The Relative Effect of Azone® and Transcutol® on Permeant Diffusivity and Solubility in Human Stratum Corneum

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Purpose. The purpose of this work was to analyse the mechanism of the enhancement of percutaneous penetration demonstrated by the known enhancers Azone® and Transcutol®.

Methods. Enhancer induced changes in the diffusivity and solubility of a model permeant (4-cyanophenol) in human stratum corneum were monitored (in-vitro) using Attenuated total reflectance Fourier transform infra-red (ATR-FTIR) spectroscopy and compared to the gross effects of the enhancers on flux as measured using simple Franz-type diffusion cells.

Results. It has been shown by both the well-established Franz diffusion cell technique and the use of ATR-FTIR spectroscopy that the enhancers studied both increase the flux of cyanophenol across human skin in-vitro by a factor of approximately two. Furthermore, it has been demonstrated by ATR-FTIR that these enhancers are likely to exert their effects by different mechanisms. It is probable that Azone reduces the diffusional resistance of the stratum corneum and that Transcutol increases the solubility of the penetrant in this barrier.

Conclusions. There is increasing interest in the apparently synergistic nature in which certain enhancers appear to work. The exact nature of these multiplicative and/or additive effects is not known although there are numerous suggestions in the current literature. The application of ATR-FTIR spectroscopy to such enhancing systems will allow the mechanisms of the observed enhancements to be probed in greater depth.

KEY WORDS: percutaneous penetration; enhancer; diffusivity; solubility; ATR-FTIR.

INTRODUCTION

There are essentially two ways in which enhancement of drug flux across membranes can be achieved in an ideal system where diffusional pathlength (h) is constant. Increasing diffusion coefficient (D) or driving penetrant activity in the upper layers of the stratum corneum (SC) will produce a rise in steady-state flux (J). The relationship between these parameters is given in the steady-state form of Ficks first law of diffusion (Eq. 1) where activity terms are replaced with those of concentration (C_m) for a fixed system.

$$J = \frac{C_m D}{h} \quad (1)$$

When utilising diffusion cell techniques to assess effects of enhancers it is not possible to directly measure the parameters D and C_m. Hence, the normal procedure is to replace C_m in Eq. 1 with KC_v where K is the membrane/vehicle partition coefficient and C_v the concentration of the permeant in the vehicle. Attempts (1) to classify enhancers by their mechanisms of action (increasing drug diffusivity and/or partitioning into the membrane) are often hindered by the fact that the permeability coefficient (P), the most commonly measured parameter, is a composite value that includes D, K and h where $P = K(D/h)$. Assessment of which of these parameters are affected by specific enhancers, and to what degree, will enable a better understanding of their mechanisms of action and aid in the design of more effective compounds.

Azone is thought to exert its effect by interacting with structured lipids of the SC. Based on evidence that Azone appears to create a more fluid environment within both artificially structured lipids (2, 3) and those of the SC (4) it appears that it may work by easing passage of a diffusant through this region i.e. by increasing the value of D.

Transcutol, a monoethyl ether of diethylene glycol, enhances penetration of several compounds (5, 6, 7). It has been suggested that Transcutol may enhance drug flux across SC by diffusing into it and altering its solubility parameter (8). Diffusion cell data shows Transcutol can increase drug flux without an apparent change in lag time (6). This implies Transcutol acts by altering the solubility of a permeant in the skin.

Attempts, with limited success, have been made to deconvolute partitioning and diffusional phenomena in both synthetic and biological membranes using diffusion cells (9). The present paper describes the utilisation of attenuated total reflectance Fourier transform infra-red (ATR-FTIR) spectroscopy to assess Azone and Transcutol induced changes in both C_m and D for a model permeant (4-cyanophenol) in human SC. 4-cyanophenol was selected as a model permeant as the CN moiety absorbs strongly in the IR-transparent region of the SC. Diffusion cell experiments were also conducted to assess the gross enhancing effects of these compounds. ATR-FTIR spectroscopy has been applied to examination of diffusion in creams (10) and synthetic membranes (11). A discussion of the underlying theory is given in a recent publication (12). The technique is particularly useful as it allows deconvolution of diffusional and solubility/partitioning phenomena (13, 14).

MATERIALS AND METHODS

4-cyanophenol (95%) [Aldrich], acetonitrile (HiPerSolv grade) and ethanol (AnalaR grade) [Merck] were used as received. Azone and Transcutol were gifts from Whitby and Gattefossé, respectively.

Diffusion Cell Studies

Diffusion profiles were determined using Franz-type glass diffusion cells. Donor chambers were filled with 0.5 ml of saturated solutions of permeant in water with sufficient excess solid present to maintain saturation throughout the experiment. Receptor phase was phosphate buffered saline (pH 7.4, 37°C), continually stirred. Full thickness human skin was used. Control

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skin samples were pretreated with 50 μl of ethanol. Azone pretreatment was 50μl of a 0.05 M ethanolic solution of Azone. Pre-treatment time was 1 hour. Five replicates were conducted for each system studied, all from a single donor. 200 μl receptor phase samples were taken at 1, 6, 10, 18, 24, and 34 hours and replaced with fresh medium. Cyanophenol content was determined by HPLC [Apex I ODS 25 cm × 4.6 mm (Jones Chromatography), acetonitrile/water 30/70 at 1ml/min, UV detection at 284 nm, retention time of cyanophenol ~4.6 minutes].

The effect of Transcutol on permeation of cyanophenol was assessed as for Azone except that cyanophenol was applied in a 1:1 mixture of Transcutol and water (0.5 mls) which was saturated with cyanophenol. Transcutol was applied differently to Azone as their utilisation in formulations is not the same. Transcutol is used as a solvent and at much greater concentration than Azone. To distinguish the mechanism of action of each compound they could not be applied simultaneously. Also, application of cyanophenol in bulk Azone would not be representative of in use conditions. It was considered that this difference in application technique would not interfere with the differentiation of the mechanistics of their enhancing actions.

Nominal flux values were first calculated by application of linear regression to the diffusion profiles where they approximated to linearity (between 12 and 34 hours). Lag times were calculated by extrapolation of these fits to where they intercepted the time axis. Pathlength normalised diffusion coefficients (D/h^2) were calculated from these lag times using $t_{lag} = h^2/6D$ (15).

However, because it is hard to accurately define the onset of steady-state, permeability coefficients, pathlength normalised diffusion coefficients (D/h^2) and values of Kh, were also calculated using a method that does not assume the achievement of steady-state (15). Equation 2 describing diffusion through a plane sheet ($u = \mu g cm^{-2}$ penetrated, K = vehicle/SC partition coefficient, h = diffusional pathlength, C = concentration difference across the membrane (which approximates to donor phase concentration), D = penetrant diffusion coefficient and t = time) has been utilised for calculation of permeability coefficients using a computer fitting routine to gain the best fit to the data collected.

$$u(t) = KhC\left[\frac{D}{h^2} - \frac{1}{6} - \frac{2}{\pi^2}\sum_{n=1}^{\infty}\frac{(-1)^n}{n^2}\cdot\exp\left(\frac{-Dn^2\pi^2t}{h^2}\right)\right] \quad (2)$$

As K, D and h are all unknown, the products Kh and D/h^2 were replaced in Equation 1 by P_1 and P_2 to give Equation 3.

$$u(t) = P_1C\left[P_2 - \frac{1}{6} - \frac{2}{\pi^2}\sum_{n=1}^{\infty}\frac{(-1)^n}{n^2}\cdot\exp(-P_2n^2\pi^2t)\right] \quad (3)$$

Values of P_1 and P_2 were obtained using a non-linear curve fitting package with n = 10 in Equation 3 (Ultrafit™, Biosoft, Cambridge, UK on an Apple Macintosh). The product P_1P_2 is then equal to the permeability coefficient (as $Kh.D/h^2 = KD/h$). This approach is as used elsewhere (16) except that steady-state is not assumed and hence all data points collected (including those on the non-linear portion of the diffusion profile) can be used.

ATR-FTIR Studies

SC membranes were prepared by heat separation and subsequent trypsinisation as previously described (17). The membranes were placed in direct contact with the surface of a ZnSe attenuated total reflectance crystal (Spectra-Tech Inc) mounted on a Nicolet 710 FTIR spectrometer. This was achieved by floating skin samples from the surface of distilled water onto the crystal. Once the membrane was in place a PVC trough was placed on top. Trough and membrane were sealed together with petroleum jelly and the join monitored for leaks throughout the experiment. The SC membrane was treated with 130 μl of either ethanol (control) or a 0.05 M (1.4% w/v) ethanolic solution of Azone. This represented an equivalent dose per unit area to that used in diffusion cell experiments. After 1 hour a saturated aqueous cyanophenol solution (plus excess solid) was placed in the trough on the SC and the trough closed by a plastic cover held in place by a weight. Nicolet Omnic software allowed automated collection and manipulation of spectra. Spectra were collected every 5 minutes with 50 sweeps for each spectrum. Five replicates were run for each treatment regimen. After each run peak areas associated with CN stretch at 2230 cm^{-1} were calculated to give measurements of cyanophenol permeation.

Equation 4 (where C = permeant concentration at the interface at any time t, D = permeant diffusion coefficient, h = film thickness and C_0 = solubility of penetrant in the membrane) describes build up of a penetrant at the lower membrane/crystal interface with time (10). There is an initial period where permeant concentration at the interface increases followed by an exponential rise to a plateau that represents saturation of the membrane with permeant.

$$C = C_0\left[1 - \frac{4}{\pi}\sum_{n=0}^{\infty}\frac{(-1)^n}{2n+1}\exp\left(\frac{-D(2n+1)^2\pi^2t}{4h^2}\right)\right] \quad (4)$$

It is possible, assuming the Beer-Lambert law applies, to replace the concentration terms in Eq. 4 with experimental absorbance values to give Eq. 5 where A = area of penetrant peak (at time t) of IR absorbance relating to the permeant and A_0 = area of penetrant peak corresponding to the situation where the membrane is saturated (in the plateau region of the curve).

$$A = A_0\left[1 - \frac{4}{\pi}\sum_{n=0}^{\infty}\frac{(-1)^n}{2n+1}\exp\left(\frac{-D(2n+1)^2\pi^2t}{4h^2}\right)\right] \quad (5)$$

Experimental values of penetrant peak areas against time were fitted using Eq. 5 and Ultrafit. Values of D/h^2 and A_0 were allowed to vary until best fit was achieved as measured by minimisation of χ^2.

ATR-FTIR experiments to assess effects of Transcutol on SC permeability were conducted as for Azone except that, as for diffusion cell studies, cyanophenol was applied as a saturated solution in a 1:1 mixture of Transcutol and water.

Statistics

All statistical comparisons were conducted using SuperANOVA on an Apple Macintosh and Fishers Protected LSD at a significance level of 5%.

RESULTS AND DISCUSSION

Diffusion profiles achieved using Franz cells (Figure 1) indicate that Azone enhances permeation of cyanophenol across human skin. There was a clear difference (p = 0.0015) in flux,

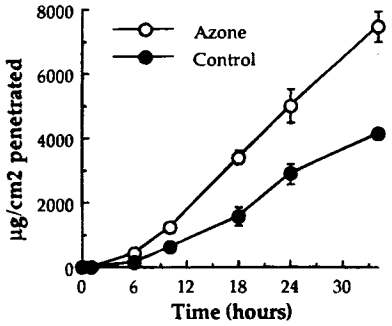

Fig. 1. Flux of cyanophenol through human skin in-vitro.

calculated by linear regression over the 12 to 34 hour period, between the control (150 ± 17 $\mu gcm^{-2}hr^{-1}$) and Azone treated samples (260 ± 41 $\mu gcm^{-2}hr^{-1}$), giving an enhancement ratio of approximately 1.7.

Application of lag time analysis to the two data sets yields values (Table 1) of pathlength normalised diffusivities (D/h^2) of 0.036 ± 0.011 hr^{-1} and 0.039 ± 0.016 hr^{-1} for control and Azone treated samples respectively (where D = diffusion coefficient and h = pathlength). These values were not statistically different, suggesting Azone had little or no effect on diffusivity of cyanophenol in SC. This implied the increase in flux demonstrated in the presence of Azone resulted from increased partitioning of penetrant into SC. i.e. as J = KDC/h the increase (assuming h and C to be constant) must come in K, if it is not in D.

By using the solution to Ficks first law shown in Equations 2 and 3, where $P_2 = D/h^2$ and $P_1 = Kh$, it is theoretically possible to separate and calculate effects of K and D on total flux across SC (14, 15, 16). Calculated average values of D/h^2 and Kh are included in Table 2. There were no significant differences between diffusional, $[D/h^2]_{fit}$, or partitioning, $[Kh]_{fit}$, terms calculated using this method. This assessment using full-curve analysis of the results contradicts the above interpretation regarding increases in flux resulting from increases in K.

The application of deconvolution techniques to Franz cell data was thus of very limited success in that the methods applied confirmed the existence of enhancement but shed little light on the mechanistics. This was possibly due to the small enhancement ratio (<2) and the contribution of two parameters (D and K) to the observed enhancing effect, leading to an inability to detect changes in them individually. When the data are compared in terms of the composite parameter P_{fit} (where $P = P_1P_2 = DK/h$), the multiplicative effect of potential changes in both D and K are highlighted and it thus becomes possible to detect significant differences between treatments.

Use of the P_1P_2 data to predict fluxes and permeability coefficients yields values extremely close to those gained with the linear regression approach indicating the mutually-consistent nature of the methods (statistical comparison yielded very high p values in all cases).

Previous results (18) indicated Azone increased SC diffusivity of diazepam but not its partition coefficient. The enhancement ratio for diazepam (2 to 15, and enhancer concentration dependent) was generally greater than that observed in this work for cyanophenol. Changes were therefore easier to detect. However, as the authors did not indicate the degree of significance associated with their results, it is difficult to make a complete assessment of them.

The results of ATR experiments are summarised in Table 3 which contains average values of D/h^2 and A_0 for Azone treated and control samples. A_0 values are a reflection of diffusant solubility in SC and D/h^2 a reflection of its diffusion coefficient. The values of both these parameters are greater for Azone treated samples, although this difference is only statistically significant for D/h^2. The implication is that, in this case, Azone produces its enhancing effect by increasing the diffusion coefficient of the permeant in SC rather than its solubility in this membrane.

Table 1. Diffusional Parameters for Cyanophenol Calculated from Linear Regression Fits to Franz Cell Data (±SE)

Treatment	J $\mu gcm^{-2}hr^{-1}$	t_{lag} hr	$[D/h^2]_{lag}$ hr^{-1}	P $cmhr^{-1}$
Control	150 ± 17	5.0 ± 1.6	0.036 ± 0.011	0.010 ± 0.0013
Azone	260 ± 41	4.8 ± 1.6	0.039 ± 0.016	0.017 ± 0.0027
p value	0.0015	0.863	0.774	0.0014

J = flux calculated from linear regression at steady-state.
t_{lag} = lag time calculated from time axis intercept of flux regression line.
$[D/h^2]_{lag}$ = pathlength normalised diffusion coefficient calculated from the lag time using $t_{lag} = h^2/6D$.
P = permeability coefficient calculated from $P = J/C_d$ where C_d = donor phase concentration = 15.1 $mgml^{-1}$.

Table 2. Diffusional Parameters for Cyanophenol Calculated from P_1P_2 Method Applied to Franz Cell Data (±SE)

Treatment	$[D/h^2]_{fit}$ hr^{-1}	$[Kh]_{fit}$	P_{fit} $cmhr^{-1}$	J_{fit} μgcm^{-2} hr^{-1}
Control	0.032 ± 0.015	0.43 ± 0.30	0.011 ± 0.0016	160 ± 24
Azone	0.030 ± 0.023	0.65 ± 0.31	0.018 ± 0.0028	270 ± 42
p value	0.843	0.323	0.0024	0.0024

$[D/h^2]_{fit}$ = pathlength normalised diffusion coefficient calculated from the non-linear curve fitting routine (P_1 in Eq. 3).
$[Kh]_{fit}$ = product of partition coefficient and pathlength calculated from the non-linear curve fitting routine (P_2 in Eq. 3).
P_{fit} = permeability coefficient calculated from $P = [D/h^2]_{fit} \cdot [Kh]_{fit} = P_1P_2$ where C_d = donor phase concentration = 15.1 $mgml^{-1}$ ·
J_{fit} = flux calculated from fitted values of $[D/h^2]_{fit}$ and $[Kh]_{fit}$.

Table 3. Diffusional Parameters for Cyanophenol Calculated from ATR-FTIR (±SE)

Treatment	D/h^2 hr^{-1}	A_0	P_{pseudo}
Control	0.71 ± 0.23	2.89 ± 0.34	2.05
Azone	1.33 ± 0.15	3.50 ± 0.43	4.66
p value	0.0498	0.296	—
ER	1.87	1.21	2.27

D/h^2 = pathlength normalised diffusion coefficient
A_0 = plateau level
$P_{pseudo} = DA_0/h^2$

The overall calculated enhancement ratio of 2.3 (Table 3) was of the same order as that derived using Franz cells (1.7). The enhancement ratio can be separated into diffusional and solubility contributions. There is little difference between control and Azone treated samples in terms of the solubility term (A_o). This is reflected in the enhancement ratio of 1.21 (Table 3). The average enhancement ratio of 1.87 for fitted diffusional term (D/h^2) is greater than enhancement ratio calculated for A_o values reiterating the point that Azone is exerting its enhancing effect by increasing the diffusion coefficient of cyanophenol in SC.

However, the absolute values of D/h^2 determined using the ATR technique differ from those calculated from Franz diffusion data. Values calculated from the ATR experiments are approximately 18-fold greater than those derived from Franz cell work (0.71 hr^{-1} from ATR data compared with 0.04 hr^{-1} from Franz data). The ATR and Franz cell experiments used SC and full-thickness skin respectively and, in-vitro, the aqueous dermis in full-thickness skin can act as an artificial barrier to lipophilic compounds (19). This could explain the smaller value of D/h^2 obtained from Franz cell data. The literature value for the octanol/water partition coefficient (logP = 1.56) of cyanophenol (20) and its relatively high water solubility (15 mg/ml) indicate its moderate hydrophobicity and imply this is a possible explanation. These differences may also be due in part to the inherent variability of human skin (21). Even if this were not the case it is the enhancement ratios that are pertinent here. The figures for these are very similar in the Franz (1.7) and the ATR (2.3) results. However, the significance of this phenomenon is far from clear and certainly requires further investigation.

Figure 2 compares permeation profiles for cyanophenol through full-thickness skin from a saturated 1:1 Transcutol/water mixture with a control. The difficulty in using simple permeation profiles for determination of enhancement mechanistics is highlighted by this data. The assessment of a steady-state region in a diffusion profile is a pre-requisite to calculation of flux and other permeation parameters. It is evident (Figure 2) that such an assessment for the Transcutol/water system is difficult and the data is open to mis-interpretation. The use of linear regression fits to often arbitrarily selected regions of linearity has recently been criticised (22) and hence use of this method for assessment of lag times (and therefore mechanistics) must also be questioned.

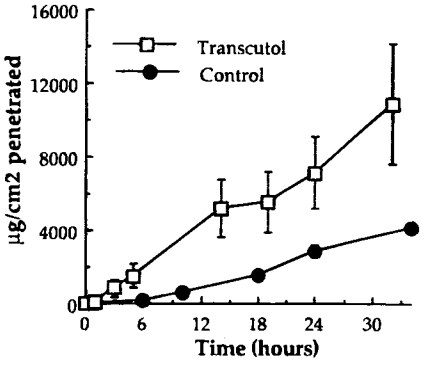

Fig. 2. Permeation of cyanophenol through human skin in-vitro from a 1:1 Transcutol/water mixture compared with control.

Table 4. Diffusional Parameters for Cyanophenol Calculated from ATR-FTIR (±SE)

Treatment	D/h^2 hr^{-1}	A_0	P_{pseudo}
Control	0.71 ± 0.23	2.89 ± 0.34	2.05
Transcutol	0.29 ± 0.04	13.7 ± 2.26	3.97
p value	0.271	0.0007	—
ER	0.41	4.74	1.94

D/h^2 = pathlength normalised diffusion coefficient.
A_0 = plateau level.
$P_{pseudo} = DA_0/h^2$.

It is evident (Figure 2) that Transcutol enhanced the flux of cyanophenol. The enhancement ratio was ~2.2 (taking the average flux of cyanophenol from the Transcutol/water vehicle as 331 µg/cm²/hr compared to 149 µg/cm²/hr for the control). These values were calculated by regression fits to the data over the best estimate of linearity but are not considered accurate enough to perform analysis similar to that conducted for the Azone experiments. The use of the P_1P_2 approach also failed to produce any meaningful results as errors associated with the fitted values were extreme. This interpretation does not, however, preclude use of Franz cell experiments for assessment of gross enhancement or vehicular effects.

Table 4 contains results of the ATR-FTIR experiments using the Transcutol/water mixture. Again, there is evidence of enhancement of cyanophenol permeation. However, the situation is somewhat different to that found with Azone. Examination of the D/h^2 and A_0 values in Table 4 shows that the enhancement that occurred with Transcutol was the result of changes in the solubility rather than the diffusional parameter (where Azone was the enhancer the reverse was the case). Further, changes in A_0 are statistically significant whereas those seen in D/h^2 are not. Also the overall enhancement ratio of 1.94 (Table 4) does not differ greatly from that (2.2) tentatively derived from Franz cell data.

CONCLUSIONS

It has been shown, by established diffusion cell techniques and ATR-FTIR spectroscopy, that both the enhancers Azone and Transcutol increase flux of cyanophenol across human skin in-vitro by a factor of ~2. ATR-FTIR spectroscopy indicates that these enhancers probably exert their effects by different mechanisms. It is likely that Azone reduces diffusional resistance of SC and that Transcutol increases the solubility of the penetrant in SC. Azone is believed to produce its enhancing effect through direct interaction with SC lipids leading to a more fluid environment. Such an increase in fluidity should produce an increased diffusion coefficient for permeating molecules. Evidence for the mechanism by which Transcutol enhances is limited. However, Transcutol does not appear to have a fluidising effect on structured lipids. For example, Transcutol has no effect on the phase transition temperature of DPPC structured lipids whereas Azone reduces this value (5). This suggests a similar mechanism of action is unlikely. There is also evidence that Transcutol increases the SC solubility of oestradiol and produces concurrent rises in the flux of this drug

across human skin (7) and that Transcutol is responsible for the formation of a hydrocortisone depot in human skin (23).

There is increasing interest in apparent synergism between certain enhancers. Azone and propylene glycol have a multiplicative enhancing effect on permeation of metronidazole through human skin (24) and a mixture of oleic acid and Transcutol produce an additive enhancing effect on theophylline permeation (25). The exact nature of these multiplicative and additive effects is not known. The application of ATR-FTIR spectroscopy to such enhancing systems will allow the mechanisms of observed enhancements to be probed in greater depth.

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