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Phase behaviour of the lidocaine-monoolein-water system

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Summary

The phase behaviour of the four-component system, lidocaine base-lidocaine HCl-monoolein-water, was investigated. It was found that the cubic phase formed in the monoolein-water system was transformed into a lamellar liquid crystalline phase on addition of lidocaine HCl, and to a reversed hexagonal liquid crystalline phase or a reversed micellar phase when the base form was added. With roughly equal amounts of the base and salt forms of lidocaine, the cubic phase persists. These findings strongly indicate not only that lidocaine in both forms participates in the lipid aggregation, but also that the interfacial curvature changes in the opposite direction in relation to the curvature of the cubic phase. These changes were rationalized by making use of the so-called packing concept of amphiphilic molecules. From a pharmaceutical point of view, the phase behaviour of the system shows a number of interesting properties. Some phases may be in equilibrium with excess water solutions, and temperature-induced transitions occur between phases of very different rheology.

Introduction

This work deals with the effect of a surface active drug, lidocaine (see Table 1), on the phase behaviour of the monoolein-water system. Monoolein (glyceryl monooleate) belongs to a class of water-insoluble lipids which swell in water and form various kinds of lyotropic liquid crystals. A sample of monoolein will take up about 80% of its own weight of water at room temperature, going through three different one-phase regions (see Fig. 1). At low water content, a reversed micellar phase (L_2) is formed, followed by a lamellar liquid crystalline phase (L_α). At higher water content, the system enters into the cubic phase region (C). In fact, this region consists of two cubic phases belonging to different cubic space groups, but this is of minor importance in the present context, since the phases have very similar structure (Hyde et al., 1984). At high temperatures, a reversed hexagonal liquid crystalline phase (H_{II}) is formed. The cubic phase of monoolein can co-exist in equilibrium with excess water (the monomer solubility in water is about 10^{-6} M).

The cubic phase formed in this system has a number of properties making it interesting from a pharmaceutical point of view. It consists of a curved bilayer extending in three dimensions, separating two congruent networks of water channels, as is schematized in Fig. 1. Thus, this cubic phase is both lipid- and water-continuous,

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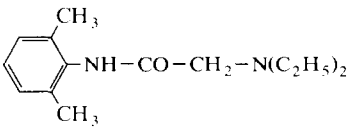
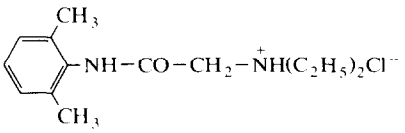
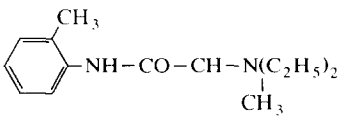
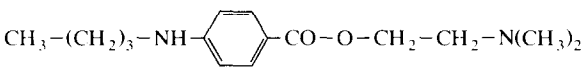
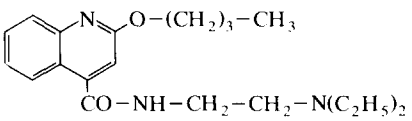
TABLE 1

Molecular formulas

$CH_3-(CH_2)_7-CH=CH-(CH_2)_7-CO-O-CH_2-CH(OH)-CH_2OH$	1-Monoolein
	Lidocaine
	
	Prilocaine
	Tetracaine
	Dibucaine

and the water pore size of the fully swelled phase is about 5 nm. The interfacial area is about 400 m^2/g cubic phase. The network structure makes the phase very viscous (in the literature, the cubic phase is sometimes referred to as the 'viscous isotropic phase'). A number of reviews of cubic phases in general have been published recently, discussing various aspects such as structure and biological relevance (Larsson, 1989; Lindblom and Rilfors, 1989; Fontell, 1990).

The cubic phase formed by monoolein in excess water has the potential of acting as an in situ

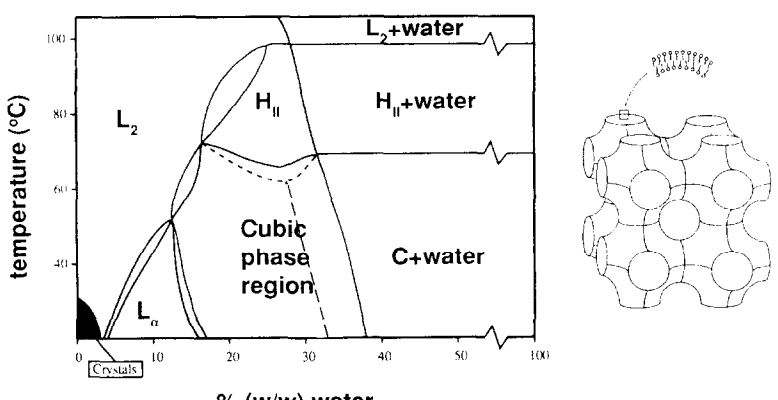


Fig. 1. Phase diagram of the monoolein-water system and a schematic picture showing the structure of the cubic phase. The composition of the monoglyceride is given in Table 2.

forming biodegradable drug delivery system. The biodegradability arises from the fact that monoolein is subject to lipolysis due to different kinds of esterase activity in different tissues. The cubic phase may play a vital role in the lipolysis, since the bicontinuous structure is optimal in that it provides both a non-polar solvent for the substrates (triglycerides) and products (fatty acids), and a water route for the hydrolysis (Patton and Carey, 1979; Eldem and Speisser, 1989). Monoolein is a common food additive and has also frequently been used in emulsions for dermatology (cf. Cosmetic ingredient review, 1986).

The monoolein-water system, and in particular the cubic phase, has previously been studied in the context of drug release. The cubic phase was shown to extend the release of bioactive substances both in vitro and in vivo (Engström et al., 1988; Löfroth et al., 1988; Ericsson et al., 1988, 1991). It was found inter alia that the cubic phase decreased the enzymatic degradation rate of an oligopeptide in simulated intestinal fluid (Löfroth et al., 1988; Ericsson et al., 1991). Moreover, the cubic phase injected intramuscularly in rabbit showed a constant high plasma level of the tetradecapeptide somatostatin for at least 6 h (Ericsson et al., 1988, 1991). It should also be mentioned that the cubic phase may be dispersed, giving rise to a so-called Cubosome formulation with a particle size distribution similar to that of the commercially available oil-in-water emulsions used for parenteral nutrition (Landh and Buchheim, 1991). A brief description of the monoolein-water system as a drug delivery system is given by Engström (1990).

If one intends to use the monoolein-water system for drug delivery, it is crucial to gain insight into how a third substance, e.g. a pharmaceutical compound, influences the phase behaviour of the system. Surface-active compounds are, in this respect, of particular interest, since one would expect them to interact with the lipid aggregates in one way or another. Among the pharmaceutical compounds belonging to this class, we have focused our interest on the local anesthetics in general, and lidocaine in particular.

Local anesthetics are surface active (Vilallonga and Phillips, 1979), probably a necessary property in order to block nerve signals. Moreover, most of the therapeutically used local anesthetics are weak bases with pK_a values around 8, and therefore exist in a charged and an uncharged form under most physiological pH conditions. These properties add up to make the local anesthetics interesting for phase studies in the monoolein-water system with its rich variety of lipid-water aggregate types.

In a previous preliminary work, the effect of lidocaine on the phase behaviour of the monoolein-water system was investigated (Engström et al., 1989). It was shown that the uncharged base form and the charged salt form influenced the phase behaviour differently. The base form promotes the formation of phases of the reversed type, H_{II} and L_2, while the effect of the salt form goes in the opposite direction, i.e. towards phases of the normal type (L_α in this case). That lidocaine influences the interfacial curvature of the lipid-water aggregates is obvious from these results.

In this work we have extended the phase studies, trying to give a more complete picture of the phase behaviour of the lidocaine-monoolein-water system, with respect to changes in both composition and temperature. X-ray diffraction studies have been performed both to show the existence of various phases, and to investigate the water swelling behaviour of some of the phases formed. Moreover, the changes in phase behaviour brought about with the two forms of lidocaine are interpreted in terms of the so-called packing concept of amphiphilic molecules (Israelachvili et al., 1980). It turns out that this approach is very useful for an understanding of the aggregation properties.

Materials and Methods

Since pure monoolein is a very expensive substance, one has to resort to monoglyceride blends based on vegetable oils, e.g. sunflower oil. The material used in this work, which we denote monoolein, was a molecular distilled product manufactured by Grindsted A/S (Brabrand, Denmark) with a fatty acid composition accord-

TABLE 2

Fatty acid composition of the monoglyceride (denoted monoolein in the text)

Fatty acid	% (w/w) [a]
Palmitic	0.5
Stearic	2.0
Oleic	92.3
Linoleic	4.3
Linolenic	trace
Arachidonic	0.5

[a] According to Grindsted A/S, Denmark.

ing to Table 2. The monoester content was 97% (w/w), the rest being 1.0% free glycerin, 1.0% free fatty acids, and 1.0% diglycerides. The product was an isomerized equilibrium mixture of the 1- and 2-isomers (about 9:1).

The fatty acid distribution of the monoglyceride, however, is not critical, as long as it contains, say 90%, unsaturated fatty acids such as oleic (18:1) and linoleic (18:2), and 5–6% saturated fatty acids. Monolinolein also gives rise to the cubic phase (Lutton, 1966). The commercially available monoglycerides based on sunflower oil, e.g. Myverol 18-99 (Eastman Chemicals, U.S.A.) and Dimodan (Grindsted, Denmark), also show a phase behaviour in water similar to that of pure monoolein. The difference shows up in the precise location of the phase boundaries.

All local anesthetics (base and salt forms) used in this work (see Table 1) – lidocaine, prilocaine, tetracaine and dibucaine – were gifts from Astra Pain Control AB (Södertälje, Sweden). The water used was doubly distilled.

The samples for the phase diagram determination were prepared in glass ampoules, which after sealing were left standing until equilibrium was reached (usually after a couple of days). The various phases were detected by means of using crossed polarizers in order to detect any anisotropy and polarizing microscopy in order to study the texture of the anisotropic phases. The more than 100 samples were studied for a period of about 3 months. The low angle X-ray diffraction studies were undertaken at 37°C with a DPT camera with K_α radiation ($\lambda = 1.542$ Å) and point focus (Stenhagen, 1951). The resulting X-ray films were examined with an image analysing system (JAVA, Germany) equipped with a Philips CDD video camera.

Results and Discussion

Phase diagrams

The phase behaviour of a four-component system (lidocaine base (L)-lidocaine HCl (L:HCl)-monoolein (MO)-water (W)) should be presented in a phase tetrahedron (see Fig. 3). However, the full description of the phase properties of the system would require a huge number of samples, and we have therefore restricted ourselves to a subsystem with up to about 20% (w/w) of L and/or L:HCl. In order to facilitate the presentation, we begin by giving the phase diagrams of the two three-component subsystems, i.e. L-MO-W and L:HCl-MO-W, respectively. These phase diagrams are given in Fig. 2A and B, respectively, and show the conditions at 37°C. It should be noted that the phase boundaries drawn in Fig. 2 (and Fig. 3) are estimates based on the samples with compositions indicated in the diagrams.

It is evident from Fig. 2A that the base form of lidocaine clearly promotes the formation of the reversed type of phases as was mentioned above, i.e. H_{II} and L_2. It is also seen in the figure that the maximum water swelling of the reversed phases is less than for the swelling of the cubic phase. In fact, the effect of lidocaine base on the MO-W system is qualitatively very similar to that shown by triglycerides from vegetable oils, e.g. sunflower oil (Lindström et al., 1981; Engström, 1990).

The salt form of lidocaine, i.e. L:HCl, behaves in a completely different manner as is evident from Fig. 2B. The cubic phase is here seen to be converted to a lamellar liquid crystalline phase as the L:HCl content increases. Moreover, the water swelling capacity of this phase is comparable to the swelling of the cubic phase. A similar phase behaviour is shown by the three-component system sodium taurocholate (a bile salt)-monoolein-water (Svärd et al., 1988). However, in that system a large micellar region is also found, which does not have its counterpart in Fig. 2B.

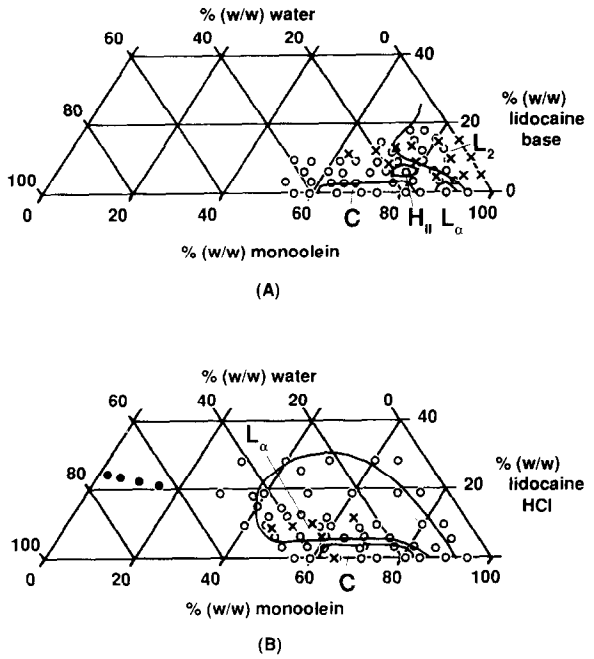


Fig. 2. Phase diagrams of the subsystems (A) lidocaine base-monoolein-water, and (B) lidocaine HCl-monoolein-water at 37°C. The circles show the compositions of the samples from which the phase boundaries are estimated. The filled circles in panel B represent the compositions of the micellar solutions formed with tetracaine HCl. Samples shown as crosses were investigated by X-ray diffraction.

The reason for the lack of a micellar region is that L:HCl does not form micelles. However, tetracaine HCl, which does form micelles, can solubilize at least 15% (w/w) monoolein in an original 25% (w/w) water solution of tetracaine HCl (compositions indicated in Fig. 2B).

It is interesting to note the pronounced effect on the phase behaviour caused by the charged amino group. From the phase diagrams it is evident that there exists a subtle balance between the forces giving rise to a particular equilibrium phase. Since pK_a for lidocaine is slightly below 8, both forms are present under most physiological conditions. Therefore, if one intends to use the system for drug delivery, it is necessary to study the phase behaviour when mixtures of L and L:HCl are present.

We replaced monoolein with mixtures of L and L:HCl at a total water content of 35% (w/w). The resulting phase behaviour (which is indicated by the shadowed plane in the tetrahedron in Fig. 3) is shown in Fig. 3A and B at two temperatures, 20 and 37°C, respectively. From the phase diagrams in Fig. 3, it is seen that in starting out from an L_α phase, increasing pH results in a phase transformation $L_\alpha \rightarrow C \rightarrow H_{II}/L_2$. Thus, it is seen that at roughly equal

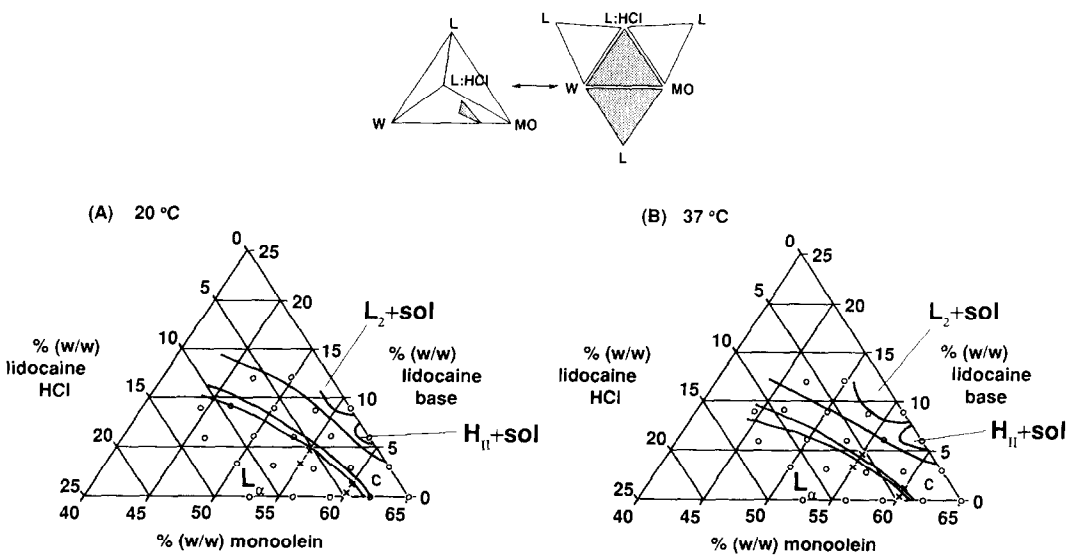


Fig. 3. Phase diagrams of the subsystem lidocaine base-lidocaine HCl-monoolein at 35% (w/w) water at (A) 20°C, and (B) 37°C. Samples shown as crosses were investigated by X-ray diffraction.

amounts of the two lidocaine forms, the individual effects cancel and the cubic phase is preserved.

X-ray studies of the water swelling behaviour of the L_α and L_2 phases

Swelling of the L_2 phase in the L-MO-W system was studied by small-angle X-ray diffraction. Different ratios of L/MO could incorporate different amounts of water in an L_2 phase (see phase diagram, Fig. 2A). Due to aggregate interaction, the L_2 phase will swell into liquid crystalline phases when the amount of monoglycerides exceeds 90%.

The L_2 phase swells in a linear fashion according to Fig. 4, indicating a lamellar structure of the micelles. The slopes are not affected by the ratio L/MO, which means that no molecules other than water give rise to the increasing repeat distance. However, the repeat distance at a given amount of water is lower for the ratio 85:15 compared to those for 90:10 and 95:5. These results represent evidence in support of a micellar structure where lidocaine base is embedded in the monoglyceride bilayers, and thus the repeat distance is reduced due to the smaller hydrocarbon chains and polar head groups for lidocaine base.

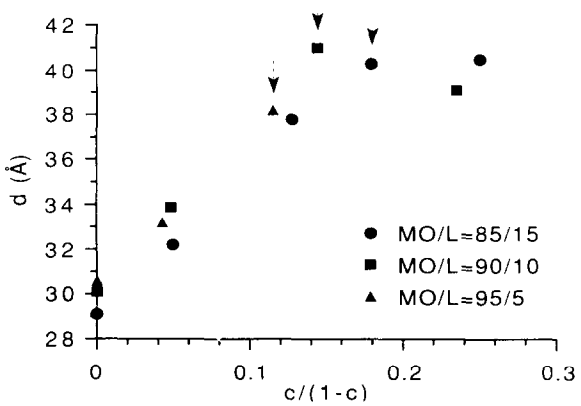


Fig. 4. X-ray spacings vs weight fraction of water to lipids. Arrows indicate maximum swelling of the L_2 phase. Uncertainties for the X-ray spacings are ±0.5 Å. The results are based on X-ray spacings recorded at 37°C.

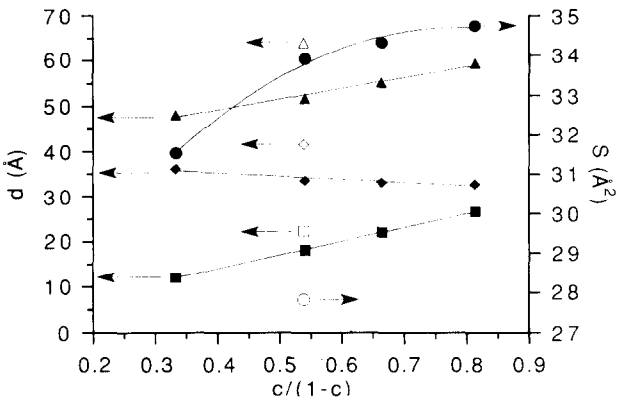


Fig. 5. The lamellar repeat distance d (▲), lipid bilayer thickness d_l (◆), water layer thickness d_w (■), and lipid surface area S (●) as a function of weight ratio of water $c/(1-c)$. Filled symbols correspond to a monoolein/lidocaine HCl ratio of 5.5, and unfilled symbols to a ratio of 9.85. Uncertainties for the X-ray spacings are ±0.5 Å. The results are based on X-ray spacings recorded at 37°C.

It is relevant to mention here a recently published study (Engström, 1990) on aggregation and structural variations in the L_2 phase in the system water-soybean oil-sunflower oil monoglycerides. The phase behaviour of this system has striking similarities with the lidocaine base-monoolein-water system. However, on swelling the L_2 phase in this system the monoglyceride bilayers were penetrated by the triglyceride oil. Further, no triglycerides could be localised in the lipid bilayer.

The repeat distance d(Å) for samples in the L_α phase region with constant L:HCl/MO weight ratio (1:5.5) but with different amounts of water was also determined by low angle X-ray diffraction. The compositions studied are shown as crosses in Fig. 2B. Fig. 5 shows d as a function of the water content in the samples, and also the lipid bilayer thickness d_l and the water layer thickness d_w as given by

$$d_l = \frac{(1-c)v_l}{(1-c)v_l + cv_w} d \qquad (1)$$

and

$$d_w = d - d_l \qquad (2)$$

where c is the weight fraction of water, and v_l and v_w the partial specific volumes of lipid and water, respectively. Since v_l is difficult to estimate, we used $v_l = v_w = 1$ ml/g as an approximation. The values obtained for d_l and d_w are also depicted in Fig. 5.

The lamellar repeat distance d increases with water content in an approximately linear fashion according to Fig. 5. The lipid bilayer thickness, however, decreases with increasing water content, which is a typical behaviour and reflects the increased carbon chain disorder in the lipid bilayer as more water is added. Moreover, due to the high water solubility of L:HCl, one cannot exclude that the local anesthetic enters into the water domain of the lamellar phase as the water content increases.

The area per lipid molecule, S (Å^2), at the water interface can be calculated according to

$$S = \frac{2V_M}{d_l} = \frac{2Mv_l}{N_A d_l} \quad (3)$$

where V_M is the lipid molecular volume, M denotes the lipid molecular weight, and N_A is Avogadro's number. The molecular weight of the lipid was calculated as a mean value where the molar ratio of lidocaine HCl to monoolein was taken into account. The data in Fig. 5 reveal an increase in lipid surface area with increasing water content, which corresponds to a reduction in the lipid bilayer thickness d_l.

Fig. 5 also illustrates the d and S values (unfilled markers) obtained at 35% (w/w) water but with a lower L:HCl/MO ratio (1:9.85). It is clearly seen that the surface area decreases probably due to lower electrostatic repulsion caused by L:HCl in the polar head group region.

Lidocaine packing in the lipid aggregates

What do the fundamentally different effects on the cubic phase reveal concerning the locations of the two forms of lidocaine in the lipid-water structure? An insightful way to discuss this matter is in terms of the so-called packing concept of amphiphilic molecules (Israelachvili et al., 1980). In this theory, which should be used qualitatively, an amphiphile is characterized by the dimensionless critical packing parameter, cpp, which is defined as v/al, where v is the volume of the hydrocarbon chain, a the polar head group area, and l the length of the hydrocarbon chain. If an amphiphile in an aggregate can be mimicked by a cylinder, then from simple geometry cpp = 1. It is assumed that amphiphiles with cpp values near 1 form aggregates with a planar interface, as in a lamellar phase. Lecithin (e.g. dioleoylphosphatidylcholine) and Aerosol OT are examples of molecules with a tendency to form lamellar phases over a wide range of lipid/water ratios.

If the value of the cpp deviates considerably from 1, two situations may arise. For amphiphiles with large polar head group areas, e.g. SDS (sodium dodecyl sulphate) and CTAB (cetyltrimethylammonium bromide), the cpp becomes less than 1 ($al > v$), and these molecules are known to form aggregates of the normal type in water, such as micelles of spherical and cylindrical shapes. If, on the other hand, the polar head group area of the amphiphile is small, the cpp is larger than 1 ($al < v$), and inverted micelles are likely to be formed. If one mixes amphiphiles with different cpp values, one may as a first approximation assume that the resulting cpp for the mixture is a weighted sum (on a molar basis) of the individual cpp values.

The cubic phase in this system consists of a curved bilayer, and monoolein's cpp is about 1.2 as estimated from X-ray data. If the packing concept is to be applied to the two forms of lidocaine, we may at first conclude that the cpp of L:HCl is smaller than that of the base form. It is also reasonable to assume, with the phase behaviour in mind, that the cpp for L:HCl is less than 1.2, and larger than 1.2 for the base form. Furthermore, the preservation of the cubic phase when the base and salt forms are mixed in roughly equal amounts clearly indicates that the deviations of the cpp values from 1.2 cancel. Fig. 6 shows a schematized picture of the packing properties of the lidocaine forms. It is more appropriate to give the phase diagram in mol% than in weight % if the packing aspect is to be visualized; nevertheless, the phase diagram does not change

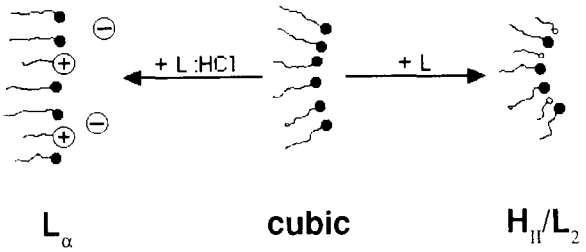


Fig. 6. A schematic picture of the interfacial curvature change as a consequence of lidocaine base and lidocaine HCl addition to the monoolein-water cubic phase.

drastically if this were to be done due to the similar molecular weights of the base form (234 g/mol) and salt form (271 g/mol).

It is thus concluded that both forms of lidocaine are associated with the monoolein bilayer, and that the difference in phase behaviour is caused by the difference in polar head group area. Some of the salt molecules may also exist in the water domain of the phases. We are presently studying the self-diffusion of the lidocaine forms in the cubic phase by means of the pulsed gradient spin echo (PGSE) NMR method (Lindman et al., 1987). The resulting self-diffusion coefficients are important for the determination of the location of the lidocaine forms in the cubic phase, and they may also be correlated with the diffusion coefficients obtained from release experiments.

How are the parameters defining the cpp, i.e. v, a and l, influenced by changes of external conditions? The volume v increases as the temperature is raised due to increasing hydrocarbon chain mobility. This condition manifests itself in the formation of reversed phase types at increasing temperatures, as demonstrated by the phase diagrams, e.g. Fig. 1. The polar head group area depends in a complicated manner on the conditions in the aqueous domain. For ionic amphiphiles, addition of salt causes the molecules of spherical micelles to pack closer to each other due to the decreased electrostatic repulsion between the polar head group charges. This effect decreases the interfacial curvature, and thus promotes the formation of rod-shaped micelles.

The temperature effect is clearly seen when comparing the phase diagrams in Fig. 3; the reversed phase types grow when the temperature is raised. This property is important to consider, since a phase transition changes not only the internal structure of a system, but also the macroscopic properties like viscosity and release characteristics. It should be noted that this behaviour is not restricted to the present system, and should be useful in other lipid systems as well.

In order to illustrate the generality of the packing behaviour of local anesthetics in the monoolein-water system, a number of samples were prepared with other local anesthetics. These molecules have similar molecular structures (see Table 1), some being slightly more lipophilic than others. At a total concentration of local anesthetic of 5% (w/w), the phase transformation pattern $L_\alpha \rightarrow C \rightarrow H_{II}$ is clearly revealed on progression from the charged form, via mixtures to the base form of the local anesthetics. For tetracaine, the most lipophilic of these local anesthetics, the tendency to form reversed phase types occurs already at relatively low levels of the base form in a given mixture.

Although monoolein is not a component of biological membranes, the present results may have some relevance for the understanding of local anesthesia. For example, it is evident that a lipid bilayer may be perturbed due to the interaction with a local anesthetic like lidocaine. This perturbation may in turn influence the membrane proteins responsible for the transport of ions across the membrane. Moreover, the local anesthetic dibucaine was shown to transform the L_α phase of the phospholipid diphosphatidylglycerol in water into a cubic phase (Lindblom and Rilfors, 1989). A discussion of the anesthetic effect in relation to the cubic phase structure was given by Larsson (1988).

Lidocaine formulations

Lidocaine is used in various kinds of formulations, in either its base or salt form. The salt form is used in injectable formulations and gels, while the base form, being poorly soluble in water, is used in ointments and cream emulsions. An example of the latter kind is the EMLA® (Eutectic Mixture of Local Anesthetics) cream (ASTRA, Sweden), which is an oil-in-water emulsion, where

the oil consists of a 1:1 low melting mixture of lidocaine base and prilocaine base, emulsified in an aqueous solution containing a thickener (Carbopol 934) (Nyqvist-Mayer et al., 1986). One advantage with the EMLA® cream is the fact that the oil droplets of the emulsion act as a depot for local anesthetics, maintaining constant activity in the aqueous medium. This gives a fairly constant release of local anesthetics over time (Nyqvist-Mayer et al., 1986).

Our system, with its various equilibrium phases containing lidocaine, ought to behave worse in this respect due to the interaction between lidocaine and the lipid aggregates. On the other hand, the ability of the various phases to be in equilibrium with excess water, which prevents the systems from being diluted (in contrast to an oil-in-water emulsion like EMLA®), makes it conceivable for them to be used in 'wet' environments, e.g. on mucosa. Moreover, monoolein, in combination with bile salts, has been shown to be an absorption enhancer for impermeable drugs in the GI tract (Muranishi, 1990).

Conclusions

We have shown how surface-active drugs, like lidocaine and similar local anesthetics, form various types of liquid crystalline and micellar phases when mixed with monoolein and water. The phase actually formed was dependent on the relative amount of lidocaine base with respect to lidocaine HCl. A large amount of the base form causes reversed phase types to be formed, while a lamellar phase occurs if the salt form dominates. With roughly equal amounts of base and salt, the cubic phase persists. The results are nicely explained in terms of the so-called packing concept of amphiphilic molecules.

One advantage of the present system is that a relatively large amount of lidocaine may be incorporated in the phases, up to 20% by weight. Moreover, the system is very flexible in the sense that one may go from one phase to another due to changes in external conditions such as temperature and pH. This fact may be utilized in an application where one might effect a change in, for example, rheology after administration of the formulation. Probably the most promising sites of application of formulations based on the results presented here are in aqueous environments, i.e. mucosa.

Finally, the phase behaviour presented should be of general interest since many drugs are weak acids or bases, and therefore behave in a similar way if used in a polar lipid-water system.

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