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Measuring the Uptake of Azone into Excised Human Stratum Corneum from Thin Polymer Films

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Abstract—The extent of uptake of Azone into excised human stratum corneum from thin polymer films has been measured using a sandwich model. With poly(dimethyl)siloxane, an Azone loading of the stratum corneum of 6–7% w/w was achieved. With Eudragit NE30D, a loading of less than 1% was reached. The extent of uptake into the stratum corneum and acceptor layers of the sandwich model depended on the Azone content of the films. The resulting enhanced uptake and flux of diazepam through the stratum corneum membrane into a polymeric acceptor layer corresponded closely to the amounts of Azone taken up and their known enhancing effects. The uptake of diazepam from the poly(dimethyl)siloxane films was ten times larger than that from Eudragit NE30D films. The extent of uptake is, therefore, controlled by the rate of release from the polymer and partitioning at the interface.

The lipophilic permeation enhancer Azone can be applied topically from ointments, creams or gel formulations to increase the rate of permeation of dissolved drug molecules through the skin (Stoughton & McClure 1983). As little as 1% Azone within a formulation produces a detectable enhancing effect on drug permeation rate (Walters 1989). It is known that Azone accumulates within the stratum corneum (Wiechers et al 1987), where it is thought to influence primarily the structure and barrier properties of the intercellular lipids (Barry 1987). To be effective it must, therefore, be taken up into the stratum corneum in adequate amounts after topical application. The magnitude of the enhancing effect depends on the concentration of Azone achieved within the stratum corneum. This has been demonstrated by pretreating excised stratum corneum membranes with known amounts of Azone, yielding specific loadings with the enhancer. The permeability of both mouse (Lambert et al 1989) and human (Schückler & Lee 1992) stratum corneum so treated has been found to increase linearly with Azone loading. In both cases, however, an apparent optimum in the Azone loading was evident, above which any further increase did not result in greater permeability.

These studies do not address the problem of the extent of Azone uptake into the stratum corneum from a topical formulation. There are, indeed, difficulties involved in measuring this. Azone has negligible solubility in water, for example, necessitating the use of non-aqueous donor formulations that are not necessarily inert to the stratum corneum. Even the use of volatile solvents such as acetone or methanol has been found to induce non-specific changes in the thermal behaviour of isolated stratum corneum (Schückler & Lee 1992). In the present study, these problems are avoided by using Azone-loaded polymer films as a donor phase, which also serve as model matrix-type transdermal devices. By using this technique, we address two questions concerning the extent of uptake of Azone into excised human stratum corneum—how much Azone can be taken up into this tissue from a polymeric thin film and what is the relation between the Azone content of the polymeric thin film, the extent of uptake of the Azone into and through the excised human stratum corneum, and the resulting enhanced rate of drug uptake into this membrane?

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Materials and Methods

Preparation of thin polymer films

Thin polymer films of poly(dimethyl)siloxane were prepared by vulcanizing dimethyl siloxane with 3% Hardner T (Wacker Chemie, Germany) at 70°C for 12 h. Films were also prepared from Eudragit NE30D (Röhm, Germany) by solvent evaporation, as described by Zierenberg (1985). In this case, the freeze-dried polymer was first refluxed in water to remove an endogeneous surfactant and yield an isotropic system (Göpferich & Lee 1992). Azone (Nelson Research, USA) was incorporated directly during film manufacture at either 5, 10 or 20% w/w. Drug-loaded films contained additionally 1% w/w diazepam (Syncopharm, Germany). All films were examined by polarizing light microscopy to confirm that the drug was present in the dissolved state. The films were 50–70 μm in thickness, as determined by an Elcometer (Elcometer Instruments, UK).

Preparation of human stratum corneum membranes

Epidermal membranes were first prepared from whole human skin excised from the inner thigh by treatment in water at 60°C for 2 min. The donor was a 62 year old male, with the skin being excised less than 24 h post-mortem. The viable epidermis was then removed by digestion with a trypsin solution to yield stratum corneum membranes (Kligman & Christophers 1963). These were stored briefly at 1°C before use.

Measurement of uptake of Azone and diazepam into excised human stratum corneum

The extent of uptake of Azone and diazepam into the stratum corneum membranes was determined at $35 \pm 0{\cdot}5$°C using the thermostatted cell illustrated in Fig. 1. A round stratum corneum membrane of approximately 15 μm thick-

ness and 20 mm diameter was sandwiched between donor and acceptor polymer films of the same shape and diameter. The stratum corneum membrane had been equilibrated beforehand at 25% relative humidity. The acceptor layer was always Eudragit NE30D, to ensure high solubility of both Azone and diazepam. The geometry of the arrangement shown in Fig. 1 ensured the minimal contact area possible between the stratum corneum and the surrounding air, thereby preventing any excessive uptake of water vapour. Over the course of 100 h, two 1·3 cm^2 round samples were cut out from the sandwich with a punch and separated into their three component layers before being weighed on a microbalance. The stratum corneum layer was then extracted by refluxing in methanol, and the acceptor layer was dissolved in acetone. Both the Azone and diazepam contents of the two layers were then determined by HPLC analysis. It proved impractical to take a greater number of samples as only quite small, intact stratum corneum membranes could be prepared, limiting thereby the size of the sandwich and smaller (and hence a greater number of) samples could not be taken without a substantial loss of accuracy when determining their Azone and drug contents. This prevented a quantitative evaluation of the results using an applicable multi-layer diffusional model. Each experiment was performed in triplicate and the results expressed as histograms of w/w Azone or diazepam within the stratum corneum or acceptor layer at the two sampling times.

Results and Discussion

Before the Azone or diazepam can be taken up into the stratum corneum of the sandwich model, they must be released from the polymer film serving as a donor phase (Fig. 1). Unless the diffusivity within the donor film is some orders of magnitude larger than that within the stratum corneum, the rate of release from this film will substantially influence the rate of uptake into the acceptor layer (Bunge 1991). The acceptor film is treated here merely as a receptacle for the molecules passing through the stratum corneum. Its influence on the extent of uptake from the donor film into the stratum corneum is, therefore, ignored in this study, and the results only considered qualitatively. The solubility of both Azone and diazepam in the Eudragit polymer is more than 5% w/w.

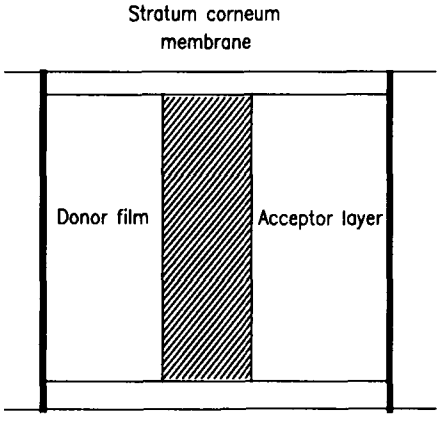

FIG. 1. Experimental arrangement for measuring uptake from a polymer film into a stratum corneum membrane and an acceptor layer.

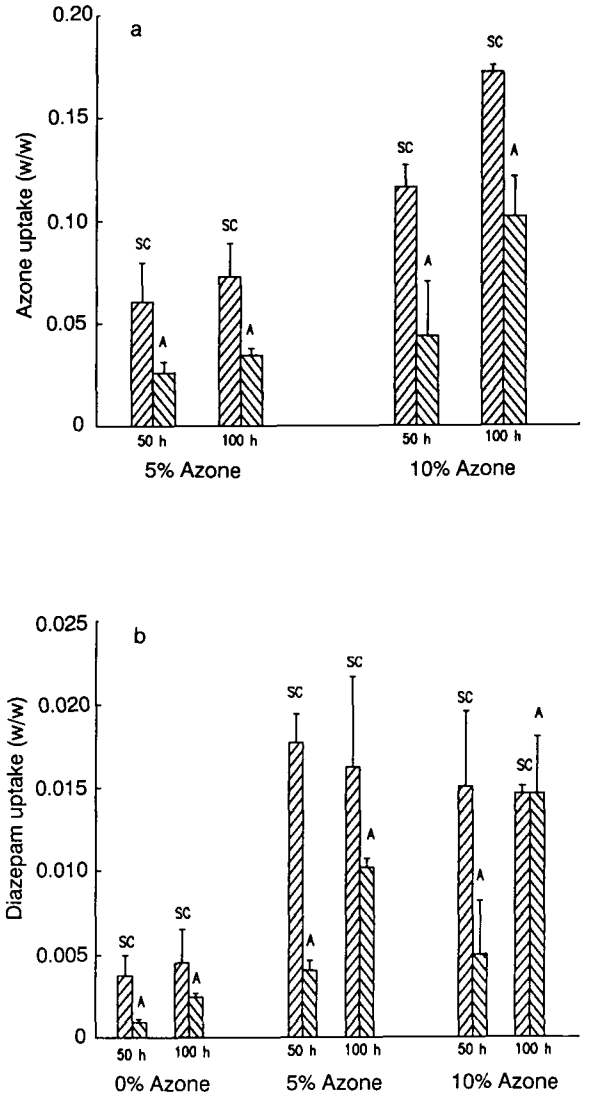

FIG. 2. Uptake from poly(dimethyl)siloxane thin films into stratum corneum membranes. a. Concentrations of Azone (w/w) taken up into stratum corneum (SC) and acceptor (A) layers. b. Concentrations of diazepam (w/w) taken up into stratum corneum (SC) and acceptor (A) layers from donor films containing 1% w/w diazepam.

Azone was taken up into the stratum corneum of the sandwich model in substantial quantities from the two poly(dimethyl)siloxane films containing 5 and 10% enhancer (Fig. 2a). From the former, some 6% w/w Azone was taken up into the stratum corneum after the first sampling time of 50 h. Substantial amounts of Azone had also passed through into the acceptor layer up to this time. After 100 h, the concentrations in both layers had increased further, resulting in an Azone loading of 7·5% for the stratum corneum. An increase in the Azone content of the poly(dimethyl)siloxane film from 5 to 10% produced an almost exact doubling of Azone concentrations with both stratum corneum and acceptor layers. This linearity implies a relatively high release

rate of Azone from the donor film into the outermost layer of the stratum corneum during this period (Göpferich & Lee 1991a). Indeed, poly(dimethyl)siloxane is known to be very permeable, with typical diffusivities of 10^{-7} cm^2 s^{-1} for moderately lipophilic molecules of mol. wt around 300 Da (Göpferich & Lee 1991b). As Azone itself acts as a plasticizer on this polymer (Pfister & Hsieh 1990), its diffusivity could be even larger. With this film it was possible to achieve an Azone loading for the stratum corneum of 12% after 50 h, which increased to 18% after 100 h. The sandwich model could thus be used to determine the extent of uptake of Azone into the excised stratum corneum membranes.

With the Azone-free but drug-loaded poly(dimethyl)siloxane film, diazepam could be identified within both the stratum corneum and acceptor layers of the sandwich model after the first sampling time of 50 h (Fig. 2b). The diazepam concentration within the stratum corneum increases only slightly between the 50 and 100 h samples, whereas that in the acceptor layer doubles in value. The lag time for diazepam permeating from an aqueous donor solution through a stratum corneum membrane is 10 h (Schückler & Lee 1992). The 50 h sample removed from the silicone thin film sandwich is, therefore, certainly post-lag time. This does not, however, indicate a pseudo-steady-state phase, as there must be a slight but continual decrease in the drug release rate from the film with time (Göpferich & Lee 1991a).

We may expect enhanced diazepam transport into and through the stratum corneum when using the poly(dimethyl) siloxane films containing both Azone and drug. The inclusion of 5% Azone into the drug-loaded poly(dimethyl)siloxane film substantially increases the diazepam concentration within the stratum corneum after 50 h and doubles that within the acceptor layer (Fig. 2b). As the stratum corneum had taken up 6% by weight Azone up to this time (Fig. 2a) this doubling of the amount of diazepam taken up into the acceptor layer corresponds well with the finding that this Azone loading leads to a twofold increase in the steady-state flux of diazepam through pretreated stratum corneum membranes (Schückler & Lee 1992). This comparison must, however, be treated cautiously, as the former experiment concerns uptake into an unstirred, solid acceptor, whereas the latter deals with permeation into a stirred fluid phase. After 100 h, the diazepam concentration in the stratum corneum has not changed, whereas that in the acceptor layer is three times that seen for the Azone-free film after this time. As the stratum corneum now contains 7·5% Azone (Fig. 2a), this finding also agrees well with that for pretreated stratum corneum membranes, where an Azone loading of 7·5% produced a threefold increase in flux of diazepam (Schückler & Lee 1992). Increasing the Azone content of the poly(dimethyl)siloxane film to 10% produces the same diazepam concentrations within the stratum corneum after 50 and 100 h seen with the 5% Azone film (Fig. 2b). As seen in Fig. 2a, the stratum corneum takes up 12% Azone after 50 h. This does not, however, lead to a greater drug concentration within the acceptor layer at this time, as observed for application of the film containing 5% Azone. Only after 100 h, when the stratum corneum had taken up 18% Azone (Fig. 2a), is the drug concentration in the acceptor layer larger than that seen with this former film. Azone loadings of the stratum corneum of 12–17% produce, for pretreated stratum

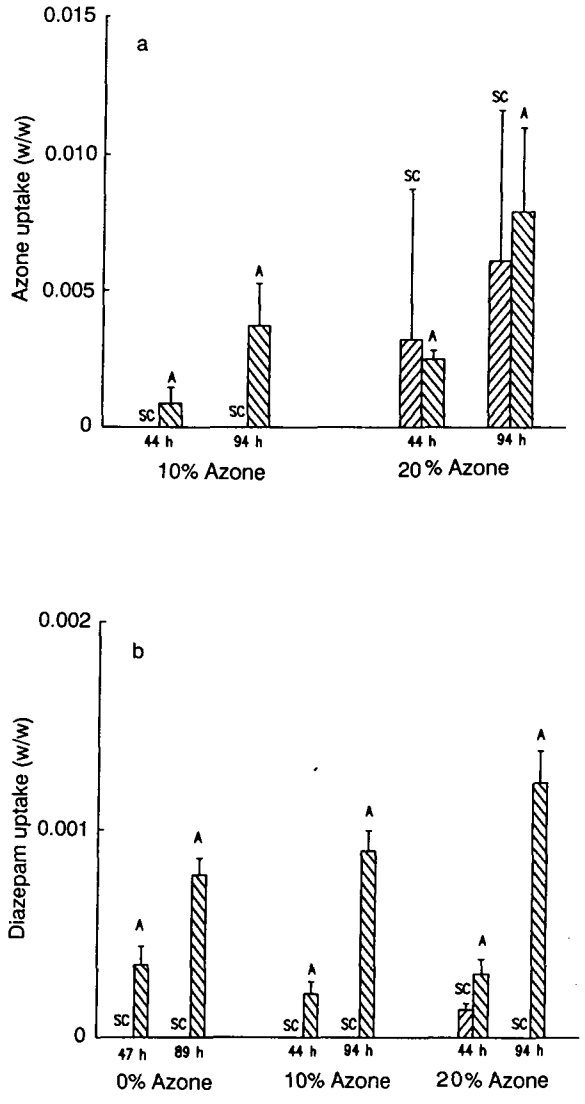

FIG. 3. Uptake from Eudragit NE30D thin films into stratum corneum membranes. a. Concentrations of Azone (w/w) taken up into stratum corneum (SC) and acceptor (A) layers of the sandwich model. b. Concentrations of diazepam (w/w) taken up into stratum corneum (SC) and acceptor (A) layers from donor films containing 1% w/w diazepam.

corneum membranes, a threefold increase in diazepam flux (Schückler & Lee 1992). This corresponds well only with the diazepam concentrations found after 100 h in the acceptor. The result obtained with the film containing 10% Azone is, however, subject to an artifact caused by the limited compatibility of Azone with the silicone polymer. At concentrations above approximately 5·5% w/w Azone, we observed that a portion of the enhancer remained as a thin fluid layer on the film surface. This finding is typical for lipophilic solvents within silicone polymers, which usually have an upper level of compatibility of approximately 5% (Pfister & Hsieh 1990), above which the solvent strongly plasticizes the polymer. The excess Azone (possibly containing dissolved diazepam) would have been immediately available for uptake into the stratum corneum on fixing the film in the sandwich and could have distorted the result.

This artifact was not seen with the more lipophilic Eudragit NE30D films, where more than 20% w/w dissolved Azone could be included without adversely affecting film formation. The change of polymer had, however, substantial effects on the extent of Azone uptake into the stratum corneum and acceptor layers of the sandwich model. It is immediately clear from Fig. 3a that the amounts of Azone taken up into these layers are an order of magnitude smaller than those found with the poly(dimethyl)siloxane polymer (Fig. 2a). Indeed, starting with the Eudragit film containing 10% Azone, we find no detectable concentrations of enhancer within the stratum corneum even after 94 h. We consider this unlikely to be a procedural or analytical error, as the result was reproducible. Indeed, it is clear that Azone had diffused out of the donor film, as it could be detected in increasing concentrations in the acceptor layer (Fig. 3a). The second film, containing 20% Azone, produces only a small Azone loading of the stratum corneum, being 0·3% after 50 h, increasing to 0·6% after 100 h. The Azone concentrations in the acceptor layer at both sampling times are just twice those seen with the film containing 10% Azone. The rate of release of Azone from the Eudragit film must, however, be substantially smaller than that for the poly(dimethyl)siloxane film, to account for the much smaller uptakes of Azone into the stratum corneum and acceptor layers. Unfortunately, the diffusivities for Azone in Eudragit NE30D and silicone are not available. It is known, however, that permeant diffusivities (mol. wt 300 Da) are much lower in Eudragit NE30D (10^{-11} cm^2 s^{-1}) than in poly(dimethyl)siloxane (10^{-7} cm^2 s^{-1}) (Göpferich & Lee 1991b). Such a difference in diffusivity for Azone would be sufficient to explain the differing rates of permeant uptake within the sandwich model (Göpferich 1991), but would have to be determined before this hypothesis could be accepted. A difference in partition coefficient for the two polymers with the stratum corneum would also have consequences for the rate of uptake into the stratum corneum. As poly(dimethyl) siloxane is relatively hydrophilic, the partition coefficients of both Azone and diazepam would be expected to favour the stratum corneum. Smaller partition coefficients with the more lipophilic Eudragit NE30D would reduce the uptake of Azone and diazepam into the stratum corneum, provided the partition coefficients do not exceed approximately 100 in value (Göpferich & Lee 1991a).

The diffusivities for diazepam in Eudragit NE30D and poly(dimethyl)siloxane are known to differ substantially, being 10^{-11} and 10^{-7} cm^2 s^{-1}, respectively (unpublished data). Thus, a reduction in the drug uptake into the stratum corneum and acceptor layers from Eudragit compared with the poly(dimethyl)siloxane is expected, and is seen with the Azone-free Eudragit film (Fig. 3b). The amounts of diazepam taken up into the stratum corneum and acceptor layers are approximately one tenth of those taken up from the poly(dimethyl)siloxane films. This is the same magnitude of change as occurred for the uptake of Azone when exchanging Eudragit for the poly(dimethyl)siloxane. Of the three films examined, diazepam could be detected within the stratum corneum only with that containing 20% Azone after 44 h. The drug concentrations in the acceptor layer are not substantially different at either of the sampling times for the Azone-free film and that containing 10% Azone; however, with the latter film no Azone was found in the stratum corneum up to 94 h (Fig. 3a). For the film containing 20% Azone, a marginally higher diazepam concentration in the acceptor layer is seen after 94 h compared with the Azone-free film. As 0·6% Azone had accumulated within the stratum corneum for this system (Fig. 3a), it is comparable with the result for the same Azone loading that produced an increase of 20% in the diazepam flux through pretreated stratum corneum membranes (Schückler & Lee 1992).

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References

Barry, B. (1987) Mode of action of permeation enhancers in human skin. J. Contr. Rel. 6: 85–97

Bunge, A. (1991) A parametric study of in vitro drug release from topical formulations across synthetic membranes. Proc. 2nd Int. Conf. Prediction of Percutaneous Penetration, Southampton

Göpferich, A. (1991) An Analysis of Transdermal Drug Delivery from a Matrix-type Device. PhD Thesis, Heidelberg University

Göpferich, A., Lee, G. (1991a) Measurement of drug diffusivity in stratum corneum membranes and a polyacrylate matrix. Int. J. Pharm. 71: 245–253

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

Göpferich, A., Lee, G. (1992) The influence of endogenous surfactant on the structure and drug release properties of Eudgrait NE30D-matrices. J. Contr. Rel. 18: 133–144

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

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

Pfister, W., Hsieh, S. (1990) Permeation enhancers compatible with transdermal drug delivery systems. Part II: system design considerations. Pharm. Technol. 14: 54–61

Schückler, F., Lee, G. (1992) 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. Int. J. Pharm. 80: 81–89

Stoughton, R., McClure, W. (1983) Azone: a new non-toxic enhancer of cutaneous penetration. Drug Dev. Ind. Pharm. 9: 725–744

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

Wiechers, J., Drenth, B., Jonkman, J., de Zeeuw, R. (1987) Percutaneous absorption and elimination of the penetration enhancer Azone in humans. Pharm. Res. 4: 496–499

Zierenberg, B. (1985) Einsatz des Nelder-Mead-Verfahrens zur Optimierung der Freigabeparameter von Polymersystemen für Arzneistoffe. Acta Pharm. Technol. 30: 17–21