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Relating the concentration-dependent action of Azone and dodecyl-L-pyroglutamate on the structure of excised human stratum corneum to changes in drug diffusivity, partition coefficient and flux *

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Summary

The concentration-dependent effects of the two lipophilic permeation enhancers Azone and dodecyl-L-pyroglutamate (dlp) on excised human stratum corneum have been examined. Stratum corneum (SC) membranes were prepared having enhancer loadings within the range 0–30% w/w, and transport and structural experiments conducted with them. The permeation of the model drug diazepam through the enhancer-loaded SC membranes was measured, and drug diffusivities, partition coefficients and fluxes determined. For this calculation, a non-steady state model for permeation through an isotropic membrane valid for non-sink boundary conditions was used. With enhancer loadings up to approx. 12% w/w, the diffusivity increased whilst the partition coefficient remained constant. The diffusivity apparently decreased at higher loadings. Careful consideration of the results indicated that the application of the model at high enhancer loadings was questionable. By using differential scanning calorimetry, it was found that the skin lipid 'melting' peak progressively disappeared with increasing loading with enhancer. This occurred with approx. 12% Azone; for dlp, more than 25% was required. At high enhancer loadings, crystallized dodecyl-L-pyroglutamate was detected within the stratum corneum preparation. The concentration dependence of these changes in stratum corneum structure and barrier properties could be compared to those found previously with insoluble monolayers of skin lipids.

Introduction

The permeation enhancer Azone is thought to accumulate within the lipid fraction of the stratum corneum. Studies of excised stratum corneum using thermal analysis (Walters, 1989) and X-ray diffraction (Bouwstra et al., 1991) indicate that its presence causes a strong disruption of the lamellar, lipid bilayer structure. Recently, the use of isolated skin lipids as an experimental model for studying the nature of this disruption has attracted some attention. The combined skin lipids can be extracted in toto from epidermis using an appropriate solvent (e.g., Walker and Hadgraft, 1991), or the individual, synthesized skin lipid

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* Dedicated to Professor Herbert Stricker on the occasion of his 60th birthday.

components can be mixed together (e.g., Friberg and Osbourne, 1985). Studies of the interaction of Azone with insoluble monolayers of either selected amphiphiles (Lewis and Hadgraft, 1990) or isolated skin lipids (Schückler and Lee, 1991a) have been performed. The presence of enhancer was found to cause, inter alia, a strongly concentration-dependent reduction in the state of condensation of the skin lipid monolayers, corresponding to increased fluidity within this two-dimensional structure.

The relevance of these results depends very much on the extent to which the effects of Azone on lipid monolayers correspond to its action on intact stratum corneum. Important structural differences clearly exist between the two-dimensional monolayers and the three-dimensional lamellar bilayer structure of the lipids within the stratum corneum. By conducting the present study, we hoped to determine if the action of Azone on the barrier properties of intact stratum corneum showed a comparable concentration dependence with that seen for the skin lipid monolayers. To that end, a relation was sought between the concentration of enhancer within excised stratum corneum and the observed changes in the rate of drug permeation through this membrane. This has indeed been attempted before, by measuring drug permeability coefficients through excised mouse stratum corneum (Lambert et al., 1989). This combined coefficient cannot, however, be readily resolved into its component parts, i.e., diffusion and partitioning. We wished to assess the barrier properties of the stratum corneum in a somewhat more quantitative manner by the direct determination of diffusivities and partition coefficients. By distinguishing between these two system coefficients, we hoped to obtain an improved picture of the effects of enhancer on the lipid fraction of the stratum corneum. The calculation of diffusivities and partition coefficients necessitates, however, the use of a model that is, at its simplest, derived for permeation through an isotropic membrane. The validity of such a model for stratum corneum treated with enhancer clearly must be carefully considered.

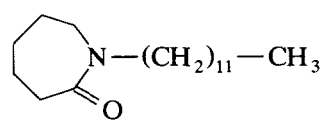
Two lipophilic permeation enhancers were examined in this study, Azone and dodecyl-L-pyroglutamate. The effects of both on insoluble monolayers of skin lipids are known from the literature (Schückler and Lee, 1991a,b). The structural changes in excised human stratum corneum treated with the enhancers were examined by thermal analysis. The barrier properties of the stratum corneum were characterized by measuring the rate of permeation of a lipophilic, non-dissociable model drug, diazepam. Viewed intuitively, this drug should diffuse as a single species through the lipid fraction of the stratum corneum and be sensitive to any structural changes induced there.

Materials and Methods

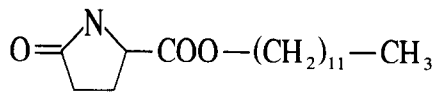
Preparation of stratum corneum membranes

Whole human skin was excised from the upper thighs of amputated legs within 24 h post operation. Sheets of stratum corneum plus attached viable epidermis (SCE) were first prepared from the whole skin by immersion in water at 60°C for 2 min (Kligmann and Christophers, 1963). Isolated stratum corneum (SC) was then obtained from this SCE by digesting the viable epidermis with a 0.001% trypsin (Sigma, Deisenhofen, Germany) solution in 0.5% sodium bicarbonate. The SC sheets were dried at room temperature overnight and subsequently stored at 4°C in a desiccator at 25% RH, according to the method of Swarbrick et al. (1982). The skin used in this study was obtained from two donors: a white male of age 55 years for the experiments with Azone, and a white male of 65 years for those with dodecyl-L-pyroglutamate.

Before experimentation, the SC was treated with either Azone (Nelson Research, Irvine, CA, U.S.A.) or dodecyl-L-pyroglutamate (dlp; Teijin, Tokyo, Japan):



Azone

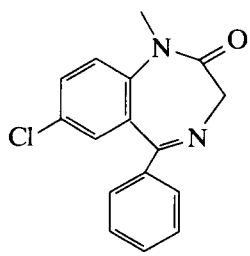


dlp

Azone is a liquid at skin surface temperature, and is insoluble in water. Dlp is a solid having a measured melting point of 45°C, and has very slight aqueous solubility. As dlp could clearly not be applied to SC using a spray technique (Lambert et al., 1989), solutions of each enhancer were prepared in methanol (pA grade, Baker, Deventer, The Netherlands), the particular concentrations being selected according to the required loading of the SC with enhancer. 30–50 μl of an enhancer solution was pipetted over the outer surface of an approx. 2 cm^2 piece of SC of known weight, which was subsequently stored overnight at room temperature. In this manner SC with fairly exact enhancer loadings in the range 0–30% w/w could be prepared. Some SC membranes were also treated with 50 μl of pure methanol, to determine the influence of this solvent alone. Each piece of SC was then used for either a permeation or a thermal analysis experiment. In the former case, the thickness of the SC membrane was measured at 20 positions using an Elcometer (Elcometer Instruments, Manchester, U.K.).

Measurement of drug permeation

The model drug diazepam (Syncopharm, Hamburg, Germany) was used to characterize the barrier properties of the SC:



Diazepam

Its permeation rate through SC membranes was determined at 35 ± 0.2°C using an all-glass, horizontal-type diffusion cell of standard design (Göpferich and Lee, 1991a). A pH 5 phosphate buffer was used as donor and acceptor medium, corresponding thereby to the pH existing within the SC (Friberg and Osbourne, 1985). Diazepam had a solubility of approx. 35 μg/ml in this buffer. The diffusion cells were operated as described before (Swarbrick et al., 1984), with samples of the acceptor medium being drawn at regular time intervals up to 50 h, and the diazepam concentration determined by HPLC analysis. At the end of each experiment, the SC was removed from the diffusion cell and an accurate enhancer loading determined by reflux extraction with methanol. Each experiment was performed in quadruplicate.

The result of each permeation experiment was expressed in the standard fashion as a permeation profile of $m_a(t)/m_0$ vs time, where $m_a(t)$ is the mass of drug in the acceptor medium at time t and m_0 the mass of drug in the donor medium at $t = 0$. Each profile was then characterized quantitatively by using a diffusional model for membrane permeation derived from the linear form of Fick's Second Law and illustrated in a preceding article (Göpferich and Lee, 1991a). Of relevance to the present study are the assumptions that the SC can be represented as a plane, isotropic membrane, and that drug partitioning occurs spontaneously at all boundaries. By virtue of the low solubility of diazepam in the acceptor medium it was necessary to employ non-sink boundary conditions. As the analytical solution to this model (Spacek and Kubin, 1967) is cumbersome for practical use, the problem was solved numerically using an implicit finite-difference method (Crank and Nicolson, 1947). The effective drug diffusivity within the SC (D) and its partition coefficient between SC and buffer (K) were determined by finding the theoretical numerical solution that best fitted each experimental profile of $m_a(t)/m_0$ vs t. An improved simplex method was used (Nelder and Mead, 1965), as described before (Göpferich and Lee, 1991a). The maximum flux of the drug, J, was determined at the point of inflexion of each profile.

Thermal analysis measurements

The SC membranes were subjected to differential scanning calorimetry (DSC) using a Mettler

model TC10A instrument (Greifensee, Switzerland). The temperature range 280–405 K was scanned at a speed of 10 K/min. Each sample was scanned twice, the second scan showing more intensive lipid phase transitions, as noted previously by Bouwstra et al. (1989).

Results and Discussion

The individual permeation profiles shown in Fig. 1 are typical examples from an untreated SC membrane (lower curve) and an SC membrane treated only with methanol (upper curve). The coordinates represent the experimentally determined values of $m_a(t)/m_0$, and the curves drawn through them are the best-fitting theoretical solutions to Fick's Second Law for the non-sink model. It is known that values for the diffusivity and partition coefficient for an intact, isotropic membrane can be independently calculated with precision from the curve fitting procedure to this model (Göpferich and Lee, 1991b). This yields here for the untreated SC a diffusivity of $5.39 \times 10^{-12} \pm 1.66 \times 10^{-12}$ cm^2 s^{-1} and a partition coefficient of 764 ± 90.2 ($n = 4$). The former lies within the range typical for drug molecules of this size (Swarbrick et al., 1984). The scatter of this result arises primarily from error in the measurement of the thickness of the SC, and also from natural

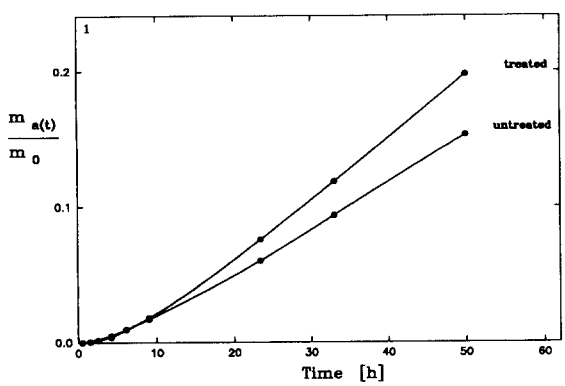


Fig. 1. Typical permeation profiles for an untreated SC membrane (lower curve) and an SC membrane treated with methanol (upper curve). The coordinates are the experimentally determined values of $m_a(t)/m_0$, and the curves the best-fitting theoretical solution.

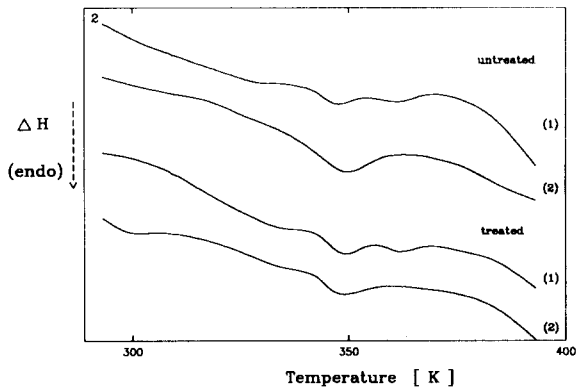


Fig. 2. DSC thermograms for first (1) and second (2) scans of untreated SC membrane and SC membrane treated with methanol.

variation in the anisotropic structure of this membrane. The large partition coefficient clearly reflects the lipophilic nature of the drug. These manifestly plausible values for D and K thus concur with the visible closeness of the fitted theoretical profile in Fig. 1 to the experimental coordinates obtained for the untreated SC.

The SC membrane treated with 50 μl of methanol yields a smaller fitted diffusivity of $2.00 \times 10^{-12} \pm 0.54 \times 10^{-12}$ cm^2 s^{-1} ($n = 4$), the scatter being of the same order as that seen for the untreated SC. Since this treatment did not affect the measured SC thickness, it evidently produced some other alteration. This conclusion is supported by the larger fitted partition coefficient of 3033 ± 76 ($n = 4$) for the SC treated with methanol, which is a consequence of the steeper slope of the permeation profile in Fig. 1. The DSC thermograms shown in Fig. 2 for the first and second scans of untreated and treated SC give, however, no indication of any alteration of substance in the thermal behaviour of the SC. The first scan for the untreated SC shows endothermic peaks at approx. 75 and 85°C, described in the literature as arising from lipid 'melting' (Bouwstra et al., 1989). These become combined in the second scan into a single broad endothermic transition at approx. 75°C. Treatment with methanol does not alter the temperature at which this transition occurs, as would be expected if the solvent had been taken up to any

extent within the SC. The enthalpy of this transition on the second scan is, however, reduced by about one third compared with untreated SC, indicating some slight change of undefined nature in the SC. As a qualitative observation, the fitted theoretical profile given in Fig. 1 still passes closely through the experimental coordinates obtained with the treated SC.

The two enhancers produced concentration-dependent alterations in the permeation profiles for SC within the range up to approx. 12% loading. Fig. 3a and b illustrates how the typical permeation profiles change with increasing Azone or dlp loading within this range. The profiles assume a very pronounced sigmoidal shape. Yet the theoretical curves of best fit pass closely through the experimental coordinates, indicating that the curvature can be ascribed to the non-sink boundary conditions (Jenkins et al., 1970) and the substantial amounts permeating up to the end of the experiment ($m_a(50\ \text{h})/m_0 = 0.4$). Two important changes in the shape of the profiles can be observed with increasing enhancer concentration. Firstly, the 'lag-time' decreases; this results in a continual increase in the fitted diffusivities up to enhancer loadings of approx. 12% (shown as D/D_0 in Fig. 4, with D_0 being diffusivity for SC treated only with methanol). Azone clearly has a much stronger influence on D than does dlp, with diffusivity increasing more sharply with Azone concentration than with dlp concentration within this range. Once the loading with either

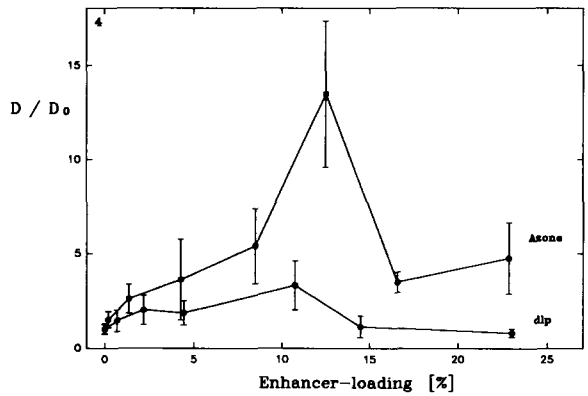


Fig. 4. Influence of enhancer loading on fitted diffusivity, D/D_0 (D, diffusivity of SC membrane treated with enhancer; D_0, diffusivity of SC membrane treated only with methanol). Mean ± SD ($n = 4$).

enhancer exceeds approx. 12%, however, the fitted diffusivity decreases. A decrease in diffusivity could arise from either an increased path length (as a result of a volume expansion of the SC after the uptake of large amounts of enhancer and the resulting disrupted order of the lipids) or a greater resistance to drug diffusion within the SC (as a result of the formation of solid lipid component (Ongpipattanakul et al., 1991) within the disrupted lipid structure). It should be borne in mind, however, that there exist two sources of uncertainty about this result for high enhancer loadings. Firstly, according to the model for membrane permeation, the diffusivity determines

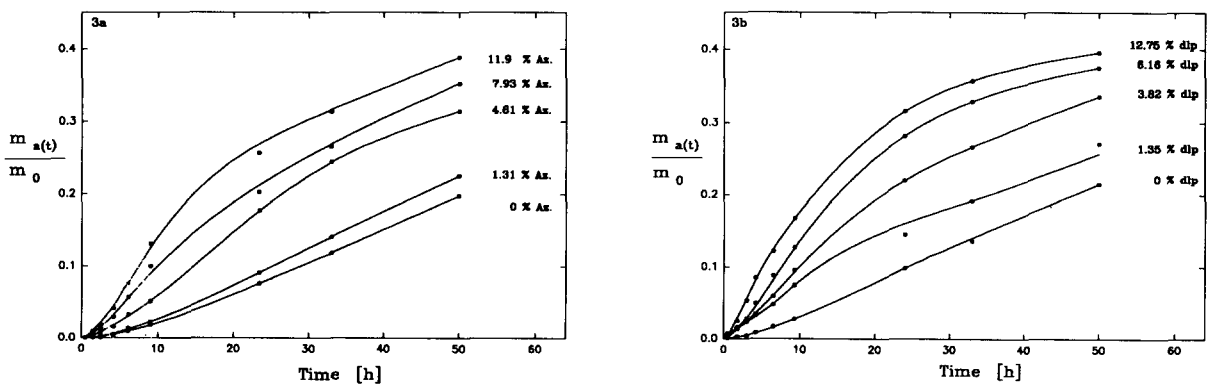


Fig. 3. Typical permeation profiles for diazepam through excised human SC. (a) Influence of Azone loading; (b) influence of dlp loading.

both the lag time and the slope of the permeation profile for known membrane thickness, whereas the partition coefficient determines only the slope (for spontaneous partitioning). If the lag time becomes very small at high enhancer loadings, the permeation profile will be almost linear at early times, and the model will not be able to fit D and K unequivocally and independently. Secondly, the model is derived for an isotropic membrane. As such, it is questionable whether it can still be used to calculate diffusivity at enhancer loadings where the structure of the SC is most likely strongly disrupted. This misgiving is corroborated by the finding that at very high enhancer loadings (above 25%) it was no longer possible to obtain consistent fits of the model to the experimental results; the sum of squares did not converge to a minimum.

The second change in profile shape to be observed in Fig. 3a and b with greater enhancer loading up to approx. 12% is an increase in the permeation rate, $dm_a(t)/dt$, during the first 20 h. This would indeed be expected as a result of the increasing diffusivities seen in Fig. 4. At later times, however, the profiles run more-or-less parallel to one another as they flatten off in compliance to the non-sink conditions. Accordingly, the fitted values for the partition coefficients, after an initial slight decrease at low loadings, show no unequivocal dependence on enhancer concentration (Fig. 5). The value for dlp, however, shows a sudden increase after approx. 12% loading.

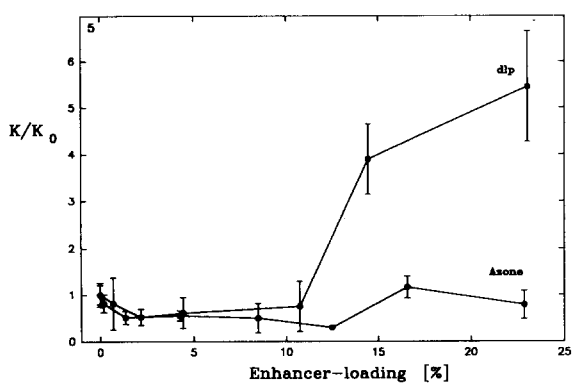


Fig. 5. Influence of enhancer loading on fitted partition coefficient, K/K_0 (K, partition coefficient of SC membrane treated with enhancer; K_0, partition coefficient of SC membrane treated only with methanol). Mean ± SD ($n = 4$).

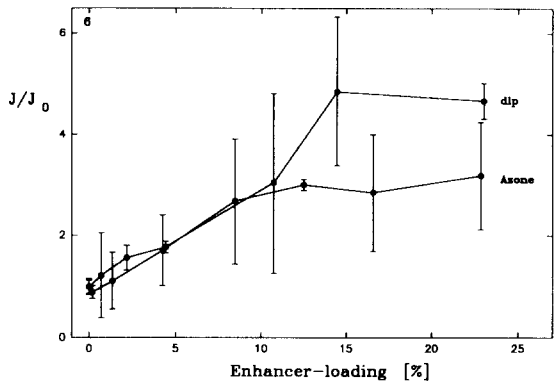


Fig. 6. Influence of enhancer loading on flux, J/J_0 (J, flux through SC membrane treated with enhancer; J_0, flux through SC membrane treated only with methanol). Mean ± SD ($n = 4$).

To clarify these complex changes in D and K, it is helpful to consider the behaviour of the measured flux, $J = dm_a(t)/Adt$. As this parameter is equal numerically to the maximum slope of the permeation profile for unit SC membrane area, its calculation is independent of the diffusional model. Fig. 6 shows how the flux becomes larger as the loading with either enhancer increases up to approx. 12%. This observation is easily explained by considering the increasing diffusivities within this concentration range (Fig. 4) and the unchanging partition coefficients (Fig. 5); according to Fick's First Law the flux must increase. We find, therefore, an unequivocal agreement between the changes in diffusivity, partition coefficient, and flux occurring for enhancer loadings up to approx. 12%. The enhancers are incorporated within the SC in a concentration dependent manner within this range, causing a progressive alteration in barrier properties. The diffusional model can apparently be used successfully to evaluate these changes in the barrier properties of the SC. Anomalies arise at higher enhancer loadings. With more than 12% Azone, for example, the flux reaches a plateau value and remains constant despite the aforementioned decrease in fitted diffusivity (Fig. 4) with constant partition coefficient (Fig. 5). This contradicts Fick's First Law, all other things being equal, and

is in contrast to the findings with Azone loadings below 12%. Similarly, with dlp loadings above approx. 12%, the decreasing fitted diffusivity (Fig. 4) does not agree with the observed sudden increase in flux (Fig. 6). The latter observation results in the simultaneous jump in the value of the fitted partition coefficient at this loading (cf. Figs 5 and 6). These results corroborate the misgiving mentioned above, that the diffusional model can no longer be applied to describe drug permeation at enhancer loadings above approx. 12%.

These findings are in agreement with previous observations that drug permeability coefficients $(dm_a(t)/Ac_0dt)$ increase up to a limiting value for SC treated with Azone (Lambert et al., 1989). We now address the relationship between these changes in barrier properties and any concentration-dependent alterations in the structure of the SC. Fig. 7a and b shows a series of DSC thermograms obtained for SC treated with Azone or dlp. These are all second scans, to show the transitions most intensely. In both cases, the temperature of the lipid melting transition decreases with increasing enhancer loading (see Fig. 8). Above

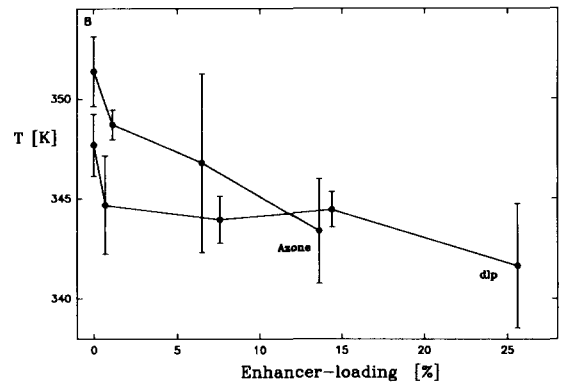


Fig. 8. Temperature of the DSC lipid melting transition for SC membranes having increasing enhancer loadings.

approx. 15% Azone loading the peak is no longer detectable. With dlp, however, the effect is weaker, with the peak still being faintly identifiable at 25% loading. Reduction in this transition temperature has been assigned to increasing disruption of the lamellar bilayer structure of the lipid fraction of the SC (Bouwstra et al., 1989). With Azone the effect is, therefore, clear: loading the SC up to approx. 12% causes a progressive,

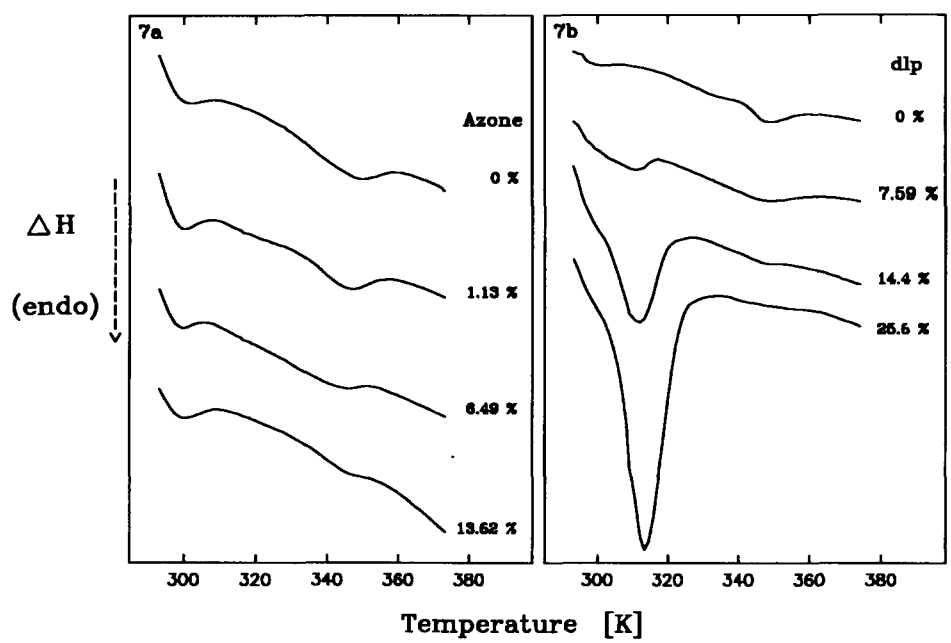


Fig. 7. DSC-thermograms obtained for SC membranes having increasing enhancer loadings. (a) Azone; (b) dlp.

simultaneous disruption in SC structure and increase in drug diffusivity. We conclude that the latter effect is a direct result of the former. The behaviour of dlp is complicated by its existence as a solid at skin surface temperature. Thus, at dlp loadings above approx. 6%, a further endothermic transition can be seen at approx. 37°C (Fig. 7b). Although this temperature lies some 8°C lower than the known melting point of dlp, this transition certainly represents melting of crystalline dlp. Indeed, microscopical examination revealed crystalline dlp present on the surface of the SC. This peak becomes very large at dlp loadings above approx. 10% (see Fig. 7b), indicating that substantial proportions of the applied dlp did not penetrate into the SC. The sharp increase in the fitted partition coefficient at this dlp loading (cf Fig. 5) may, therefore, be caused by the presence of crystalline dlp on the outer surface of the SC. The amount of dlp that did penetrate into the SC is evidently sufficient to disrupt the SC lipids, although at least 25% dlp loading is required before the lipid melting transition effectively disappears (cf. Fig. 7b). The weaker influence of dlp on drug diffusivity compared to Azone (Fig. 4) may partly be due to this smaller uptake of dlp at equivalent loadings.

Increasing concentrations of these two enhancers are already known to produce a progressive reduction in the state of condensation of two-dimensional monolayer films of isolated skin lipids. It has been found that mole fractions of enhancer as low as 0.02 clearly reduced film condensation, with the effects becoming very pronounced up to a mole fraction of 0.25 (Schückler and Lee, 1991a,b). In the present work, a concentration-dependent influence of the two enhancers is also found for intact SC. Thus, a progressive reduction in the order of the lipid structure of the SC is observed with increasing concentrations of Azone or dlp within the range 1–12%/25% w/w, and is accompanied by an increase in drug diffusivity within the SC. This range corresponds to mole fractions of either Azone or dlp within the skin lipids of 0.1–0.4/0.5, and is, therefore, somewhat higher than that observed with the monolayer experiments. For this calculation the assumptions were made that the lipid fraction comprises 10% w/w of the SC; that its composition is that given by Elias (1990); and that it contains 32% w/w water. The two- and three-dimensional systems are therefore, certainly qualitatively comparable, but show quantitative differences.

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References

Bouwstra, J., Peschier, L., Brussee, J. and Bodde, H., Effect of *n*-alkyl azocycloheptan-2-ones including Azone on the thermal behaviour of human stratum corneum. *Int. J. Pharm.*, 52 (1989) 47–54.

Bouwstra, J., De Vries, M., Gooris, G., Bras, W., Brussee, J. and Porrec, M., Thermodynamic and structural aspects of the skin barrier. *J. Controlled Release*, 15 (1991) 209–220.

Crank, J. and Nicolson, P., A practical method for numerical evaluation of solutions of partial differential equations of the heat conduction type. *Proc. Camb. Phil. Soc.*, 43 (1947) 50–67.

Elias, P., The importance of epidermal lipids for the stratum corneum barrier. In Osbourne, D. and Amann, A. (Eds), *Topical Drug Delivery Formulations*, Dekker, New York, 1990, pp. 13–28.

Friberg, S. and Osbourne, D., Small-angle X-ray diffraction patterns of stratum corneum and a model structure for its lipids. *J. Disp. Sci. Technol.*, 6 (1985) 485–495.

Göpferich, A. and Lee, G., Measurement of drug diffusivity in stratum corneum membranes and a polyacrylate matrix. *Int. J. Pharm.*, 70 (1991a) 245–253.

Göpferich, A. and Lee, G., A study of multi-dimensional drug diffusion in matrices and membranes. *J. Biopharm. Sci.*, 2 (1991b) 45–64.

Jenkins, J., Nelson, P. and Spirer, L., Calculation of the transient diffusion of a gas through a solid membrane into a finite outflow volume. *Trans. Faraday Soc.*, 66 (1970) 1391–1401.

Kligman, A. and Christophers, E., Preparation of isolated sheets of human stratum corneum. *Arch. Dermatol.*, 88 (1963) 702–710.

Lambert, W., Higuchi, W., Knutson, K. and Krill, S., Dose-dependent enhancement effects of Azone on skin permeability. *Pharm. Res.*, 6 (1989) 798–803.

Lewis, D. and Hadgraft, J., Mixed monolayers of dipalmitylphosphatidylcholine with Azone or oleic acid at the air/water interface. *Int. J. Pharm.*, 65 (1990) 211–218.

Nelder J. and Mead, R., A simplex method for function minimisation. *Comput. J.*, 7 (1965) 308–313.

Ongpipattanakul, B., Burnette, R., Potts, R., and Francoeur, M., Evidence that oleic acid exists as a separate phase within stratum corneum lipids. *Pharm. Res.*, 8 (1991) 350–354.

Schückler, F. and Lee, G. The influence of Azone on monomolecular films of some stratum corneum lipids. *Int. J. Pharm.*, 70 (1991a) 173–186.

Schückler, F. and Lee, G., Einfluss von Dodecyl-L-pyroglutamat auf ein zweidimensionales Modell für die Lipide im Stratum Corneum. *Pharm. Ztg-Wiss.*, 5/6 (1991b) 219–222.

Spacek, P. and Kubin, M., Diffusion in gels. *J. Polym. Sci.*, C16 (1967) 705–714.

Swarbrick, J., Brom, J. and Lee, G., Drug permeation through human skin. I: Effect of storage conditions of skin. *J. Invest. Dermatol.*, 78 (1982) 63–66.

Swarbrick, J., Lee, G., Brom, J. and Gensmantel, N., Drug permeation through human skin. II: Permeability of ionizable compounds. *J. Pharm. Sci.*, 73 (1984) 1352–1355.

Walker, M. and Hadgraft, J., Oleic acid – a membrane 'fluidizer' or fluid within a membrane? *Int. J. Pharm.*, 71 (1991) R1–R4.

Walters, K., Penetration enhancers and their use in transdermal therapeutic systems. In Hadgraft, J. and Guy, R. (Eds), *Transdermal Drug Delivery*, Dekker, New York, 1989, pp. 197–246.