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# Azone® and the formation of reversed mono- and bicontinuous lipid-water phases

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## Summary

The interfacial properties of the skin penetration enhancer Azone® were investigated as well as the effect the substance has on the phase behaviour of two bilayer forming lipid-water systems, i.e., lecithin-water and monoolein-water. Interfacial studies revealed a change in Azone packing on the water surface as the area per molecule decreased below 62 Å$^2$. It is suggested that this change reflects a situation where the polar amide bond of Azone loses its direct contact with water and the molecule adopts a straighter conformation normal to the surface. Phase studies show that Azone promotes the formation of reversed types of lipid-water phases such as bicontinuous cubic, reversed hexagonal and reversed micellar phases. From this phase behaviour and recent literature data, we suggest that the formation of reversed types of phases should be considered as one important mechanism behind the increased skin penetration of drugs caused by lipophilic substances such as Azone.

## Introduction

Transdermal administration of drugs suffers from the fact that the stratum corneum constitutes such an effective barrier. One way to overcome this problem is by using so-called penetration enhancers. Several types of penetration enhancers have been discussed in the literature, such as solvents (ethanol, propylene glycol) and amphiphilic substances (detergents, fatty acids, monoglycerides). One of the most extensively studied molecules in this respect is Azone® (Fig. 1), and it has been shown to increase the penetration of drugs, both polar and non-polar, through skin (Wiechers and De Zeeuw, 1990; Wiechers, 1992). Even though Azone has been used in many studies, little has been discussed as to its and similar enhancer's effects at a molecular level (Barry, 1987a,b; Potts et al., 1991; Schückler et al., 1993).

In this work, the interfacial properties of Azone were investigated, as well as the effect the molecule has on two bilayer forming lipid-water systems, lecithin-water and monoolein-water. These model systems are not directly applicable to stratum corneum lipids, which have a unique composition, particularly with respect to the low concentration of phospholipids and the presence

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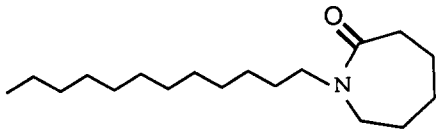


Fig. 1. The molecular structure of Azone.

of ceramides. The lecithin-water and monoolein-water systems, however, are well-characterized with respect to their phase behaviour, and are therefore suitable model systems in order to illustrate the general effects caused by lipophilic penetration enhancers such as Azone. The results from this study can then be used in the interpretation of the effects caused by Azone in the more complicated skin lipid systems.

An important tool in the understanding of the molecular behaviour of Azone and similar molecules is the phase diagram, the graphical representation of the phase behaviour of a system under study. Hundreds of phase diagrams, more or less detailed, have been published over the years describing systems including amphiphilic molecules in water. In spite of the several hundreds of substances studied, their phase behaviour can be rationalised by taking into account the shape (in a broad sense) of the molecules. The so-called packing concept (Israelachvili et al., 1980) is one way of approaching the problem; another is by means of the curvature of the interfacial region. A conclusion is that one may, with reasonable accuracy, predict the phase behaviour of an amphiphilic compound in water, if its molecular structure is known.

The lipids forming the extracellular matrix of skin belong to a class of water (insoluble) lipids, some of which may swell in water to form liquid crystals. In the stratum corneum they form a lamellar structure with polar and non-polar regions, which implies that any drug is hindered in its transport, since it has to diffuse over regions of opposite polarity to itself. If the lamellar structure ($L_\alpha$) at some location is altered to a bicontinuous cubic structure (Q) and/or a reversed hexagonal structure ($H_{II}$) (by, for example, a suitable penetration enhancer), then the transport should be less hindered for both polar and non-polar drugs in the bicontinuous cubic case, and for non-polar drugs in the hexagonal case. This situation is schematized in Fig. 2.

A particularly important property of the phase behaviour of lipid-water systems is that a temperature increase promotes the formation of reversed types of phases, i.e., to the right in Fig. 2. A second property is that the same effect can be achieved by adding selected (amphiphilic) compounds to the system. In a recent paper by Abraham and Downing (1991, 1992), it was elegantly illustrated that reversed hexagonal phases were formed in skin lipid model systems when the temperature was increased to above 60°C. In this work we will show that addition of Azone to a soya lecithin-water and a monoolein-water system will give rise to the same effect at room temperature. Moreover, we discuss the possibility that Abraham and Downing have also given evidence for the occurrence of a cubic phase in their skin lipid model systems. We base our argumentation

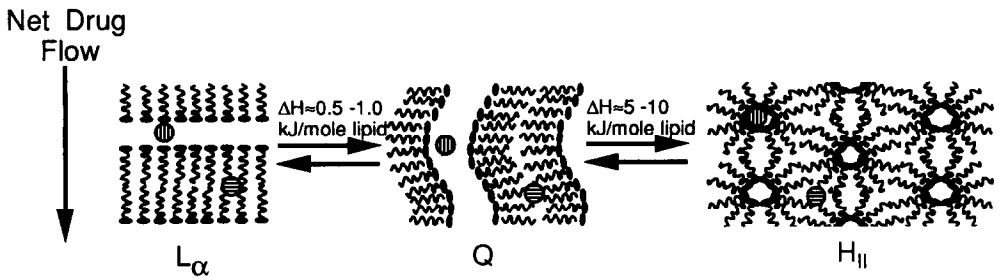


Fig. 2. A schematic picture illustrating the effect of transitions from lamellar to bicontinuous cubic to reversed hexagonal structures on drug penetration. It should be noted that the sketch representing the cubic phase Q, does not fulfill the symmetry requirements but merely illustrates the bicontinuous structure.

on the deuterium NMR spectra and on the electron micrographs published in the paper by Abraham and Downing (1991).

## Materials and Methods

*Materials*

Azone® was a gift from Whitby Research Inc. (Richmond, VA, U.S.A.), and phosphatidylcholine from soya bean (lecithin, SPC) was a gift from Karlshamns Lipidteknik AB (Stockholm, Sweden). Glycerol monooleate (monoolein, GMO) was purchased from Grinstead Products A/S (Denmark). The water used was of double 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 examined by using crossed polarizers in order to detect any anisotropy, polarizing microscopy in order to study the texture of the anisotropic phases, and X-ray diffraction.

*X-ray diffraction* For the X-ray measurements a DPT camera (Stenhagen, 1951) and a Guinier camera after Luzzati et al. (1960) with a quartz monochromator were used. The resulting X-ray films were examined by eye and with an image analysing system (JAVA, Germany) equipped with a Philips CDD video camera.

*Surface balance* For the surface film measurements an automatic recording surface balance based on the vertical Wilhelmy plate principle was used. The monolayers were obtained by spreading the appropriate amount of Azone dissolved in hexane on the subphase, which was double distilled water. After spreading, the system was allowed to equilibrate for 30 min at 24°C.

## Results and Discussion

*Interfacial properties*

Azone is a combination of a water-insoluble hydrocarbon chain and $\epsilon$-caprolactam, the latter

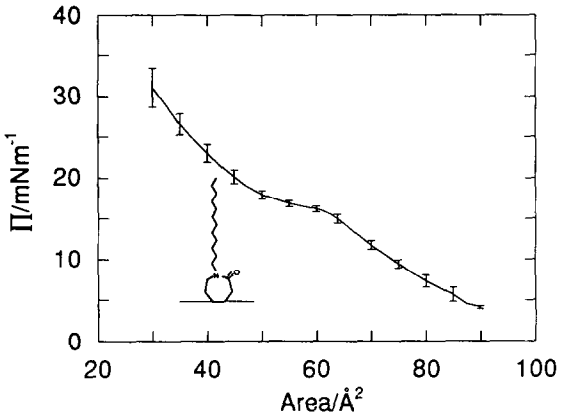


Fig. 3. $\pi$-$A$ compression isotherm for Azone on double distilled water at 24°C. The proposed conformation of Azone below 62 Å$^2$ is shown in the diagram.

freely soluble in water as well as in organic solvents. Azone itself is practically insoluble in water but dissolves readily in dodecane. It is surface active and Beastall (1987) has demonstrated that it lowers the interfacial tension between an aqueous buffer solution and isopropylmyristate. From Beastall's data it is possible to determine the limiting interfacial area of Azone at high concentrations to be about 69 Å$^2$ (1 Å $= 10^{-10}$ m), a number implying that a substantial part of the molecule occupies the interfacial region.

We have studied the surface properties of Azone in surface balance experiments. Fig. 3 shows the $\pi$-$A$ curve for Azone on distilled water. The curve reveals a conformational change around $62 \pm 1$ Å$^2$ followed by a monotonic increase in surface pressure. Film rupture appears slightly above 30 Å$^2$. These data agree fairly well with results obtained by Lewis and Hadgraft (1990), one difference being the conformational change around 62 Å$^2$.

We propose that the hump in the $\pi$-$A$ curve around 62 Å$^2$ reflects a conformational change where the amide group of the caprolactam ring loses its direct contact with water as sketched in Fig. 3. A similar mechanism has been proposed for esters of short chain alcohols ($\leq$ amyl) and long-chain fatty acids (Larsson, 1973). The closely packed structure below 62 Å$^2$ is then more easily explained.

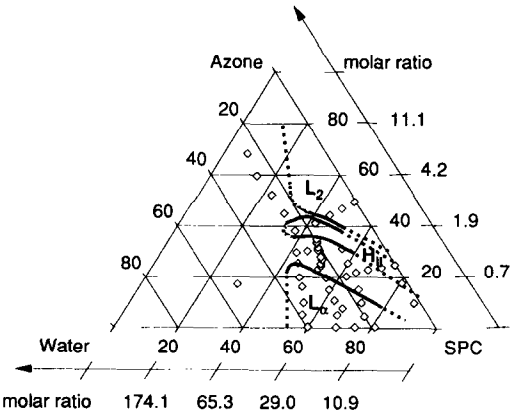


Fig. 4. Phase diagram of the Azone-soya lecithin-water system at 20°C. The markers show the compositions (wt%) of the samples from which the phase boundaries are estimated. The molecular weight of lecithin is assumed to be that of dilinoleoylphosphatidylcholine for the calculation of the molar ratio.

*The effect of Azone on bilayer forming lipids*

Figs 4 and 5 show the phase diagrams obtained for Azone in the lecithin/water and monoolein/water systems, respectively. It is obvious that Azone promotes the formation of reversed types of phases (i.e., reversed hexagonal, $H_{II}$, and reversed micellar phases, $L_2$) in both systems. This has been shown by polarizing microscopy and confirmed by X-ray diffraction. Another finding is that it takes more Azone to form an $H_{II}$ phase in the lecithin system than in the monoolein system.

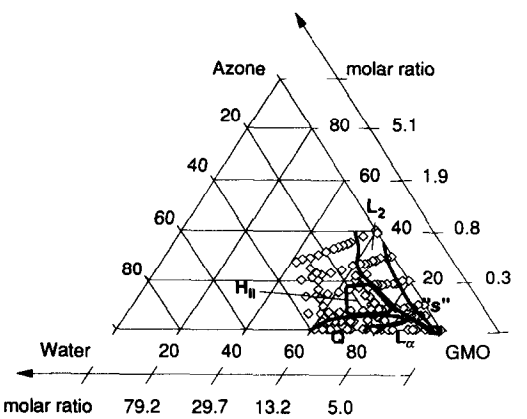


Fig. 5. Phase diagram of the Azone-monoolein-water system at 20°C. The prisms show the compositions (wt%) of the samples from which the phase boundaries are estimated.

In the lecithin-water system with 25 wt% water, the diffraction pattern changes around 20 wt% Azone, where a pattern corresponding to the hexagonal symmetry occurs. Analysis of the diffraction pattern reveals that Azone is not only anchored in the interfacial region, but also distributes in the whole hydrocarbon region. It takes a relatively large amount of Azone to induce the $L_\alpha \rightarrow H_{II}$ change, but this is to be expected, since the lamellar phase formed by lecithin is stable as shown by the high temperature at which this change occurs (> 200°C) (Small, 1967) in the lecithin/water system at 25 wt% water. Nevertheless, the effect of adding Azone is qualitatively the same as increasing the temperature.

The monoolein-water system is somewhat different in comparison with the lecithin-water system, since the latter forms a cubic liquid crystal in excess water. Azone affects the monoolein-water system in lower concentrations than the lecithin-water system. This point is clearly illustrated by the replacement of monoolein by Azone at a water concentration of 15 wt%. The monoolein-water system forms a lamellar phase at this water content. Increasing amounts of Azone lead to the phase transitions $L_\alpha \rightarrow Q \rightarrow H_{II} \rightarrow L_2(+H_2O)$ as shown in the phase diagram in Fig. 5.

The bicontinuous cubic structure (Q) may give the impression of being a very curved and complicated structure. This bicontinuous cubic phase, however, shows more similarity with the lamellar phase than the lamellar phase does with the reversed hexagonal phase. The cubic phase consists of a congruent lipid bilayer extending in three dimensions separated by water channels (Hyde et al., 1984). The similarity is further reflected by the relatively low enthalpy change involved in the $L_\alpha \rightarrow Q$ transition ($\approx 0.5$ kJ/mol lipid; Engström et al., 1992) compared to the $L_\alpha \rightarrow H_{II}$ transition (typically 5–10 kJ/mol lipid; Seddon et al., 1983). The reason for this is the small interfacial curvature shown by the cubic phase since each point on the interface is a saddle point. In fact, the mid-plane between the hydrocarbon chains is a so-called infinite mini-

mum periodic surface (IPMS) with zero curvature. The interfacial region has a slight negative curvature, i.e., it tends to circumvent water.

The phase diagrams given above clearly show that Azone promotes the formation of reversed types of phases in the monoolein and soya lecithin systems. This indicates that Azone plays the same role as a temperature increase as regards the phase behaviour. The reason for Azone promoting reversed phases is related to its molecular structure, being only weakly polar. The same phase behaviour is seen, for example, when adding oleic acid and triglycerides (Lindström et al., 1981). The local anesthetic lidocaine base, used in the EMLA® cream (Nyqvist-Mayer et al., 1986), also gives rise to the same phase behaviour when mixed with monoolein and water (Engström and Engström, 1992).

One difference between Azone and the latter substances is that the $H_{II}$ phases with Azone can take more water. This is probably due to the fact that Azone can distribute both at the interface as well as in the middle of the hydrocarbon region, filling the space between cylinders with a large radius. This effect has also been observed in the lecithin-dodecane-water system (Sjölund et al., 1987).

Our conclusion regarding the effect of Azone on the phase behaviour of lipid-water systems differs from the interpretation made by Schückler et al. (1993) on a lipid model system composed of fatty acids. One of their studied systems consists of six different, saturated and unsaturated, fatty acids partly neutralized in water. Based on X-ray data, they claim that a reversed hexagonal phase is formed by this fatty acid/fatty acid salt system, and that Azone induces a transformation to a lamellar phase. Since the system used by Schückler et al. is more complex, it is difficult at this stage to resolve this discrepancy, but work is in progress in our laboratory to clarify this point.

*A possible mechanism of action of Azone in skin*

Abraham and Downing (1991) have investigated skin lipid model systems by means of deuterium NMR and electron microscopy. $\alpha$-$CD_2$ labelled palmitic acid was used in the systems to probe the existence of anisotropic phases. One of their systems (38 mol% ceramide from stratum corneum, 38% cholesterol, 19% palmitic acid ($\alpha$-$CD_2$) and 5% cholesteryl sulphate, where the lipid blend was mixed with 50 wt% distilled water) gave rise to a $^2$H-NMR spectrum showing two quadrupolar splittings above 60°C implying the existence of a lamellar and a reversed hexagonal phase in equilibrium. In this spectrum, a central peak also occurred at all temperatures, implying an isotropic environment of the palmitic acid on the time scale of the NMR experiment.

The central peak could arise in at least three different ways. One is the possibility that the palmitic acid is partly ionised to form micelles, which would give rise to an isotropic deuterium signal. Since distilled water was used, the formation of micelles is impossible. Another possibility is that the palmitic acid molecules form a hydrocarbon liquid domain between the monolayers of the lamellar phase or the rods of the hexagonal phase. Moreover, these palmitic acid molecules have to exchange slowly with the fatty acid molecules with their carboxyl head groups situated at the interface of the two liquid crystalline phases. The formation of two well separated domains in the same phase seems unlikely, and the exchange rate between interior and interface should have at least resulted in a broadening of the peaks.

The existence of a reversed micellar phase could also give rise to an isotropic peak, which could be the case at temperatures above 70°C (the melting point of palmitic acid is 63°C) but probably not at lower temperatures. The third plausible interpretation of the central peak is therefore that it reflects the existence of a cubic liquid crystalline phase in the system. Two of the electron micrographs (Fig. 3A and B in the work by Abraham and Downing (1991)) also reveal the existence of a cubic liquid crystal. These micrographs were interpreted as reversed hexagonal phases, but their 'dotty' texture implies the existence of a cubic structure as well.

Our hypothesis is, therefore, that penetration enhancers like Azone and oleic acid have the possibility of inducing structural transitions of the $L_\alpha \rightarrow Q \rightarrow H_{II} \rightarrow L_2$ type in the extracellular lipid matrix of the stratum corneum. Since the compo-

sition of the intercellular skin lipid matrix varies both parallel and perpendicular to the skin surface, we have to assume that these structural changes occur locally. Several features support this hypothesis: (i) these kinds of transitions occur in biological lipid and living systems such as fat digestion and cell fusion (Larsson, 1989; Lindblom and Rilfors, 1989; Fontell, 1990, 1992; Seddon, 1990); (ii) it is a reversible low-enthalpy process; and (iii) the structures explain the increased permeation of lipophilic drugs (Q, $H_{II}$ and $L_2$), and hydrophilic drugs (Q).

In conclusion, investigations are needed in order to demonstrate the existence of cubic and other liquid crystals in skin lipid systems due to changes in temperature or addition of suitable penetration enhancers such as Azone. Investigation of the importance of such structural changes with respect to drug penetration is also required.

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