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# The influence of Span®20 on stratum corneum lipids in Langmuir monolayers: comparison with Azone®

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**Abstract**

Recently we have proved that Span 20 has the same enhancer effect as Azone on in vitro percutaneous penetration of lipophilic compounds (log $P_{oct}$ from 1.34 to 2.33). The purpose of this work is to study the interactions of Span 20 with stratum corneum lipids monolayers and to compare them with Azone. The surface pressure–area characteristics of Span 20 in mixed monolayers with different model lipids (ceramides, cholesterol, free fatty acids and two mixtures of ceramides + cholesterol, and ceramides + cholesterol + free fatty acids) in similar proportions to that which exists in human stratum corneum lipids were recorded as compression isotherms at 25°C. Azone was also investigated on monomolecular films of some of these lipids. The results indicate that the effect exerted upon lipid packing by the Span 20 correspond, as in the case of Azone, to increased fluidity within monolayers. To quantify and compare the effect of Span 20 and Azone, the compressibility of enhancer–lipid model mixed monolayers was calculated, and expressed as a function of mole fraction of enhancer present on the films. Statistical comparison of the results obtained from both enhancers shows that they are equally potent in their interaction with the lipid models assayed. These models, if restricted, seem to be good for predict the activity and potency of percutaneous enhancers on the fluididity of the lipidic structure of the stratum corneum. 

*Keywords:* Percutaneous enhancers; Azone; Span 20; Monolayer; Stratum corneum lipids

## 1. Introduction

The lipids found in the multilamellar lipid matrix of the stratum corneum act as the vital barrier in mammalian bodies (Elias, 1983; Menon et al., 1992). Because the skin provides an excellent barrier against the absorption of drugs, penetration enhancers can be used in order to improve both local topical therapy and transdermal delivery. The mechanism by which these enhancers operate is not fully understood but has usually been interpreted as an increase in fluidity, i.e. acyl chain disorder, which facilitates diffusion of molecules through the hydrocarbon region of the lipid bilay-

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ers in the intercorneocyte space (Knutson et al., 1985; Barry, 1987; Golden et al., 1987; Walters, 1989).

Nonionic surfactants are widely used in topical formulations. They can, however, have an effect on the permeability characteristics of several biological membranes, including the skin (Florence et al., 1984; French et al., 1993). We previously studied the effect of Span 20 (sorbitan monolaurate) on in vitro percutaneous absorption of compounds with different lipophilicities and compared it with a well known penetration enhancer, Azone (Llinares et al., 1998; López et al., 2000). Our results indicated that for the compounds with lipophilicity values ranging from log $P_{oct}$ 1.34 to 2.33, Span 20 has the same efficacy as Azone as an enhancer. The effect of Azone on the fluidity of the lipid fraction of the stratum corneum has been thoroughly studied (Barry, 1987; Lewis and Hadgraft, 1990; Schückler and Lee, 1991; Engblom, 1996; Harrison et al., 1996; Engblom et al., 1998) but there is little information on the lipophilic surfactant Span 20. Since the effects of Azone are considered to be partially due to its C12 alkyl chain, which gives it the capacity to insert among the acyl chain of lipids in the bilayers of the intercorneocyte space, and Span 20 has also a C12 alkyl chain (Fig. 1), we decided to ascertain whether both lipophilic compounds, Span 20 and Azone, interact in a similar way with intercellular stratum corneum lipids, and if so, to compare the magnitude of their respective effects.

To test this hypothesis we analysed the interactions of Span 20 with different models of stratum corneum lipids using mixed monolayers spread on an aqueous subphase in a Langmuir trough and compared the results with those obtained using Azone. Langmuir technique is generally recognised to be a suitable method for preparing monolayers of amphiphilic molecules (Lödgren and Pascher, 1977). Given the relative inaccessibility of the stratum corneum lipids to a direct experiment, the Langmuir technique offers a way to examine the interaction between percutaneous enhancers and the lipids of the stratum corneum (Lewis and Hadgraft, 1990; Schückler and Lee, 1991). Although lipid content and composition change gradually as a function of depth in the stratum corneum (Bonte et al., 1997; Wertz and van den Bergh, 1998) it is well known that the intercellular lipids that form lamellar bilayers consist mainly of ceramides, cholesterol and free fatty acids (Wertz and Dowing, 1989; Schürer and Elias, 1991; Wertz and van den Bergh, 1998). By varying the composition of the monolayers the role of each lipid class can be examined in detail. In our study, we used the major components of the stratum corneum lipids that are capable of forming monolayers, i.e. ceramides, cholesterol and free fatty acids. Several models of stratum corneum lipids were used: two of them were pre-

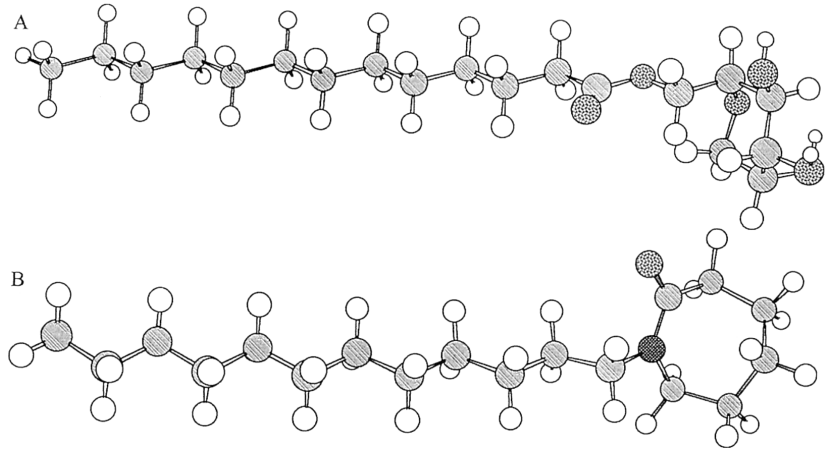


Fig. 1. Molecular structures of Span 20 (A) and Azone (B).

pared with pure lipids (ceramides or cholesterol) and the others were mixtures of lipids (free fatty acids, ceramides + cholesterol and ceramides + cholesterol + free fatty acids). The relative proportions of the components were calculated to imitate those existing in the human stratum corneum (Elias, 1990). Azone was also investigated in some of these lipids for comparative purposes.

The results obtained permits us to propose that the correlation between the mole fraction of enhancer incorporated into the monolayer and the compressibility values of the corresponding monomolecular film may reflect the efficacy of a molecule as a percutaneous enhancer.

## 2. Materials and methods

### 2.1. Materials

All of the lipids used in this study, i.e. ceramides (Sigma type III), cholesterol, and free fatty acids (oleic, palmitic and linoleic acids), were used as received from Sigma Chemical, Co. (Madrid, Spain) with a stated purity of $>99\%$.

Span 20 was also purchased from Sigma Chemical Co. (Madrid, Spain) and had a stated purity of $>99\%$. Azone was a gift from Whitby Research (USA). Both were used as received.

Chloroform (HPLC grade) was used to prepare the monolayers. The sub-phase consisted of double distilled water from an all glass apparatus, further purified by a Milli-Q Plus water de-ionising system (Millipore) that delivers a product with a resistivity of 18.2 MΩ cm.

### 2.2. Monolayer studies

Lipid solutions were prepared in chloroform, to a concentration of 1.4 mM. Solutions containing lipids and either Span 20 or Azone, were prepared with gradually increasing mole fractions of enhancer from 0 to 1. Surface pressure ($\Pi$) vs. molecular area ($A$) compression isotherms were recorded at 25 ($\pm 0.5$)°C using a $48 \times 21$ cm teflon Langmuir trough fitted with a motorised compression barrier (2011 Nima Technology, Coventry, UK) equipped with a pressure sensor and filter paper Wilhelmy plate capable of an accuracy of measurement of 0.1 mN/m. Cleanliness was scrupulously observed to avoid contamination of the monomolecular film. To prepare monolayers, 100 μl of chloroform solutions was spread and the solvent was allowed to evaporate for 10 min before beginning compression at a rate of 50 $cm^2$/min.

For each combination of enhancer–lipid monolayer, three sets of isotherms were obtained, and plots of $\Pi$ vs. $A$ were drawn using a PC computer. The steeply sloping linear section of each isotherm was extrapolated back to zero surface pressure to determine the area per molecule, $A$ ($Å^2$), of each monolayer. The slope of this linear extrapolation was used to calculate the compressibility, C (m/mN), of the monolayer (Gaines, 1966; Hann, 1990).

## 3. Results and discussion

As the monolayer is compressed on the water surface, it undergoes several phase transformations. These are almost analogous to the three-dimensional states or phases of matter: gases, liquids and solids. The phase changes may be readily identified by monitoring $\Pi$ as a function of $A$ occupied by the film as gaseous (G), liquid expanded (LE), liquid condensed (LC) and solid (S).

At first, Span 20 like Azone showed a monolayer of the LE type (Figs. 2–6). Moreover, they presented a very similar compressibility values (Span 20: $C = 0.019$ m/mN, and Azone: $C = 0.018$ m/mN, Table 1). The area occupied per molecule was higher for Azone (Span 20: $A = 56.68$ $Å^2$, and Azone: $A = 69.70$ $Å^2$, Table 1).

Since ceramides make up a large portion of the lipids of the stratum corneum (Elias, 1990), the first model assayed to investigate interactions with Span 20 was based on this kind of lipid. However, ceramides display a wide structural variation within the human stratum corneum and most of the varieties are not commercially available. In our study, a commerciallised mixture of ceramides (Sigma type III) produced enzymatically from

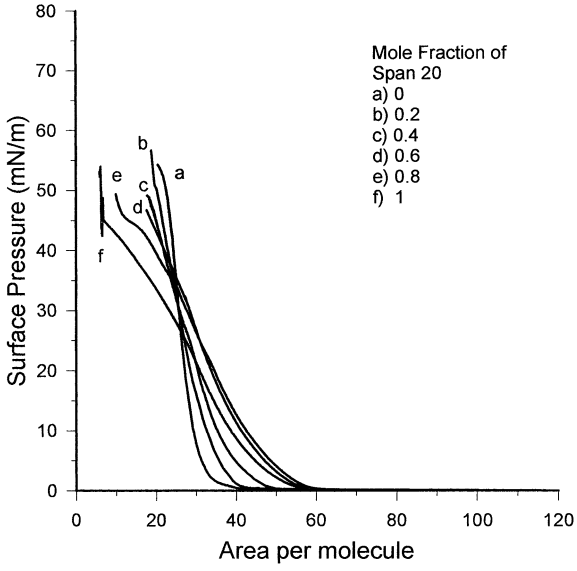


Fig. 2. $\Pi-A$ diagrams for ceramide film (a) and mixed ceramide films in the presence of increasing mole fractions of Span 20. Mole fraction ceramide:Span 20; (a) 0, (b) 0.2, (c) 0.4, (d) 0.6, (e) 0.8, (f) 1.

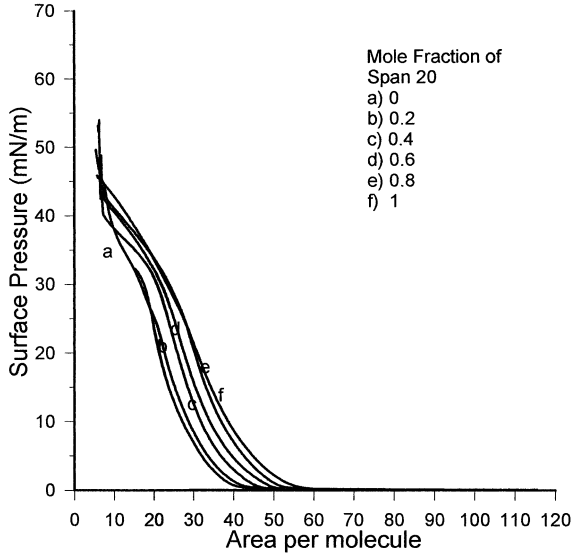


Fig. 4. $\Pi-A$ diagrams for fatty acids mixture (a) and mixed fatty acids films in the presence of increasing mole fractions of Span 20. Mole fraction fatty acids:Span 20; (a) 0, (b) 0.2, (c) 0.4, (d) 0.6, (e) 0.8, (f) 1.

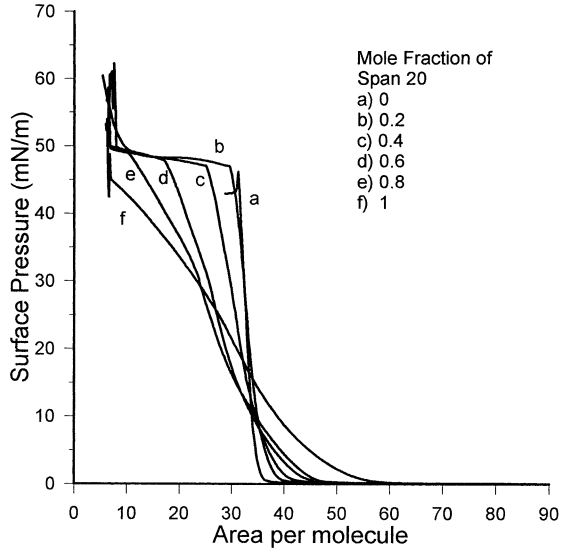


Fig. 3. $\Pi-A$ diagrams for cholesterol film (a) and mixed cholesterol films in the presence of increasing mole fractions of Span 20. Mole fraction cholesterol:Span 20; (a) 0, (b) 0.2, (c) 0.4, (d) 0.6, (e) 0.8, (f) 1.

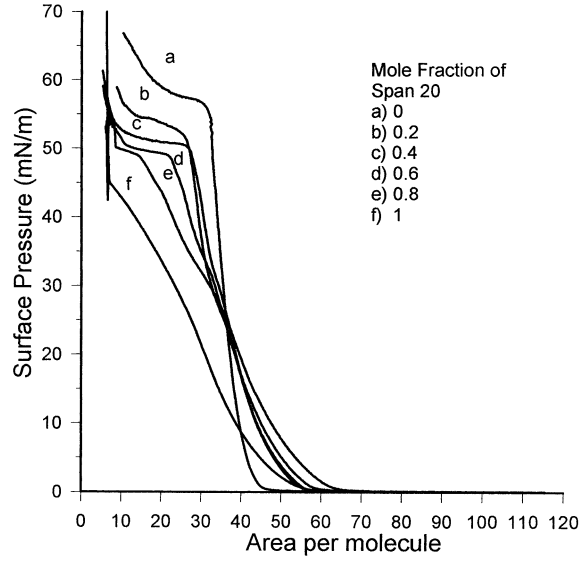


Fig. 5. $\Pi-A$ diagrams for mixed ceramides + cholesterol film (a) and mixed ceramides-cholesterol films in the presence of increasing mole fractions of Span 20. Mole fraction ceramides + cholesterol:Span 20; (a) 0, (b) 0.2, (c) 0.4, (d) 0.6, (e) 0.8, (f) 1.

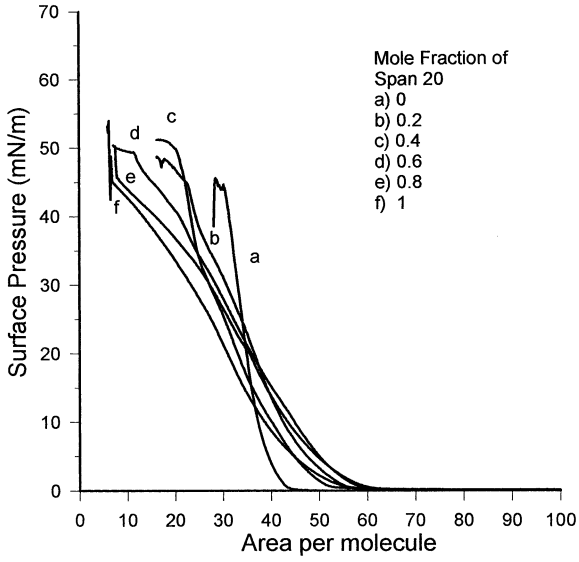


Fig. 6. $\Pi-A$ diagrams for mixed ceramides + cholesterol + fatty acids film (a) and mixed ceramides + cholesterol + fatty acids films in the presence of increasing mole fractions of Span 20. Mole fraction ceramides + cholesterol + fatty acids:Span 20; (a) 0, (b) 0.2, (c) 0.4, (d) 0.6, (e) 0.8, (f) 1.

bovine brain sphingomyelin (model I) was selected because it has been used with good results by others authors (Schückler and Lee, 1991; Parrot and Turner, 1993; ten Grotenhuis et al., 1996). The ceramide molecule contains primarily stearic (18:0) and nervonic (24:1) amide link fatty acids. Its head group is a sphingosine group, similar to that of ceramide 2 present in large quantities in stratum corneum. Fig. 2 shows $\Pi$ vs. $A$ isotherms of pure ceramide film and mixed Span 20-ceramide films in which the mole fraction of Span 20 increased from 0 to 1. Pure ceramide showed a typical LC film, with an area per molecule of 29.32 Å$^2$ and low compressibility ($C = 0.0056$ m/mN, Table 1). When Span 20 was incorporated into the ceramide monolayer it had a profound effect on the shape of the isotherm that resulted in a concentration-dependent transition from LC behaviour to LE. As it can be observed in Table 1, there was a substantial increase in area per molecule, from 29.32 to 49.57 Å$^2$. These changes in the shape and position of the isotherms were accompanied by changes in the compressibility of the monolayers containing the enhancer, which can be expressed by a linear relation between the mole fraction of enhancer in the film and the respective compressibility value, $r > 0.985$.

Cholesterol also forms a considerable proportion of the lipids of the stratum corneum. For this reason mixed monolayers of Span 20 with cholesterol as the lipid model II were prepared. Fig. 3 shows $\Pi$ vs. $A$ isotherms of cholesterol film and mixed Span 20-cholesterol films for mole fractions of Span 20 from 0 to 1. The behaviour of cholesterol monolayers has been studied a great deal (Ries et al., 1976). This lipid forms a highly condensed monolayer at the air–water interface, in the form of a typical solid film, with an area per molecule of 34.59 Å$^2$ and a compressibility ($C = 0.0021$ m/mN) that is even lower that of the pure ceramide film (Table 1). These values are consistent with those cited in the literature (Lance et al., 1996). The addition of increasing proportions of Span 20 modified the isotherms, which became more and more similar in shape to the isotherm of the enhancer; i.e. Span 20 produced a transition from S behaviour to LE. While increasing the mole fraction of Span 20 in mixed cholesterol monolayers produced small changes in the area occupied per molecule (from 34.59 to 39.23 Å$^2$, Table 1) the compressibility of the film increased substantially and there was also a good linear correlation between the mole fraction of Span 20 and the compressibility values of the mixed monolayers, $r > 0.985$.

The other major components of stratum corneum lipids are the free fatty acids. Aside from the small amount of cholesterol sulphate fatty acids are the only ionisable lipids in the stratum corneum and this may be important for the formation of lamellae. A decrease in the unsaturated/saturated chain ratio of free fatty acids with depth has been recently described (Bonte et al., 1997; Wertz and van den Bergh, 1998). In our study a mixture of three free fatty acids (oleic, palmitic and linoleic acids) were investigated, as lipid model III, in proportions of 47:38:15 (Elias, 1990).

The combined free fatty acids produced a LE film with high compressibility because of the presence of unsaturated acids in the mixture (Gaines, 1966). The area occupied per molecule in this film was 29.72 Å$^2$. The incorporation of Span 20 into fatty acids monolayers did not produce a substantial

change in the shape of the isotherms; all of the mixed monolayers were also LE films (Fig. 4). In fact, as can be observed in Table 1, the compresibility values were practically the same regardless of the proportion of Span 20 present in the film. However, there was an increment in the area occupied per molecule as the mole fraction of Span 20 increased (Table 1).

Although the information obtained with lipid models made up of only one kind of components is useful, several studies have been examined the utility of lipid models composed of a mixture of lipids more similar to the lipid composition of the stratum corneum (Schückler and Lee, 1991; ten Grotenhuis et al., 1996). X-ray diffraction studies on the phase behaviour of intact human, mouse and pig stratum corneum revealed that two lamellar phases with typical periodicities are present (White et al., 1988; Bouwstra et al., 1991, 1994, 1995). On this basis the phase behaviour of isolated skin lipids was also studied by the authors to examine the role that various kinds of stratum corneum lipids play in the proper organisation of lamellar bilayers in the intercorneocyte space. The results of this research indicate that the mixtures of ceramides + cholesterol and ceramides +

Table 1
Area per molecule and compressibility of monolayers formed with lipids models, and mixed monolayers formed with enhancers (Span 20 and Azone) and lipids models

| Lipids | Mole fraction of enhancer $X$ | Area per molecule (Å$^2$) | | Compressibility $C$ (m/mN) | |
|---|---|---|---|---|---|
| | | Span 20 | Azone | Span 20 | Azone |
| Ceramides | 0 | 29.32 | | 0.0056 | |
| | 0.2 | 35.04 | | 0.0088 | |
| | 0.4 | 39.66 | | 0.0121 | |
| | 0.6 | 45.79 | | 0.0136 | |
| | 0.8 | 49.57 | | 0.0148 | |
| | 1 | 56.68 | | 0.0190 | |
| Cholesterol | 0 | 34.59 | | 0.0021 | |
| | 0.2 | 36.08 | | 0.0035 | |
| | 0.4 | 36.76 | | 0.0065 | |
| | 0.6 | 37.19 | | 0.0106 | |
| | 0.8 | 39.23 | | 0.0136 | |
| | 1 | 56.68 | | 0.0190 | |
| Fatty acid mixture | 0 | 29.72 | | 0.0138 | |
| | 0.2 | 38.96 | | 0.0201 | |
| | 0.4 | 43.65 | | 0.0224 | |
| | 0.6 | 49.33 | | 0.0200 | |
| | 0.8 | 57.42 | | 0.0198 | |
| | 1 | 56.68 | | 0.0190 | |
| Ceramides + Cholesterol | 0 | 41.77 | 41.77 | 0.0045 | 0.0045 |
| | 0.2 | 38.96 | 41.60 | 0.0061 | 0.0082 |
| | 0.4 | 43.65 | 45.65 | 0.0075 | 0.0101 |
| | 0.6 | 49.33 | 64.22 | 0.0112 | 0.0162 |
| | 0.8 | 57.42 | 69.04 | 0.0151 | 0.0160 |
| | 1 | 56.68 | 69.70 | 0.0190 | 0.0180 |
| Ceramides + Cholesterol + Fatty acid mixture | 0 | 38.68 | 38.68 | 0.0047 | 0.0047 |
| | 0.2 | 33.97 | 36.62 | 0.0077 | 0.0090 |
| | 0.4 | 41.33 | 39.83 | 0.0102 | 0.0137 |
| | 0.6 | 51.44 | 54.08 | 0.0148 | 0.0150 |
| | 0.8 | 70.11 | 57.78 | 0.0194 | 0.0157 |
| | 1 | 56.68 | 69.70 | 0.0190 | 0.0180 |

cholesterol + long-chain free fatty acids closely mimic the lipid phase behaviour in intact stratum corneum (Bouwstra et al., 1996).

Many studies have tried to elucidate the mechanism by which laurocapram (Azone), a well-known lipophilic permeation enhancer, acts (Barry, 1987; Lewis and Hadgraft, 1990; Schückler and Lee, 1991; Allan, 1995; Engblom, 1996; Harrison et al., 1996; Engblom et al., 1998). In one of them the effects of the enhancer on the monolayers of simple lipids such as ceramides and cholesterol, a mixture of six fatty acids, and mixed monolayers of these lipids were examined (Schückler and Lee, 1991). The results obtained by the authors could be considered qualitatively similar to those we found for Span 20 on the aforementioned lipid models. Therefore, in order to obtain new experimental data that would permit us to quantify and compare the magnitude of the effects of both enhancers (Span 20 and Azone), mixtures of ceramides + cholesterol were prepared (model IV) in proportions similar to what is found in human stratum corneum (65:35) (Elias, 1990). Mixtures of ceramides + cholesterol + free fatty acids (model V) were also prepared (35:20:25) (Elias, 1990). Both Span 20 and Azone were tested on these mixtures of lipids.

Fig. 5 shows the effect of Span 20 when it was incorporated into monolayers prepared with the ceramides + cholesterol mixture (model IV). As can be observed the shape of the isotherms of this lipid model is very similar to that of pure ceramide, but the presence of cholesterol induces a decrease in compressibility; ($C = 0.0045$ m/mN) and an increase in the area occupied per molecule ($A = 41.77$ Å$^2$, Table 1). Mixed monolayers prepared with this lipid model and Span 20 show a behaviour similar to that observed on mixed monolayers of this enhancer with model I formed by simple ceramides. Therefore, it seems that the addition of Span 20 also induces a concentration-dependent transition from LC to LE behaviour. Eq. (1) shows the linear relationship obtained between the mole fraction of Span 20 ($X$) present in the monolayers of model IV and the respective compressibility values ($C$).

$$C = 0.015(0.001)X + 0.003(0.001) \quad (r > 0.981) \tag{1}$$

Similar effects were observed when Azone was incorporated into monolayers prepared with this lipid model (model IV). There is also a linear relationship between the mole fraction of Azone present and the compressibility of the film, as well as an increment in the area occupied per molecule. Eq. (2) shows the correlation between the mole fraction of Azone present in the monolayers of model IV and the respective compressibility values.

$$C = 0.014\ (0.002)\mathrm{X} + 0.005(0.001) \quad (r > 0.967) \tag{2}$$

In order to compare the magnitude of the effect of both enhancers on the compressibility of monolayers of this lipid model, statistical comparison of the two equation slopes using Student's $t$-test was done. This analysis shows that the slopes are not significantly different ($P < 0.05$).

Finally, Fig. 6 shows the effects of Span 20 when it was incorporated into monolayers prepared with the mixture of ceramides + cholesterol + free fatty acids (model V). As can be seen in Table 1 the monolayer free of enhancer has a compressibility similar to that of the ceramides + cholesterol mixture (model IV). The presence of fatty acids, then, does not alter the compressibility. When Span 20 and Azone were added, the area occupied per molecule and the compressibility of the monolayers increased. The relationships obtained between the mole fraction of enhancer present and the compressibility of the respective monolayers were linear in both cases; for Span 20 the equation is:

$$C = 0.016(0.002)X + 0.005(0.001) \quad (r > 0.979) \tag{3}$$

and for Azone:

$$C = 0.013\ (0.002)X + 0.006\ (0.001) \quad (r > 0.955) \tag{4}$$

The slopes of both regression lines are not significantly different ($P < 0.05$).

In conclusion, the results obtained indicate that Span 20 reduces the state of condensation

of monomolecular films prepared with ceramides, cholesterol, ceramides + cholesterol, and ceramides + cholesterol + free fatty acids. On the other hand, the incorporation of the Span 20 into fatty acid monolayers does not produce a substantial change in the shape of the isotherms. Therefore, it seems that Span 20, like Azone, modifies the state of condensation of ceramides and cholesterol, thus increasing fluidity within the monolayers.

Furthermore, statistical comparison of the results obtained with both enhancers, Span 20 and Azone, using models IV and V shows that their effects are not statistically different and that consequently they seem to have the same potency in their effect on monolayers compressibility. For this reason, Span 20 could be considered a percutaneous enhancer that has an effect similar to that of Azone on intercellular stratum corneum lipids — i.e. it increases the diffusivity of the molecules. At the same time, the models assayed, if restricted, seem to be good models for predicting the activity and potency of percutaneous enhancers on the fluidity of the lipid structure of the stratum corneum.

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