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# PENETRATION ENHANCER INCORPORATION IN BILAYERS

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## ABSTRACT

A preliminary study has been made of the interaction and location of the penetration enhancing agent dodecyl-1-aza-cycloheptane-2-one (Azone) in a system which models certain elements of the lipid arrangements found in the stratum corneum. The model host comprises a nonionic surfactant which forms associated multimolecular structures through hydration interactions with the aqueous component of the system.

Azone incorporation into the lamellar bilayer phase leads to a marked increase in ability of the phase to take up water. Small-angle X-ray diffraction and Deuterium NMR measurements indicate the degree of water and azone penetration into the bilayers to be inter-dependent. Similar behaviour is observed for oleyl alcohol but not by hydrotropic materials such as isopropyl alcohol or propylene glycol.

The mechanism by which azone promotes the penetration of hydrophilic drugs is indicated to be related to its ability to increase the water capacity of the lipid matrix of the stratum corneum.

## INTRODUCTION

A clear understanding of the unperturbed mechanism of drug transport through the stratum corneum does not exist. A major part of the uncertainty is related to the wide range of molecular characteristics associated with penetration enhancing substances. Such an understanding of the role of

penetration enhancing agents on a *molecular scale* would enable predicitive design of such molecules. This should radically reduce the time for screening for such agents and lead to efficient optimization of their use in topical drug delivery formulations. The aim of this preliminary study is to obtain information about the type of interactions found between commonly used penetration enhancers and lipid systems, since it is the lipid matrix of the stratum corneum (SC) which provides the main barrier function of the skin.

Progress has been made (1-4) in the understanding of the barrier function of the skin and the role of stratum corneum lipids. The molecular organization of thelipids in the intercellular matrix is essentially *lamellar* ie. a bimolecular arrangement of the molecules in infinite sheets separated by aqueous layers. Such bilayer structures are formed by phospholipids, soaps and surfactants and have been well-described (5,6) in basic physical chemical terms. The incorporation of molecules into the bilayer structure and the resultant perturbations have been studied spectroscopically (7-10) and from the phase behaviour (5, 11,12).

A similar development in the molecular description of penetration enhancement has not yet been achieved, although the criteria associated with good penetration enhancing capability have been discussed (13). A variety of different molecular types have been identified as enhancing agents ranging from simple solvents (13,14), surfactants (15,16) to various miscellaneous types (17-20). It was decided , therefore, to investigate the incorporation of Azone into bilayers produced by aqueous polar surfactant systems. This molecule has been shown (19-22) to promote the penetration of various types of molecules. The choice of bilayers of a polar surfactant was determined by the known variation of the composition of lipids in the epidermis: thus, the lipid composition varies with location through the epidermis (23-26) from a high phospholipid content near the basal layers to predominately single chained polar molecules and partially neutralized fatty acids in the surface layers of the stratum corneum. Particular reference is made in this study to the use of bilayers produced by nonionic surfactants. These are essentially polar molecules which form associated structures through solvation interactions between the aqueous component and their headgroups. It is in these respects that they may be thought of as simplified analogues to the type of lipids predominating in the outer SC layers.

## EXPERIMENTAL

### Methods

Samples of different composition were prepared by weight with vigorous mixing followed by centrifugation to remove air bubbles. This procedure was performed in sealed tubes several times until sample homogeneity was achieved as judged by observation through crossed polars. The samples were stored at 298K and the phase diagrams constructed from the equilibrium states.

Small-angle X-ray diffraction measurements were made using a Kiessig low-angle camera from Richard Siefert. Nickel-filtered Copper radiation was used and the reflections determined by a position sensitive detector (Tennelec, Model PSD-1100). The lamellar phase samples were sealed into thin glass capillary tubes (0.7 mm diameter) for the X-ray determinations.

$^2H$ NMR measurements from samples made with $^2H_2O$ (MSD Isotopes) were made at a resonant frequency of 13.71MHz at 298K using a multinuclear FT NMR spectrometer (JEOL FX90Q). Sweep widths in the range 1-5KHz were used with a pulse width of 21us.

### Materials

n-Dodecyl pentaoxyethylene glycol ether ($C_{12}EO_5$) from Nikko Ltd. (Japan) was used as received. Gas chromatographic analysis indicated the headgroup homogeneity to be better than 98%.

Azone was used as received as a gift from the Upjohn Company (Kalamazoo, Michigan) and was of research grade.

Oleyl alcohol (Sigma) and isopropyl alcohol (Aldrich) were of highest available purity and used without further purification.

## RESULTS & DISCUSSION

The lipid composition in the SC varies with depth in the epidermis becoming mainly those which are uncharged with single hydrophobes in the uppermost layers. Such lipids as the ceramides can have strong interactions with water in similarity to nonionic surfactants such as the alkyl polyoxyethylene glycol ethers. The nature of the interaction with water in the presence of azone is indicated from the behaviour of the lyotropic lamellar phase. A large increase in the ability

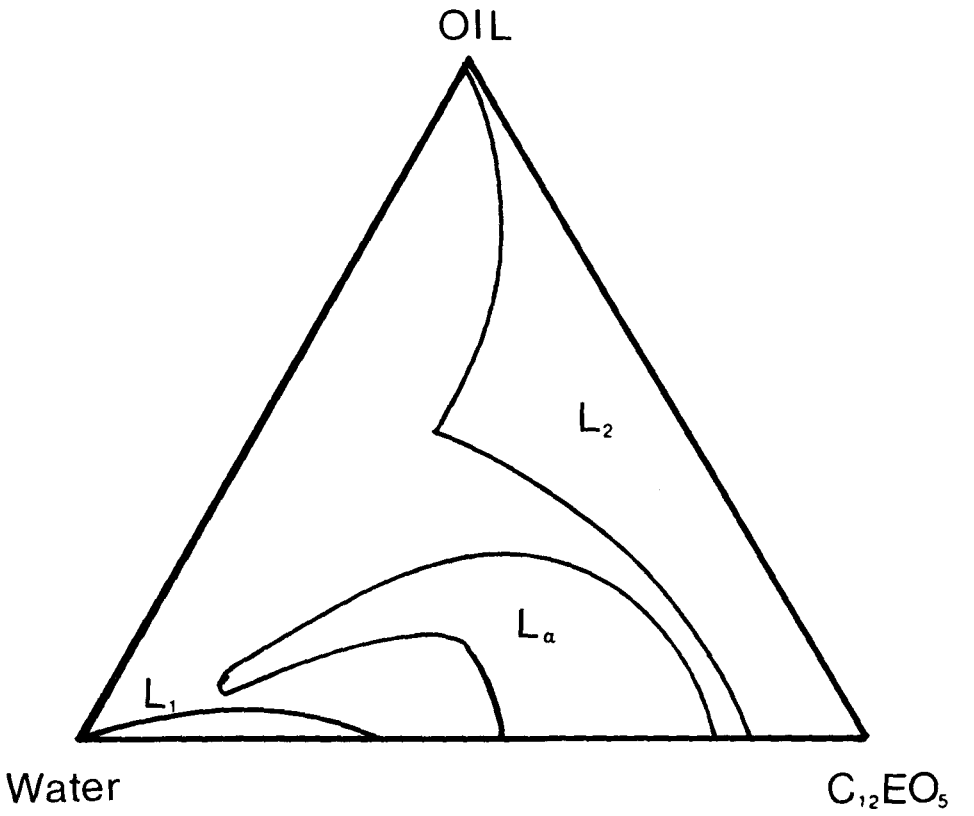


**Figure 1**: Phase diagram at 298K for the Water/Azone/$C_{12}EO_5$ system: $L_1$ - normal micelles; $L_2$ - W/O microemulsions; $L_\alpha$ - lamellar liquid crystal.

of the bilayer structure to accomodate water is seen (Figure 1) as well as the ability to incorporate a large amount of the enhancing agent. Here the lamellar phase incorporates up to 35% (w/w) of azone and the water capacity is increased to ca.85% (w/w) from 50% in the simple two-component system. This behaviour is also found for oleyl alcohol (Figure 2) and is similar to that previously observed (27) for the interaction of benzene with the lamellar phase of this system. The X-ray data for this system was interpreted in terms of effects of the solubilized benzene on the organization of the oxyethylene chains in the bilayer/water interfacial region making it more accessible to the aqueous phase. This contrasts the effect of molecules such as isopropyl alcohol and propylene glycol which displace bound headgroup water (28) to form a mixed solvation layer. In these cases, a decrease in the water capacity of the lamellar phase is observed (Figure 2) in the presence of the incorporating molecules.

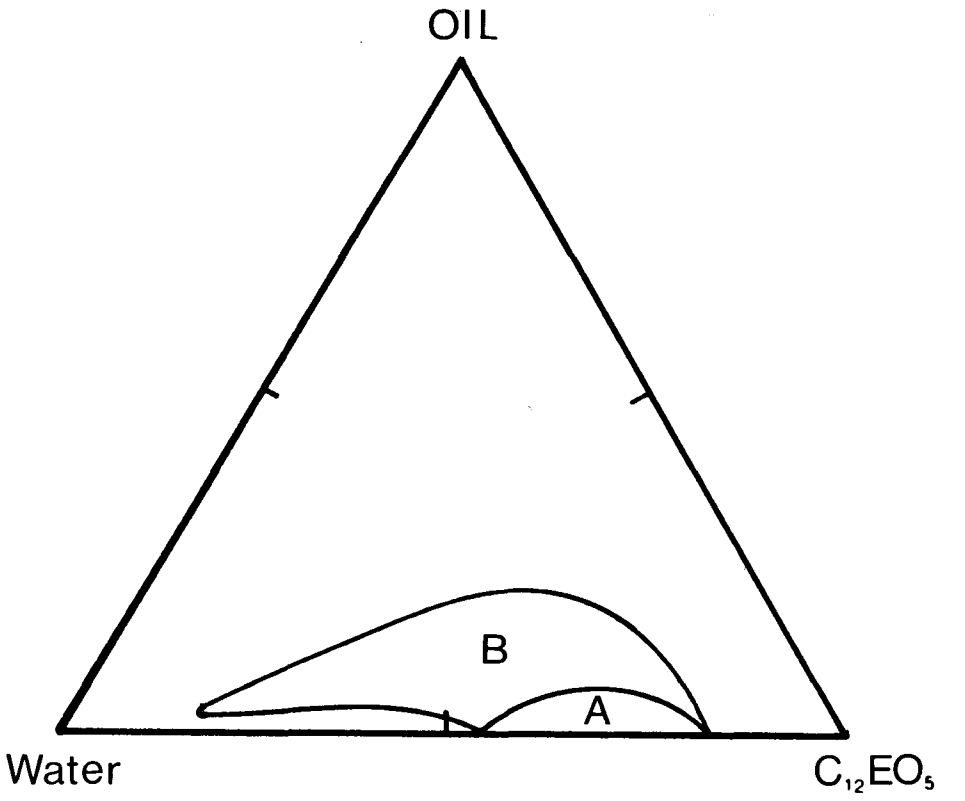


**Figure 2**: Phase diagrams at 298K for Water/Oil/$C_{12}EO_5$ systems:A - Isopropyl alcohol; B - Oleyl alcohol.

Small-angle X-ray (SAXS) reflections were obtained for the lamellar $C_{12}EO_5$/water/azone system and the d-spacings characteristic of the repeat distance of the bilayer-water units derived (Figures 3 and 4). The expected linear increase is found with increasing water content at fixed azone:surfactant ratios. The slopes of these plots decreased (Figure 4) with increasing azone:surfactant at fixed surfactant:water ratios after an initial increase compared with the azone-free system. The decreasing d-spacing values at larger azone contents occurred at different azone:surfactant ratios dependent upon the water:surfactant ratio of the system.. A tentative interpretation of this data is that the degree of penetration of azone between the surfactant molecules of the host bilayer increases up to a certain azone:surfactant ratio where the degree of

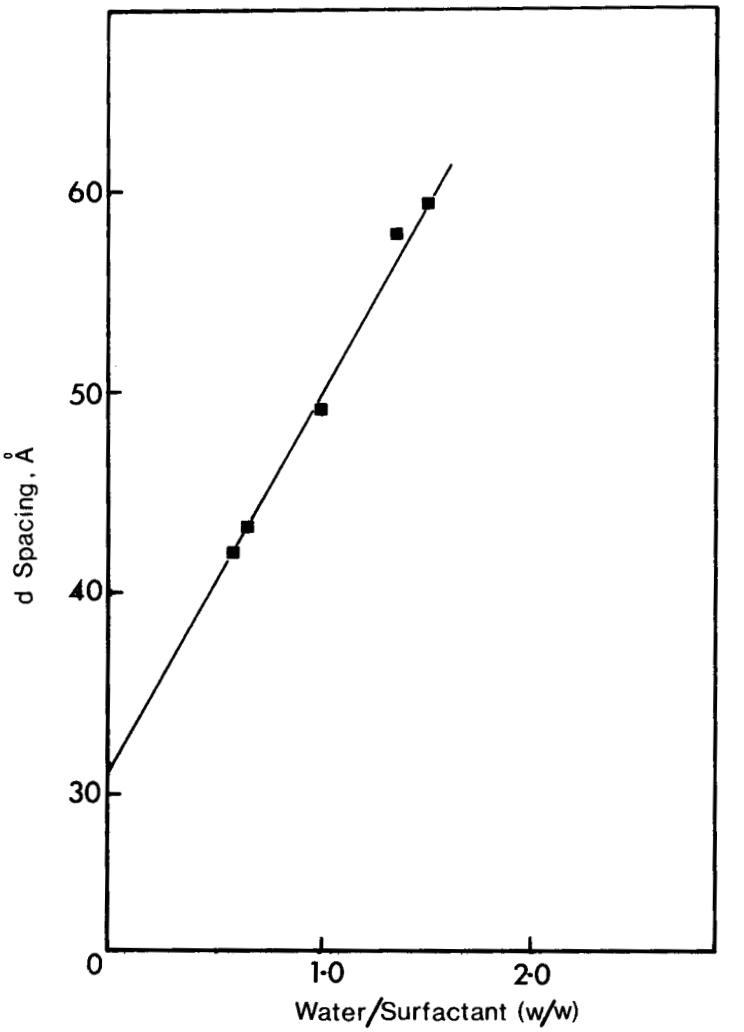


**Figure 3**: Effect of water content on the d-spacing of an Azone/$C_{12}EO_5$ system (35% w/w).

penetration is 100%. This is followed by either increased penetration of the water component into the bilayer or extraction of surfactant molecules into the interior of the bilayer. Both of these possibilities will lead to decreasing d-spacing values, but the SAXS data alone does not allow distinction between the possibilities to be made. However, further information relevant to this interpretation can be obtained from NMR data.

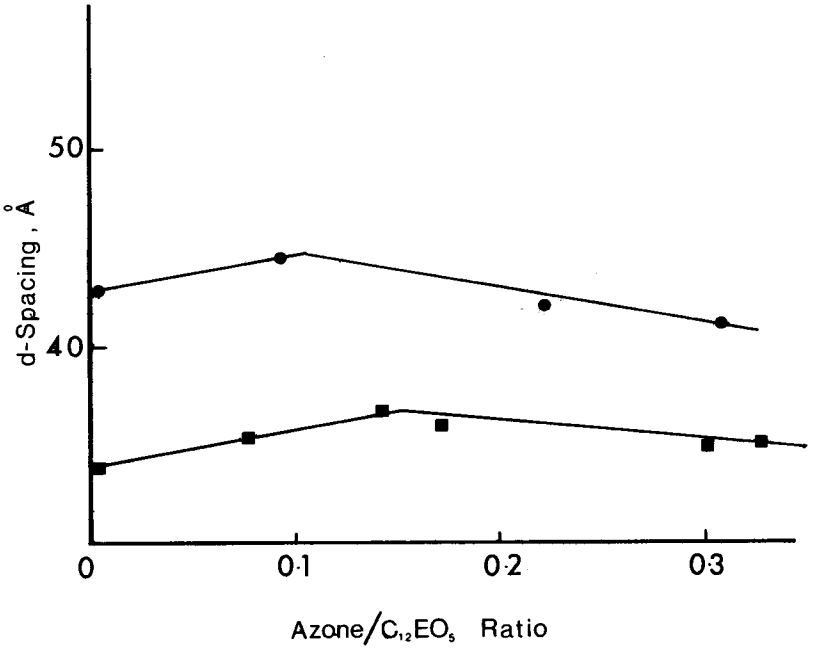


**Figure 4**: Effect of Azone addition on the d-spacing of Water/$C_{12}EO_5$ mixtures: ● - 53.9% and ■ - 70% (w/w) water content.

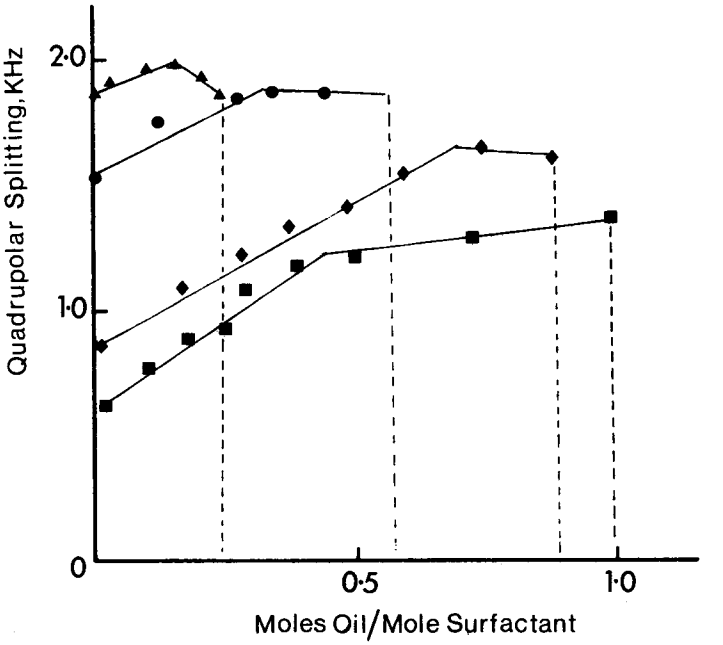


**Figure 5**: Effect of addition of Azone on the Quadrupolar Splitting of water in the lamellar phase of $C_{12}EO_5$: ▲ - 78:22 ; ● - 70:30 ; ◆ - 60:40; ■ -56:44 ratio of $C_{12}EO_5$:water.

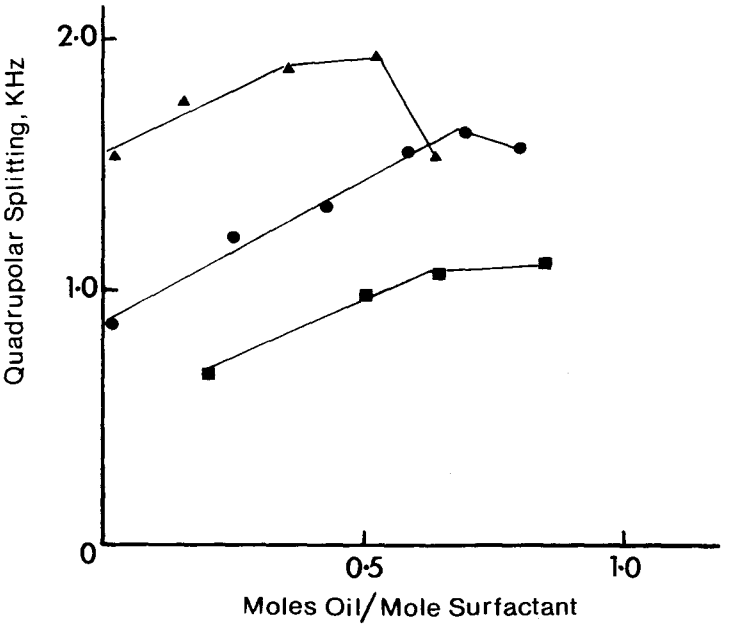


**Figure 6:** Effect of addition of Oleyl alcohol on the Quadrupolar splitting of water in the lamellar phase of $C_{12}EO_5$: ▲ - 70:30 ; ● - 60:40 ; ■ - 50:50 ratio of $C_{12}EO_5$:water.

The $^2H$ NMR spectrum of water in the unoriented lamellar dispersions studied has the characteristic "powder" (29) form with an associated quadrupolar splitting, $\Delta\upsilon$ . Measurements of $\Delta\upsilon$ were made on samples with fixed surfactant:water ratios with increasing amounts of added third component (ie. the total water content remaining constant). Addition of azone (Figure 5) and oleyl alcohol (Figure 6) produced increased splittings at all surfactant:water ratios studied; whereas addition of IPA (Figure 7) yielded an approximately linear decrease in the water splitting, Since the observed splitting has been shown (30,31) in this type of system to be described as the weighted average of the splittings from bound water molecules in fast exchange with essentially isotropic free water molecules, such a decrease can be understood in terms of a decreased amount of bound water. The results obtained here for IPA are in agreement with those found previously (28).

Comparison of the compositions at which the values of $\Delta\upsilon$ and the d-spacing stop increasing with added azone shows that they occur at the same composition.

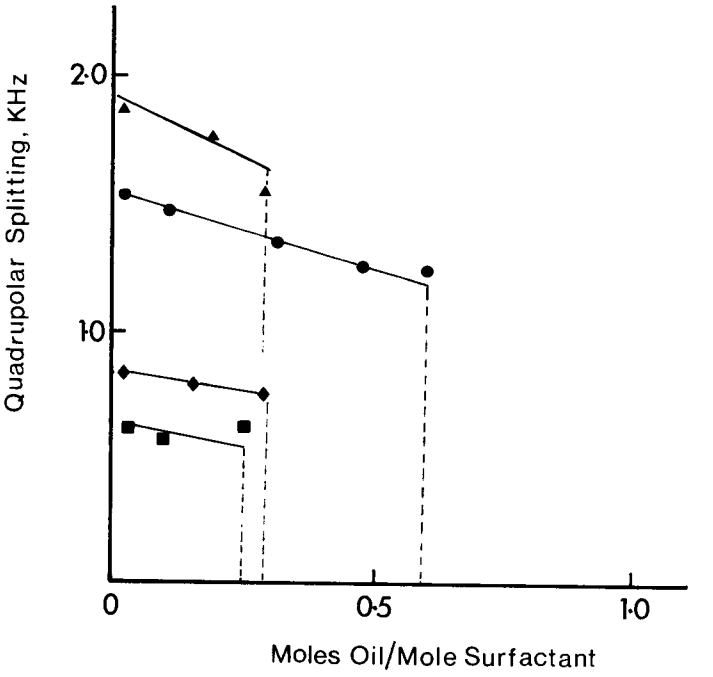


**Figure 7**: Effect of addition of Isopropyl alcohol on the Quadrupolar splitting of water in the lamellar phase of $C_{12}EO_5$: ▲ - 78:22 ; ● - 70:30 ; ⬮ - 60:40 ; ■ - 56:44 ratio of $C_{12}EO_5$:water.

This indicates that the degree of penetration of azone between the surfactant molecules, which increases from 79% to 100% at ca.10% added azone, leads to a decrease in the area occupied per hydrophobic chain at the oxyethylene/alkyl chain interface ie. the effective interface between the bilayer and the aqueous compartment for this type of system (40). It is the water molecules associated with the EO-groups adjoining this interface which contribute most (37,38) to the observed quadrupolar splitting from the bound water. Thus an increase in order in this region is as expected and is reflected in the increased $\Delta\nu$ values.

The opposite effect is manifested upon addition of IPA, which has been shown (28) to alter the degree of headgroup hydration. These molecules replace some of the water molecules from the hydration shell giving rise to a greater fraction of unbound water (with effectively zero splitting) contributing to the observed splitting , thereby reducing its value. This reduces the effective area/headgroup and increases the surface curvature in the system promoting the transition to reversed micellar structures (33).

The behaviour of oleyl alcohol (Figure 2 and 6) is the same as that of azone. It is an amphiphilic molecule which undoubtedly tends to intercalate between the surfactant molecules in the bilayers. Given the high degree of interfacial flexibility (32) found for bilayers of this type of surfactant, it would not be surprising to also find the alcoholic group located in the plane between the headgroup chain and the hydrophobe. This point is to be pursued in a further SAXS study. The incorporation of azone into the bilayer with increasing water retention capacity provides a greater potential resevoir for hydrophilic drug molecule absorption. Furthermore, the increased volume of the hydrophobic compartment of the bilayer produced by a certain amount of the azone loacting in the region of the surfactant methyl groups provides an enhanced potential resevoir for hydrophobic molecule solubilization.

## CONCLUSIONS

The NMR and SAXS data may be interpreted in terms of the distribution of the azone molecules within the bilayer structure and its consequences on the bilayer/water interaction. Enhancement of the ability of the lipid bilayer to incorporate water results in the cases of azone and oleyl alcohol incorporation. This would facilitate the penetration of hydrophilic drug molecules.

A distribution of azone or oleyl alcohol molecules exists between the bilayer center and intercalated between the lipid molecules providing a potential solution property in the hydrophobic compartment of the system. This would provide a potentially enhanced pathway for absorption of hydrophobic molecules. These features are not provided by molecules such as isopropyl alcohol which act primarily on the hydration layer of the lipids.

These types of molecular considerations provide an approach to understanding the mechanisms of penetration enhancer action and aiding, therefore, design of such systems in topical applications.

**ACKNOWLEDGEMENTS**

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