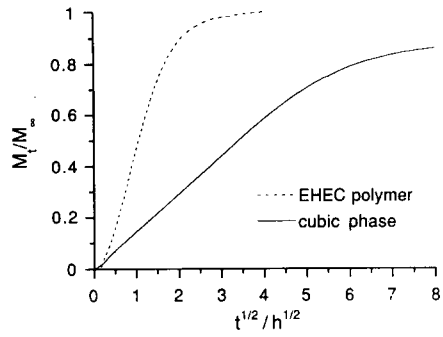


Fig. 5. The release of timolol maleate from the B-TM and EHEC (adapted from Lindell et al., 1991) systems at 37 °C. The diffusion coefficients ($D_{cubic} \approx 4.3 \times 10^{-11}$ m$^2$ s$^{-1}$ and $D_{EHEC} \approx 2.7 \times 10^{-10}$ m$^2$ s$^{-1}$) were determined from fits to the linear part of the curves according to Eq. 2.

mined from linear least-squares fits of the release data to the equation.

$$M_t/M_\infty = 2 \cdot \left(Dt/\pi h^2\right)^{1/2} \qquad (2)$$

where $M_t$ and $M_\infty$ are the released amount of drug at time $t$ and the total drug content, respectively, and $h$ is the depth of the cylindrical hole from which the release takes place. The diffusion coefficients most probably reflect the difference in interaction between the drug and the lipid and polymer system, respectively. The obstruction to drug transport in the highly ordered lipid system ought to be larger than for the less dense polymer network.

## Discussion

In this section we will discuss the lipid systems investigated with respect to their flexibility in the choice of drug and possible site of administration.

*The budesonide or hydroxyapatite-GMO-water system*

According to the phase diagram in Fig. 1a, the GMO-water system with 16 wt% water transforms from a lamellar to a cubic phase upon change from room to body temperature. Moreover, if additional water is present, the system swells to the cubic phase. We have utilized this property in two cases. One is where the system acts as a matrix for hydroxyapatite granules, a ceramic material which promotes bone growth. The idea was to form a suspension of the granules in the lamellar phase, which after being poured in a body cavity, fills the space and becomes stiff due to the formation of the cubic phase as the temperature increases and/or water penetrates into the suspension. An advantage with the present formulation is that one is able to rinse the filled cavity with water, which is impossible if dry granules are used.

The second system consists of, in addition to GMO and water, budesonide, a lipophilic anti-inflammatory steroid. Since budesonide is a potent drug, a typical commercial formulation consists of a low amount of drug. In our study we used 0.1 wt% budesonide in the GMO-water system, an amount which did not affect the phase transition to any noticeable extent. A possible route of administration of such a system may be rectally, since the system is able to swell to about 35 wt% water; one therefore has reason to believe that it may adhere to wet surfaces like the mucosa.

*The timolol maleate or ethoxylated vitamin E-Brij 96-GMO-water system*

The system described in the preceding section has the ability to swell in the presence of water, which can be a disadvantage in some cases since the swelling may cause local irritation. Therefore, it can be of value to use a polar lipid system which is fully swollen. If the GMO-water system is to be used one must include a second amphiphilic substance in order to transform the system from a cubic phase to a lamellar phase. The choice of amphiphile is determined by its effect on the phase behaviour, and a good guess can be made by means of the so-called packing concept of amphiphilic substances. This theory claims that substances with relatively large polar head groups promote the formation of phases of the 'oil-in-water' type, while substances with relatively small polar head groups have the opposite effect.

Using the packing concept in this case means that a more polar amphiphile must be added to the GMO-water system than GMO itself. We used Brij 96, since it turns out that this substance

forms a lamellar phase at 35% (w/w) water (Jousma et al., 1989). It was therefore reasonable to assume that a proper balance between GMO and Brij96 would lead to a lamellar phase which has a curvature lying between the cubic and the hexagonal phase. It turns out that if 25 wt% of GMO is replaced by Brij 96 at 35% water, the cubic phase is transformed to a lamellar phase. However, the cubic phase exists at temperatures above 30 °C, due to increased hydrocarbon chain mobility which in turn promotes the formation of phases of the reversed type (i.e., the GMO-water cubic phases, reversed hexagonal and reversed micellar phases). It should be noted that the choice of Brij 96 is one out of many possibilities; we could also, for example, have chosen polar lipids such as phospholipids (e.g., lecithin) and soaps of fatty acids.

*The lidocaine base-lidocaine HCl-GMO-water system*

Lidocaine differs from the other drugs used in this work in that it has a pronounced surface activity. This means that it will interact with the GMO-water interface, and, depending on pH, transform the cubic phase to either a lamellar phase (low pH) or a reversed hexagonal phase (high pH). In an earlier work it was shown that by mixing the base and salt form of lidocaine in certain proportions, it was possible to obtain a system which turned out to be lamellar at room temperature and cubic at body temperature (Engström and Engström, 1992).

A common property of all lamellar phases studied in this work is that they are easily injected through a 0.45 mm needle (except that with hydroxyapatite granules). Since the systems are thermodynamically stable phases, their storage stability should be excellent. A lipid system according to the present principle should also be well-suited for prodrugs, e.g., for a drug with a carbon chain which can be anchored in the lipid bilayer. In the work reported here, we focused our attention on systems where the desired phase transition is caused by a temperature increase, but it should be noted that a change in pH may be used as well, for example in the case of long-chain fatty acids.

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## References

Bohlin, L. and Fontell, K., Flow properties of lamellar liquid crystalline lipid-water systems. *J. Colloid Interface Sci.*, 67 (1978) 272–283.

Bohlin, L., Ljusberg-Wahren, H. and Miezis, Y., Oscillatory shear flow measurements on liquid crystalline phases with hexagonal and cubic structures. *J. Colloid Interface Sci.*, 103 (1985) 294–295.

Carlsson, A., Karlström, G. and Lindman, B., Thermal gelation of nonionic cellulose ethers and ionic surfactants in water. *Colloids Surfaces*, 47 (1990) 147–165.

Engström, S., Drug delivery from cubic and other lipid-water phases. *Lipid Technol.*, 2 (1990) 42–45.

Engström, S. and Engström, L., Phase behaviour of the lidocaine-monoolein-water system. *Int. J. Pharm.*, 79 (1992) 113–122.

Ericsson, B., Eriksson, P.-O., Löfroth, J.-E. and Engström, S., Cubic phases as delivery systems for peptide drugs. *ACS Symp. Ser.*, 469 (1991) 251–265.

Hyde, S.T., Andersson, S., Ericsson, B. and Larsson, K., A cubic structure consisting of a lipid bilayer forming an infinite periodic minimum surface of the gyroid type in the glycerylmonooleat-water system. *Z. Kristallogr.*, 168 (1984) 213–219.

Jousma, H., Jousten, J.G.H., Gooris, G.S. and Junginger, H.E., Changes of mesophase structure of BRIJ 96/water mixtures on addition of liquid paraffin. *Colloid Polym. Sci.*, 267 (1989) 353–364.

Lee, V.H.L., New directions in the optimization of ocular drug delivery. *J. Ocular Pharmacol.*, 6 (1990) 157–164.

Lindell, K., Engström, S. and Carlsson, A., A novel in situ gelling system for drug delivery. 2. In vitro release. *Proc. Symp. Control. Rel. Bioact. Mater.*, 18 (1991) 265–266.

Miller, S.C. and Donovan, M.D., Effect of Poloxamer 407 gel on the miotic activity of pilocarpine nitrate in rabbits. *Int. J. Pharm.*, 12 (1982) 147–152.

Rozier, A., Mazuel, C., Grove, J. and Plazonnet, B., Gelrite: A novel, ion-activated, in-situ gelling polymer for opthalmic

vehicles. Effect on bioavailability of timolol, *Int. J. Pharm.* 57 (1989) 163–168.

Seddon, J.M., Cevc, G. and Marsh, D., Calorimetric studies of the gel-fluid ($L_\beta$-$L_\alpha$) and lamellar-inverted hexagonal ($L_\alpha$-$H_{II}$) phase transitions in dialkyl- and diacylphosphatidylethanolamines. *Biochemistry*, 22 (1983) 1280–1289.

Whorlow, R.W., *Rheological Techniques*, Ellis Harwood, Chichester, 1980.