International Journal of Pharmaceutics, 29 (1986) 147–159
Elsevier

IJP 00975

Interaction of lidocaine and lidocaine-HCl with the liquid crystal structure of topical preparations

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(Received November 9th, 1984)
(Modified version received September 19th, 1985)
(Accepted November 4th, 1985)

Key words: lidocaine – soyasterol–PEG-5–ether – liquid crystals – in vitro drug release – small angle X-ray diffraction

Summary

The interaction of lidocaine and lidocaine-HCl with liquid crystal structures of preparations suitable for topical use was studied by polarized light and transmission electron microscopy and small angle X-ray diffraction. Furthermore, in vitro release studies were performed to determine if possible differences in the microstructure of the systems may be recognized by different release patterns. It has been found that both lidocaine and lidocaine-HCl participate in the liquid crystalline structure which primarily consists of hydrated surfactant bilayers in a system consisting of soyasterol–PEG–ether, water and drug. Completely lipophilic components like paraffin, however, are not built into the microstructure as individual molecules but are dispersed as droplets in the system. The incorporation of 1% drug results in a slight increase of the interlayer spacings as measured by X-ray. From the release experiments, it can be concluded that not only the method of preparation but also the amount of the ratio of water/(water + surfactant), the presence of paraffin, and the form of the drug incorporated, may determine the release. In case of lidocaine base, the apparent diffusion coefficients increase with increasing amount of water. The diffusion of the drug through the system is independent of the method of preparing the systems. On the other hand, incorporating lidocaine after preparation of the vehicle itself leads to systems containing a surplus of undissolved drug, while melting lidocaine together with the surfactant before adding water and forming lamellar liquid crystals results in complete solubilization of lidocaine. Because of the higher solubility of lidocaine-HCl salt the systems prepared according to both methods contain dissolved drug. However, from the release profiles can be inferred, that the location in the liquid crystalline structure of the salt is different from that of the free base. Adding the drug after preparation of the vehicle did not permit the drug to enter the water containing liquid crystalline bilayers. The drug is loosely bound to the surface of vesicular liquid crystal structures and hence is easily released. In situ preparation of drug containing liquid crystals leads to entrapment of the salt within the aqueous region of the liquid crystal lamellae. The release of lidocaine-HCl from this type of microstructure is slow because the ability of the salt to penetrate the more lipophilic regions of the bilayers is low. The different options described for a drug participating in the microstructure of these liquid crystal systems which can be applied to the skin show the important role, knowing about the interactions between the various components, because they may affect the release of drug and the subsequent effect on topical therapy.

Introduction

In previous studies (Mueller-Goymann, 1984, 1985; Mueller-Goymann and Fuehrer, 1982; Us-

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selmann and Mueller-Goymann, 1984) liquid crystals have been found to be a part of the microstructure of a variety of preparations suitable for topical use. The liquid crystalline structures cover a wide range from lamellar to hexagonal to cubic. They are not only formed by the surfactants in such preparations, but other components, e.g. water and/or oil, also participate in building highly organized microstructures.

Topical preparations for the treatment of skin diseases contain drugs which are able to penetrate the different layers of the skin to a certain degree. A criterion for membrane penetration is that the penetrant has both lipophilic and hydrophilic moieties. Because of their lipophilic and hydrophilic characteristics, certain drug molecules may be destined to interact with the colloidal structures of a vehicle or of the skin, which in turn may play an important role in membrane penetration (Larsson and Lindblom, 1982). In a larger sense, drug diffusion itself through biological membranes, which have bilayers of amphiphilic molecules arranged in a liquid crystalline state (Unwin and Henderson, 1984), is, however, not yet fully understood (Sackmann et al. 1984).

It was of primary interest to study the possible interaction of a drug with liquid crystal structures, to determine whether it is part of a liquid crystal structure, and secondly how it is distributed within the total system, which may not necessarily be a monophasic liquid crystal. It was further expected that such an interaction would potentially affect release of drug.

The goal of this research was to determine the nature of drug interactions with components of mesomorphic vehicles. The vehicles were studied by freeze-fracture electron microscopy, polarized light microscopy and small angle X-ray diffraction. Although valuable information was gathered by these methods, they have limitations. In an attempt to understand these systems further, drug release studies were used as a tool for comparative purposes between several vehicles. Lidocaine and lidocaine hydrochloride were chosen as the model drugs.

Lidocaine is a local anaesthetic frequently used in various types of topical systems for the treatment of minor skin disorders such as sunburn, insect bites or hemorrhoids, all of which are accompanied by itching and pain. Previous studies have shown (Chen-Chow and Frank, 1981) that lidocaine release from Pluronic Polyol F127 gels, which were believed to be viscous isotropic liquid crystals, was dependent on the concentration of both the drug and the surfactant. The rate of release was determined by the microviscosity of the extramicellar fluid, the dimensions of the aqueous channels and the equilibrium relationship of drug between the micelles and the external aqueous phase.

Materials and Methods

Materials

Lidocaine and lidocaine hydrochloride were provided by Astra Läkemedel AB, Södertälje, Sweden. The surfactant, soyasterol–PEG-5–ether (Generol^R 122 E5; the abbreviation E5 is used in the text) was a gift from the Henkel Corp., Kankakee, IL, USA. Paraffin oil USP XX (Fisher Scientific Co.) was used. The water was distilled and then treated in a MILLI-Q2 system, Millipore Corp. The final resistivity of the water was greater than 10 M$\Omega \cdot$ cm.

Preparation of mixtures

The drug-containing preparations were prepared according to three different methods. Water which had evaporated during the preparation process was not replaced and the final water content was determined gravimetrically. The concentration of drug was 1% for all samples. The systems were stored for 15 h prior to drug release studies; exceptions to this are mentioned in each case.

Methods used

(A) Incorporation of drug after preparation of the vehicle. The surfactant and paraffin, as specified, were melted together. Water of the same temperature (about 70–80°C) was added and the preparation stirred until cool. As soon as room temperature was reached, lidocaine or lidocaine hydrochloride was incorporated in the form of crystalline powders.

(B) Direct incorporation of lidocaine base. The

surfactant and paraffin, as specified, were melted together, following which lidocaine base was added so that a homogenous melt of all three components was obtained. Immediately afterwards, water at the same temperature as the melt was added and the preparation stirred until room temperature was reached.

(C) Simultaneous incorporation of lidocaine hydrochloride and water. The surfactant and paraffin, as specified, were melted together, a hot aqueous solution of lidocaine hydrochloride was added and the preparation was stirred until room temperature was reached.

Characterization of the microstructure

The methods for studying structural changes in the systems caused by the incorporation of drug were bright-field and polarized light microscopy (Leitz), transmission electron microscopy (Philips EM 300) of replicated samples by the technique of freeze-fracture (Balzers BAF 400), and small angle X-ray diffraction according to the method of Kiessig and Kratky (Paar, Philips).

In vitro release studies

The release experiments were performed in two similar release models provided by Astra Läkemedel AB, Södertälje, Sweden. This model consisted of a cylindrical Teflon donor compartment (volume 8 ml) which was combined with a two-neck round-bottom flask so that the donor compartment was in connection with the acceptor compartment containing water or 0.01 N HCl solution. The acceptor compartments in the two devices used contained 577 ml and 628 ml, respectively. If lidocaine hydrochloride was the incorporated drug, pure water was used as the acceptor phase. For lidocaine base, 0.01 N HCl was used in the acceptor compartment, because the base is less soluble in water than is lidocaine hydrochloride. When 0.01 N HCl was the acceptor phase, the diffusing lidocaine molecules were protonated so that the concentration gradient of lidocaine base did not decrease and the acceptor compartment could be considered as a perfect sink. Back flux of the acid into the donor compartment was monitored by pH-measurement and was negligible for single-phase liquid crystalline systems. The compartments were separated by a dialysis membrane (Spectrapor membrane 1, MWCO 6000–8000, Spectrum Medical Industries, Los Angeles, U.S.A.). The effective membrane area was determined by diffusion experiments with 0.05 N HCl, with the diffusion coefficient of HCl at 25°C of 2.93×10^{-5} cm^2/s taken from the literature (Jost, 1960). From this the membrane area was calculated as 15.15 cm^2 according to the transformed Higuchi equation (Higuchi, 1962) which was also used for analysis of the data from the drug release studies. The equation is as follows:

$$A = (Q/2c_0)\sqrt{\pi/(Dt)}$$

where A = membrane area (cm^2); Q = amount of drug released (μg); c_0 = initial concentration of drug in the donor compartment ($\mu g/cm^3$); D = apparent diffusion coefficient (cm^2/s); t = time (s).

In order to calculate the apparent diffusion coefficient of lidocaine in the different vehicles, the density of the mixture was assumed to be 1 g/cm^3. All experiments were performed at 25 ± 0.1°C, and the acceptor compartment was stirred at 400 rpm. All experiments were run for 6 h. Longer times did not prove to be convenient, because back flux from the sink could change the properties of the vehicle. The sample size was 500 μl, which was replaced by fresh acceptor phase each time a sample was taken. Sink conditions were maintained at all times.

Assay of lidocaine

Since the amount of lidocaine released from these semi-solid preparations was very small, a direct UV-assay could not be used. Instead the samples were analyzed by HPLC. An existing method did not prove to be useful since the column (IBM C8 reverse-phase, silica based) was degraded after 200–300 injections due to a sample buffer of pH 8 that was 0.5 pH units above the recommended range. A methanol–phosphate buffer pH 8.0, $\mu = 0.05$ (70:30) had been chosen as the mobile phase because the pK_a of lidocaine is 7.9. The use of a buffer of pH lower than 8 was not possible, because the lidocaine salt had too

short a retention time and even appeared together with the solvent front.

To prolong column life, a new method was developed which proved to be sucessful for serial assays, as it extended the useful life-time of the column (for at least 700 injections). The conditions for this assay were as follows.

An isocratic liquid chromatograph, model 330, Beckman Instruments, was used, with a flow rate of 1 ml/min, loop volume of 100 μl, and sample injection volume of 50 μl. A Hamilton PRP I resin-based column was chosen because it has a working pH range from 1 to 13. This column is more lipophilic than the C8 silica-based column, hence it was necessary to change the mobile phase to avoid long retention times and poor peak shape. A mobile phase consisting of a methanol–phosphate buffer of pH 3.0, $\mu = 0.05$ (45:55) was used. The pH of 3.0 was selected because it assured the presence of the lidocaine-H^+ ion. The ratio of methanol/buffer was varied until a retention time of 5 min and good peak shapes were achieved. The sensitivity of this assay (800 ng/ml) was lower than that obtained with the C8 reverse-phase column (300 ng/ml). By reducing the detection wavelength from 254 nm to 240 nm, a final sensitivity of 400 ng/ml was obtained along with an evidently prolonged life-time of the column.

Results and Discussion

Structural studies

Examination of the preparations containing 1% lidocaine or 1% lidocaine-HCl by both macroscopic observation and polarized light microscopy did not show any differences in appearance when compared to the vehicles themselves. There were neither the formation of new phases nor the disappearance of any phases found previously for these vehicles within their ternary phase diagram. Incompatibilities such as a change of colour, flocculation, sedimentation or creaming were not found. Both lidocaine base and lidocaine-HCl could be incorporated into the vehicles without difficulty. It should be mentioned, especially in the case of the free base, that the solubility of the drug increased as expected in the presence of the surfactant (E5). For example, lidocaine at concentrations > 0.25% in water formed suspensions at 35°C. In the presence of a sufficient amount of surfactant, 1% of the drug was solubilized in the system prepared by method B. In this system, microscopy indicated the absence of lidocaine crystals. However, if the E5 concentration in the preparations dropped below 25%, lidocaine crystals were detected in all mixtures independent of the procedure of preparation (method A or B). In addition, the presence of lidocaine crystals was detected by microscopy in systems prepared by method A even if surfactant concentration was between 25 and 50%. In such systems, there was insufficient solubilization, which did not increase further during storage for several weeks. The solubility of lidocaine-HCl, however, did not depend on the method of preparation due to its much higher solubility in these aqueous systems.

Submicroscopic analysis was performed by small angle X-ray diffraction. The reflections indexed on a lamellar lattice and could be detected up to the fourth order. The sequence of the reflections was 1:1/2:1/3:1/4. Ternary mixtures of E5, water and lidocaine or lidocaine-HCl showed a slight increase of the interlayer spacings compared with binary systems of the same water content, but without drug. Fig. 1a represents the interlayer spacings versus the percent ratio of water/(water + surfactant). It is obvious that incorporation of the drug increases the swelling ability of the lamellar liquid crystals.

Three different possibilities for the interaction between drug, surfactant and water molecules may be discussed individually.

Fig. 2 shows possible explanations for the increase of the interlayer spacings. In the case of lidocaine, this increase may be caused by incorporating the drug molecules in the surfactant bilayers. By this mechanism, the thickness of the bilayers could increase (Fig. 2a). It is also possible that incorporation of the drug molecules in the bilayers does not affect the bilayer thickness itself, but results in an increase of the water layer thickness (Fig. 2b). The third possibility (Fig. 2c), in which drug is incorporated only in the water layer, thereby causing an increase of its thickness, is less probable for the free base than for the hydrochlo-

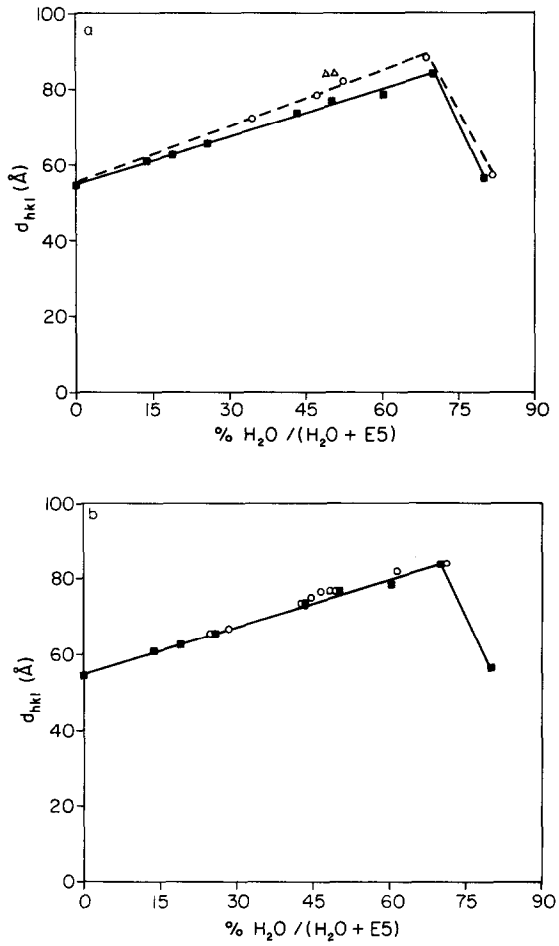

Fig. 1. Interlayer spacings (Å) as functions of the percent ratio of water/(water + surfactant). (a) ■, in binary systems of E5 and water; ○, in ternary systems of E5, water and lidocaine; △, in ternary systems of E5, water and lidocaine-HCl (drug concentration was 1% w/w). (b) ■, in binary systems of E5 and water; ○, in ternary systems of E5, water and paraffin.

ride salt. Which structural conditions occur in the preparations cannot be determined from the X-ray data alone.

Fig. 1b represents the interlayer spacings as functions of the ratios of water/(water + surfactant) in ternary systems with paraffin. It can be concluded from the graph that the presence of paraffin does not influence the thickness of the lamellar liquid crystalline layers. Paraffin does not participate in the structures on a molecular basis, but rather is dispersed in the mixtures as droplets.

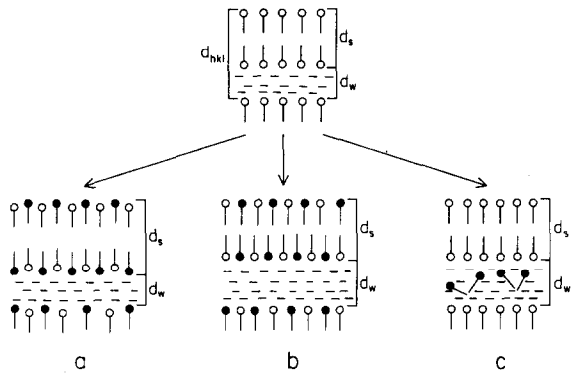

Fig. 2. Model of the increase of interlayer spacings of lamellar liquid crystals consisting of: ○, E5; ●, lidocaine or lidocaine-HCl; and ▣, water. d_{hkl} = interlayer spacings of the hydrated surfactant bilayer determined by small angle X-ray diffraction; d_w = thickness of the hydration layer; d_s = thickness of the nonhydrated surfactant bilayer from ternary systems with 1% lidocaine or lidocaine-HCl (w/w).

Whether the incorporation of such "guest" molecules is possible may be determined primarily by steric considerations and entropic changes, as well as by the nature of hydrophilic and lipophilic interactions between all molecules in the system.

The interlayer spacings in Fig. 1a increase up to 90 Å for a water content of about 70%, and then decrease at 80% water. Water concentrations above 80% did not show any X-ray diffraction pattern. The decrease of the interlayer spacings took place when increasing amounts of water caused the monophasic lamellar liquid crystal to transform into a biphasic system of multilamellar vesicles (MLV) dispersed in an aqueous medium. The monophasic lamellar liquid crystal system did not appear in a completely planar arrangement (see Fig. 2 in Mueller-Goymann, 1984) but formed vesicle-like structures which represent defect structures in lyotropic smectic phases according to Kleman and Williams (1976). At water concentrations of more than 70%, three-dimensional order between the layers swollen to maximum degrees had disappeared. Instead, the surfactant molecules and, if present, lidocaine molecules as well, arranged in a new manner consisting of multilamellar vesicles of unswollen layers dispersed in an outer aqueous phase. This new arrangement was detected by freeze-fracture electron microscopy as shown in Fig. 3. The layer thickness measured by electron

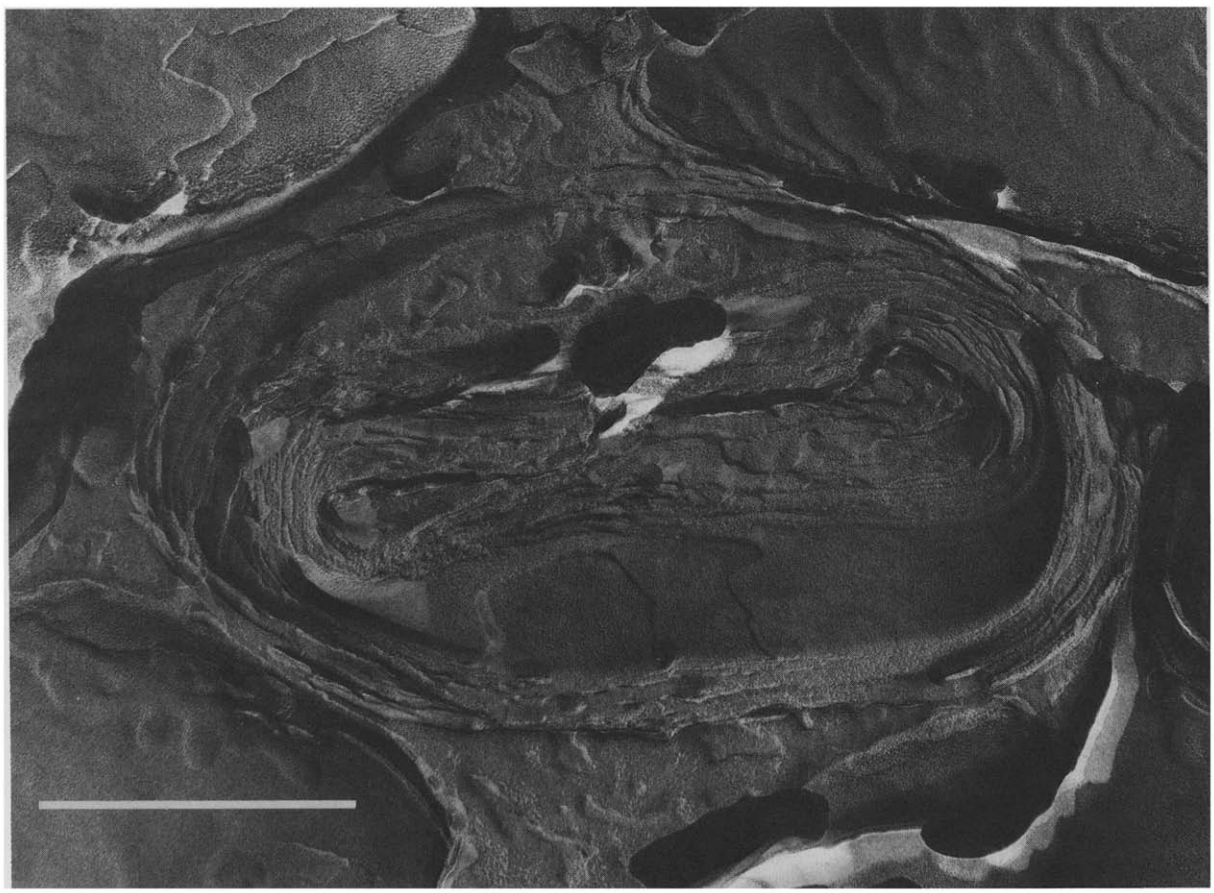

Fig. 3. Freeze-fracture electron photomicrograph of a binary system consisting of 20% E5 and 80% water. Bar 500 nm.

microscopy is in good agreement with the interlayer spacing detected by small angle X-ray diffraction. Therefore, the biphasic system could be understood as an aqueous dispersion of vesicular liquid crystalline components because of a low saturation concentration of the surfactant in the aqueous medium.

The results of the small angle X-ray studies did not show any differences with regard to the method of preparing the systems by methods A, B or C. It should be mentioned that the X-ray studies were performed after storage of the samples for more than 15 h up to 1 week, hence the preparations should have been in thermodynamic equilibrium. Lidocaine preparations by method A contained crystals in addition to solubilized drug, while lidocaine preparations prepared by method B remained as solutions and recrystallization did not occur. Preparation of lidocaine hydrochloride systems resulted in drug completely in solution, independent of whether method A or C was used.

In vitro release studies

The release of the drug from the different preparations was studied to determine variations that depended on: (1) the percent ratio of water/(water + surfactant) in the lamellar liquid crystals; (2) the method of preparation; (3) the presence of the totally lipophilic paraffin, which was dispersed in the mixtures as an o/w emulsion; and (4) the

dissociation of the drug, ie., whether lidocaine base or lidocaine hydrochloride was used.

(1) Influence of water content on lidocaine release

Fig. 4 shows typical examples of the amount of drug released as a function of time. As can be seen, the release of lidocaine from mixtures with liquid crystalline structure was rather slow. After 6 h, the maximum amount of lidocaine released from single-phase systems consisting of liquid crystals was 4% of the total lidocaine concentration in the preparation. The amount released increased to 6% when the preparation became a two-phase system of liquid crystalline multilamellar vesicles (MLV) dispersed in an outer aqueous phase. However, if the concentration of the surfactant dropped below ~25%, release increased to more than 10% over the same period of time. In order to quantify the rate of release, it is useful to determine the apparent diffusion coefficients. For this purpose, the data were analyzed by the method of Higuchi (Higuchi, 1962), which describes the release of drug from ointments containing up to 30% drug. The Higuchi relationship is valid if the drug is completely dissolved in the vehicle, which ought not to be changed over the duration of the experiment and which is the only rate-controlling factor for release.

Analysis of the data by the Higuchi equation shows that there is a linear relationship between the amount of drug released and the square root of time, as long as the preparations were liquid crystalline in nature (Fig. 5). The apparent diffusion coefficients were then calculated from the slopes. Fig. 6 represents the apparent diffusion coefficients as functions of the percent ratio of water/(water + surfactant). A linear increase of the diffusion coefficients was found also with increasing water content, as long as the preparations were single-phase systems of liquid crystals. The greater the amount of water bound within the layers, the faster was the diffusion of lidocaine through the lamellar liquid crystal phase, resulting

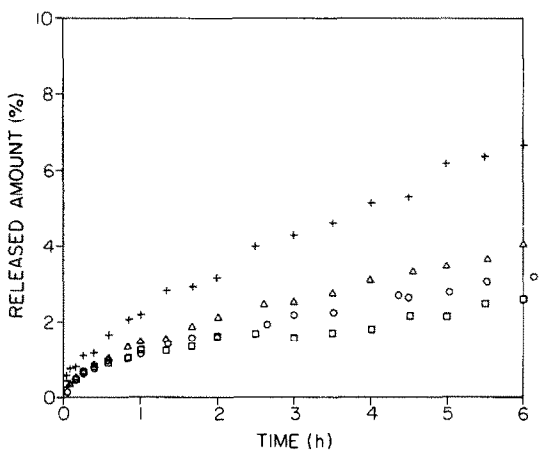

Fig. 4. Lidocaine release as a function of time at 25°C from ternary systems containing 1% lidocaine (w/w). The systems were prepared according to method A.

□ { E5 70.2% $H_2O/(H_2O+E5)$ 29%; H_2O 28.8%; Lid. 1.0%

○ { E5 49.1% $H_2O/(H_2O+E5)$ 50.4%; H_2O 49.9%; Lid. 1.0%

△ { E5 37.8% $H_2O/(H_2O+E5)$ 61.8%; H_2O 61.2%; Lid. 1.0%

\+ { E5 24.3% $H_2O/(H_2O+E5)$ 75.5%; H_2O 74.7%; Lid. 1.0%

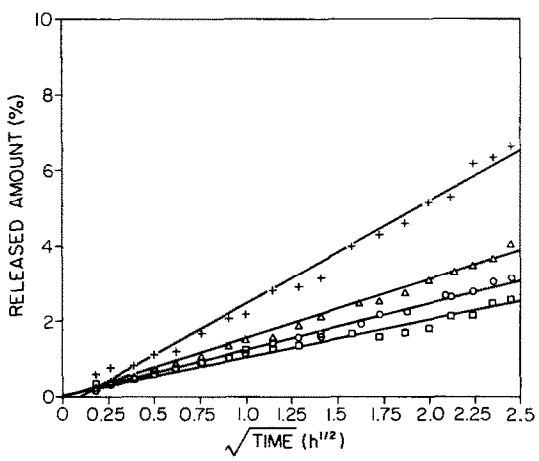

Fig. 5. Lidocaine release versus the square root of time at 25°C from ternary systems containing 1% lidocaine (w/w) and prepared according to method A. For description of the symbols see Fig. 4.

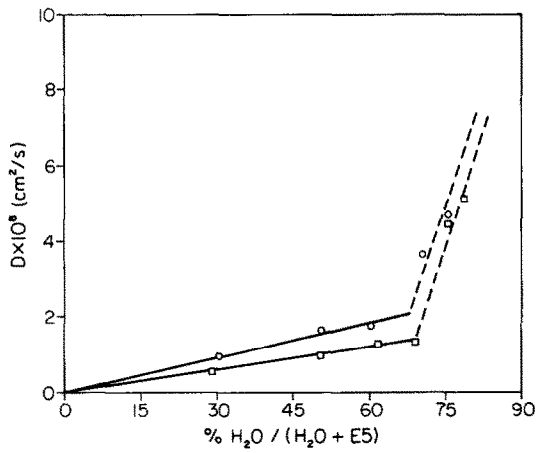

Fig. 6. Apparent diffusion coefficients for lidocaine release from ternary systems as functions of the percent ratio of water/(water + surfactant). Lidocaine concentration was 1% w/w. □, prepared according to method A; ○, prepared according to method B.

thereby in a more rapid appearance in the acceptor compartment.

The linear relationship between the apparent diffusion coefficients and the water content is analogous to the results of the X-ray studies, which indicated a linear increase of the thickness of the hydrated interlayer spacings with increasing water content (Figs. 1a and 6). A sudden change of slope occurs in both graphs at about 70% water content. The single-phase lamellar liquid crystals, which exist up to water contents of about 70%, transformed at higher water concentrations into two-phase systems of multilamellar vesicles (MLV) dispersed in the outer aqueous phase. While the apparent diffusion coefficients of the two-phase systems increased sharply because of the low viscosity of the aqueous phase, the interlayer spacing decreased to the d_{hkl}-value of the non-hydrated surfactant bilayer. A liquid crystalline arrangement of the molecules could not be detected by X-ray if the surfactant content dropped below 20%. The formation of anisotropic vesicles was still detected, however, by polarized light microscopy which is a more sensitive method, but of course, it could not give any information about interlayer spacings. Also, freeze-fracture electron microscopy indicated the existence of MLV in systems of 80% water content (Fig. 3). It should be noted further, that the swelling behaviour of these MLV seems to be different from that of the single-phase liquid crystals. Both from the X-ray data and freeze-fracture electron microscopy, it can be concluded that the two-phase dispersions of MLV consist of unswollen surfactant bilayers. This means that the mixtures are in essence suspensions of surfactant in water because of their limited solubility. Lidocaine could not be incorporated into these MLV to the same extent as in the case of the single-phase liquid crystals. Hence, a certain amount of the incorporated drug remained in crystalline form without being solubilized, as was observed by microscopy. That was not only true for all mixtures with 90% and greater water content, but even for single-phase liquid crystal mixtures and the MLV dispersions at water contents of 50% or more which were prepared by method A.

If the amount of the outer aqueous phase increased relative to the amount of the dispersed MLV and thus became the release determining factor, the application of the Higuchi equation to the data proved to be difficult or even impossible. Plots of the amount released versus the square root of time resulted in at least a biphasic trend of the data. At first the slopes were low, but then increased with time. The biphasic tendency of the amounts released as functions of the square roots of time can be recognized even in the uppermost graph in Fig. 5. The best fit of all data shown in the figure intersects the $t^{1/2}$ axis. However, separating the data in two groups would result in even better fits of the straight lines with different slopes. As expected, the straight line with the smaller slope would start at point 0. This biphasic type of release pattern was all the more marked as the water content of the two-phase system increased. Estimations of the diffusion coefficients from the final portions of typical plots for dispersions of MLV at water contents of 90% were 10–20 times higher than those from single-phase liquid crystal systems.

One of the possible explanations for the biphasic behavior of the $Q/\sqrt{t}$ plots is the fact that the dispersions of MLV, which are low viscosity emulsions, changed during the experiments so that

TABLE 1

APPARENT DIFFUSION COEFFICIENTS FOR LIDOCAINE AND LIDOCAINE-H^+ FROM PREPARATIONS CONTAINING 1% DRUG

% $H_2O/(H_2O + E5)$	% Composition of the systems, E5/paraffin/water/lidocaine (*) or lidocaine-HCl (**)	Method of preparation (see text)	Acceptor-phase, $D \times 10^8$ (cm^2/s)	
75.3	24.5 – 74.5 1.0 *	B	0.01 N HCl	4.630 4.831
75.8	24.0 – 75.0 1.0 *	B	H_2O	1.443 1.671
75.4	19.5 19.5 60.0 1.0 *	B	0.01 N HCl	3.124 3.933
74.9	19.85 19.85 59.3 1.0 *	A	0.01 N HCl	0.755 1.143
75.9	19.2 19.2 60.6 1.0 **	A	H_2O	8.434 8.896
75.9	19.2 19.2 60.6 1.0 **	C	H_2O	0.531 0.558
50.4	49.1 – 49.9 1.0 *	A	0.01 N HCl	0.993
50.6	48.9 – 50.1 1.0 *	B	0.01 N HCl	1.669
50.8	48.7 – 50.3 1.0 **	A	H_2O	3.714 3.989
51.0	48.5 – 50.5 1.0 **	C	H_2O	0.240 0.340

the Higuchi equation no longer held. Ions from the acceptor compartment, which consisted of 0.01 N HCl (in the lidocaine release experiments), apparently penetrated the membrane and diffused into the donor compartment. The resulting change of pH would cause a change in the mixture itself, since lidocaine was no longer in the free base form, but transformed into the salt. Because of the higher hydrophilicity of the salt compared with the more lipophilic base, penetration of the hydrophilic salt through the hydrophilic membrane would be faster than penetration of the base. Direct comparisons of the release of lidocaine into acceptor compartments of water and of 0.01 N HCl showed that diffusion of lidocaine, which was completely solubilized in the vehicle, was much slower if the acceptor phase consisted of water (Table 1). The reason for this may be that diffusion of lidocaine-H^+ ions through the hydrophilic membrane is facilitated when compared to that of lidocaine base. Release experiments with lidocaine-HCl as the incorporated drug also confirm that lidocaine-H^+ ions diffuse faster into the aqueous acceptor compartment than does lidocaine base (Table 1).

The possible diffusion of HCl molecules is of course a factor which should be taken into consideration for all of the preparations. The back flux of acid was restricted, however, to the layer of the vehicle adjacent to the membrane because of the high viscosity of the lamellar liquid crystalline systems. Thus, it may be assumed that back flux did not have a significant influence on the release profiles of the single-phase liquid crystal systems, which were described by the square root of time equation.

Although the membrane itself exercises some influence over the diffusion process, penetration of drug through the membrane is not the rate-controlling (limiting) factor. However, this can happen, as in the cases of the fluid mixtures containing 90% water, as well as with the aqueous solutions of lidocaine and lidocaine hydrochloride. The plots for these studies showed linear relationships from $t = 0$, indicating that the membrane chosen for these studies was not suitable for these systems. An estimate of the diffusion coefficient for lidocaine from aqueous solutions was 10^{-6} cm^2/s. If, however, the diffusion process was slow, as for example in the case of drug incorporated within liquid crystals, drug release would not be membrane-controlled and the experimental model would be valid for these studies.

There is a second explanation for the biphasic behaviour of the Q versus $\sqrt{t}$ plots which were observed for systems at water contents of 70% or more prepared by method A (which contained lidocaine crystals). As mentioned earlier, diffusion of HCl ions from the acceptor to the donor compartment caused a change of pH from alkaline to acid as determined experimentally before and after a release experiment. At the same time, the solubility, as well as the dissolution rate of the crystalline lidocaine increased. As a result, both the concentration and the concentration gradient of the dissolved or solubilized drug—now as lidocaine hydrochloride—increased. Since the concentration gradient was determining the rate of release, the apparent diffusion coefficient increased during an experiment. Again this effect became apparent only if the vehicle was not a single-phase system of liquid crystals, but a disperson of MLV. The lower viscosity of the MLV dispersion compared to the single-phase lamellar liquid crystals facilitated the rapid change of pH in the systems. Preparations containing lidocaine crystals dispersed in a lamellar liquid crystalline phase gave a release pattern which was well described by Higuchi equation.

(2) Influence of the method of preparation on lidocaine release

As can be seen in Fig. 6, the slope of a plot of the apparent diffusion coefficients as functions of the percent ratio of water/(water + surfactant) differs depending on the method of preparation. Although the opposite effect had been expected, the apparent diffusion coefficients were lower if the system was prepared by method A. On the other hand, X-ray data had shown that the method of preparation did not appear to influence the microstructure of the systems.

If release of lidocaine from the liquid crystalline vehicle is possible, diffusion of the drug will of course take place within the vehicle. As a consequence, a homogenous distribution of drug throughout the liquid crystal phase should be as-

sumed. However, as demonstrated by microscopy, a certain amount of lidocaine crystals remained undissolved and in suspension when the system was prepared by method A. The direct incorporation of lidocaine (method B) resulted instead in the complete solubilization of lidocaine at a concentration of 1%. With method A, drug was solubilized only up to the saturation limit. From these observations, it can be concluded that preparation of these systems according to method B resulted in supersaturated solutions. Delayed recrystallization of the drug was prevented by the presence of the highly organized surfactant molecules. This phenomenon is analogous to the method of preventing recrystallization from supersaturated polymer solutions of Francois (1983). Considering this, the difference in Fig. 6 between the preparations from methods A and B can be interpreted. Since dissolved and solubilized lidocaine diffuses, and since it was assumed for calculation purposes that the amount of solubilized lidocaine equalled 100% in all mixtures, lower apparent diffusion coefficients were obtained for method A when compared with method B, where in the latter case, the entire amount of lidocaine was solubilized.

Provided that diffusion from the liquid crystal structure is in fact independent of the method of preparation, calculation of the saturation concentration of lidocaine in the lamellar liquid crystals is then possible. The saturation concentration of lidocaine was calculated from the transformed Higuchi equation:

$$c = Q(\pi/(Dt))^{1/2}/2A$$

assuming that the diffusion coefficient was the same in the systems prepared by methods A and B. For this purpose, the diffusion coefficients were taken from the upper graph in Fig. 6 for each water concentration.

The saturation concentrations for various liquid crystalline systems prepared by method A are given in Table 2. The data show a slight increase in the concentration of solubilized lidocaine at saturation with increasing water content, as long as the preparations were monophase systems of lamellar liquid crystals. When the ratio of water/(water + surfactant) reached 70%, so that the monophasic system starts to transform into a biphasic dispersion of MLV, the maximum concentration of lidocaine which can be solubilized decreased. As mentioned earlier, even the biphasic systems prepared according to method B did not result in complete solubilization.

TABLE 2

CALCULATED CONCENTRATION OF SOLUBILIZED LIDOCAINE IN TERNARY SYSTEMS PREPARED BY METHOD A (INITIAL CONCENTRATION OF LIDOCAINE WAS 1% (w/w))

% $H_2O/(H_2O+E5)$	% Composition of the systems, $E5/H_2O$/lidocaine	% Concentration of solubilized lidocaine at 25°C
29.0	70.2	0.71
	28.3	
	1.0	
50.4	49.1	0.78
	49.9	
	1.0	
61.8	37.8	0.86
	61.2	
	1.0	
69.0	30.7	0.65
	68.7	
	1.0	

Finally, it can be concluded from Fig. 6 that the apparent diffusion coefficients depend on the concentration of solubilized lidocaine, as had been shown by others (Chen-Chow and Frank, 1981).

(3) Influence of the presence of paraffin on lidocaine release

Quaternary mixtures of E5, paraffin, water and lidocaine base showed the same dependence of the apparent diffusion coefficients on the method of preparation (Table 1), as described above. However, the magnitude of the coefficients was lower than those from the ternary mixtures. One reason for this difference may be the method of calculating water content, i.e. water/(water + surfactant), because this ratio does not consider the additional partitioning of the rather lipophilic lidocaine base into paraffin phase. Taking this into consideration and calculating the water content as water/(water

+ surfactant + paraffin) corrects the 75% value to 60%. Nevertheless, the apparent diffusion coefficients still deviate from the data obtained for the ternary mixtures. For a complete understanding, it would be necessary to determine the partition coefficient of lidocaine between the different phases, and further studies in this context are planned. With regard to quaternary preparations containing lidocaine-HCl, the results were similar to those obtained for the ternary systems which will be discussed in the following section.

(4) Influence of the incorporation of lidocaine base or salt on release

The incorporation of lidocaine hydrochloride instead of the free base would be expected to result in a dependence of the apparent diffusion coefficients on the method of preparation. However, just the opposite phenomenon was obtained as compared with systems containing the free base. Method A gave higher diffusion coefficients than the systems prepared according to method C. Since the solubility of the salt is much higher than 1%, there were no problems with regard to undissolved drug crystals. The different results of the release experiments may therefore come from differences in the structure of the systems. In this context, incorporation of the hot lidocaine hydrochloride solution according to method C could be expected to lead to hydration of the surfactant bilayers. Because of the more hydrophilic character of the lidocaine-H^+ ion, the microstructure of this system might then resemble the model given in Fig. 2c. By this model, drug is more or less 'entrapped' between the surfactant bilayers and hence its tendency to diffuse through the more lipophilic regions of the surfactant bilayers is low, as reflected by the extremely low apparent diffusion coefficients. On the other hand, the incorporation of lidocaine hydrochloride after vehicle preparation (method A) obviously does not result in entrapment of the drug within the surfactant layers. The higher diffusion coefficient indicates that lidocaine-H^+ ion is not bound to the liquid crystal structure as strongly as in the first example. It may be possible that drug molecules are loosely bound to hydrophilic moieties at the 'surface' of the liquid crystal structures. Such lamellar liquid crystals usually do not occur in a completely planar arrangement, but rather form vesicle-like structures. These vesicles are thought to bind ionized drug only at their 'surface' without having the ions penetrate into the more lipophilic regions of the surfactant bilayers. As a result, release of drug should be facilitated.

Conclusion

From the results presented, in vitro drug release studies appear to be valuable tools for obtaining information about the interaction of drug molecules with components of liquid crystalline vehicles. A model for drug distribution within liquid crystal structures was developed which would not have been possible by X-ray studies alone. Depending on different parameters such as water content, salt or base form of the drug, method of preparation and presence of dispersed paraffin droplets, drug may participate in the microstructure of mesomorphic systems to different extents. While lidocaine base is able to diffuse freely through the liquid crystalline system, diffusion of the salt is hindered it the drug is incorporated at the same time as the liquid crystal structure forms. The salt form is presumed to be 'entrapped' within the water layers of the lamellae and hence not able to diffuse with the same ease as the more lipophilic base. Although the release experiments in this study were primarily considered as a tool for understanding drug interactions with mesomorphic vehicles, they are also important by themselves. It should be mentioned, however, that in vitro studies obviously cannot substitute for in vivo experiments because they do not consider the influence of the biological system.

Acknowledgement

This research was supported by a U.S.A. fellowship of the Deutsche Forschungsgemeinschaft (Mu 684/1-1) for C. M.-G.

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