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Comparison of permeability data from traditional diffusion cells and ATR-FTIR spectroscopy Part I. Synthetic membrances

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Abstract

Diffusion cells and attenuated total reflectance Fourier transform infra-red (ATR-FTIR) spectroscopy have been used to monitor the permeation of a model compound, 4-cyanophenol (CP), across silicone membranes. CP permeation was measured across water saturated silicone membranes and untreated silicone membranes. In all cases the donor phase consisted of a saturated aqueous solution of CP. The receptor phase used in the diffusion cells was distilled water. After calibration of the ATR-FTIR system, it was found that the water saturated membranes yielded permeability co-efficients for CP identical to those found using diffusion cells. In contrast, it was found that the permeability coefficient determined from the ATR-FTIR spectroscopy data for the untreated membrane yielded a value almost two-fold greater than those obtained from both the diffusion cells and the ATR-FTIR spectrometer with the pre-saturated membranes. It is likely that the untreated membrane used in the diffusion cell study contained a substantial amount of water before application of the donor solution due to the inevitable uptake of the water receptor medium. Therefore, as a result of the experimental techniques and procedures used, the only membrane that was not hydrated to some degree before the donor phase was applied was the untreated one analysed by ATR-FTIR spectroscopy. It has been demonstrated that, provided all initial conditions are effectively the same, there is a good correlation between permeability co-efficients measured using diffusion cells and ATR-FTIR spectroscopy. © 1997 Elsevier Science B.V.

Keywords: Fourier transform infrared spectroscopy; Attenuated total reflectance; Diffusion co-efficient; Partition co-efficient; Permeability co-efficient; Silicone membranes

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1. Introduction

Steady-state diffusion across an inert membrane, and into a sink, can be described by Fick's first law (Eq. (1)) where J is the flux (mass per unit time per unit area crossing a membrane), D is the diffusion co-efficient, K is the membrane-vehicle partition co-efficient, h is the diffusional pathlength, and C_v is the concentration of the permeant in the vehicle.

$$J = \frac{DK}{h} C_v \tag{1}$$

The diffusion of a vehicle, or other formulation excipients, into a membrane can alter the diffusion characteristics of the active ingredient of a formulation. This effect has been exploited (serendipitously or otherwise) to enhance the penetration of drugs across the skin, and other biological membranes. Such enhanced penetration may be the result of increased permeant partition (K) and/or diffusion (D) coefficients. However, it is often difficult to separate the individual effects of enhancement on K and D and the matter is complicated further as diffusional pathlengths (h) are not always known. To ease the analysis of permeation data gained from diffusion cells, D, K and h are often quoted in terms of a composite parameter, the permeability co-efficient, k_p, defined in Eq. (2).

$$k_p = \frac{DK}{h} \tag{2}$$

A knowledge of the individual parameters that form the permeability co-efficient would aid in the design of formulations in the pharmaceutical, agrochemical and cosmetic sectors. Furthermore, the classification of penetration enhancers into diffusion co-efficient modifiers or partition co-efficient modifiers would also facilitate formulation design so that optimal co-enhancer systems could be developed. For example, the incorporation of a diffusion co-efficient modifier and a partition co-efficient modifier in a formulation will result in a multiplicative effect on flux. It has been postulated (Wotton et al., 1985) that propylene glycol (a possible partition co-efficient modifier) and Azone® (a possible diffusion co-efficient modifier) act in a synergistic manner in modifying the permeation of metronidazole across human skin in vitro. However, it is unlikely that the classification of enhancers will be absolute in terms of their mechanism of action and it may be reasonable to expect a certain degree of overlap between the two categories.

Simple diffusion cells are frequently used to determine the diffusional parameters of compounds, where diffusion co-efficients are determined by Eq. (3) in which t_{lag} is the lag time associated with the diffusion process.

$$t_{lag} = \frac{h^2}{6D} \tag{3}$$

However, some doubt has been cast on the accuracy of diffusion co-efficients determined by this method which requires an extrapolation from the steady-state region which may not always be reached. The onset of steady-state in a diffusion process is often difficult to assess and this has led to some criticism of the subjective nature of this approach for measuring diffusion co-efficients (Potts and Guy, 1994). This method of data analysis also necessitates the exclusion of data prior to the attainment of steady-state. These pitfalls of subjectivity and data loss can be circumvented to some extent by the application of an appropriate solution (Eq. (4)) to Ficks second law of diffusion in which P_1 and P_2 represent Kh and D/h^2, respectively (Watkinson et al., 1994). The derivation of Eq. (4) is based on the assumption of certain boundary conditions, not all of which will be exactly fulfilled in practice. However, its use provides a good approximation in the case where donor depletion is small, receptor phase concentrations are low and the membrane is homogeneous in nature. Diffusion cell data, mass per unit area entering the receptor phase, $u(t)$, can be fitted using Eq. (4) by varying P_1 and P_2 until a best fit is obtained. The product P_1P_2 equates to DK/h and thus allows calculation of the permeability co-efficient.

$$u(t) = P_1 C_v \left[P_2 t - \frac{1}{6} - \frac{2}{\pi^2} \sum_{n=1}^{\infty} \frac{(-1)^n}{n^2} \exp(-P_2 n^2 \pi^2 t) \right] \tag{4}$$

It has already been pointed out that a further complication in the measurement of D and K in some membranes is that the diffusional pathlength is not known. In some biological membranes, e.g. skin, the permeant is thought to take a tortuous route around the cells and diffuse largely within the intercellular domains, rather than take a more direct route across the cells and the intercellular matrices (Albery and Hadgraft, 1979). Therefore, in such cases, the diffusional pathlength is often longer than the thickness of the membrane. However, for synthetic silicone membranes where the polymer does not present a tortuous route for diffusion, the membrane thickness can be considered to be the diffusional pathlength.

The application of attenuated total reflectance Fourier transform infra-red (ATR-FTIR) spectroscopy to diffusion studies in polymers and semi-solids has been described by various authors (Farinas et al., 1994; Wurster et al., 1993; Harrick, 1967). Briefly, a membrane is sandwiched between an impermeable ATR crystal, and a saturated reservoir of permeant, providing an essentially constant concentration, C_m, of permeant in the upper surface of the membrane. The membrane is initially devoid of permeant. As diffusion into the membrane occurs, there will be a build up of permeant at the membrane/crystal interface. This build up will continue until the membrane in this region is saturated with permeant, at which time the build up will cease and a plateau level will be reached. The rate at which the plateau is attained is related to the speed of permeant movement within the membrane (D) and the plateau level itself is related to the solubility of the permeant in the membrane (which is related to K). This process can be examined in real time by reflecting an IR beam along the inside of the crystal. The IR beam has a limited depth (2–3 μm) of penetration into the membrane resting on top of the crystal and, therefore, the molecular environment that approximates the membrane-crystal interface can be probed. By using a permeant that absorbs in the IR transparent region of the membrane, changes in the concentration of the permeant at the interface can be monitored. This is performed by following increases in the IR absorbance associated with the permeant over the experimental time frame. An analytical solution describing the build up of diffusant concentration at the membrane/crystal interface with time can be obtained using Fick's second law and the relevant initial and boundary conditions (Watkinson et al., 1994), and is given by Eq. (5) where C is the concentration at the crystal–membrane interface at any time, t. The boundary conditions applied to derive Eq. (5) are similar to those for Eq. (4) except that in the former case no receptor phase is present to remove material from the membrane, hence the eventual saturation of the membrane with permeant.

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

Harrison et al. (1996) attempted to deconvolute enhancer-induced changes in K and D using ATR-FTIR spectroscopy and diffusion cells. In their work, the effects of Transcutol® and Azone® on the permeation of a model compound, 4-cyanophenol, through isolated human stratum corneum were assessed. Transcutol was shown to increase the solubility of the permeant within the membrane whereas Azone increased the diffusion co-efficient. However, because the ATR-FTIR system was not calibrated it was not possible to draw a direct comparison between diffusion cell data and ATR-FTIR data.

In a recent publication (Watkinson et al., 1995) it was shown by ATR-FTIR spectroscopy that the permeation of vehicles into a silicone membrane affected both the diffusion and partition co-efficients of 4-cyanophenol. In an attempt to correlate the ATR-FTIR data with that obtained from diffusion cell experiments, it was assumed that the receptor phase fluid in the diffusion cells did not diffuse into the membrane. However, if receptor fluid penetrates into the dividing membrane, diffusion cells have the added complication that the barrier properties of the membrane may change during the experiment unless sufficient equilibration time is allowed. In contrast to diffusion cell experiments ATR-FTIR spectroscopy involves the positioning of a membrane on an ATR crystal, which replaces the receptor phase, so that the

problems associated with the back-diffusion of receptor fluid cannot occur. In order to compare data from these two techniques the membranes used should be in an equivalent physical state at the beginning of each of the experiments. One possible way of achieving this may be to pre-saturate the membranes with the receptor fluid (water) before use with both ATR-FTIR spectroscopy and diffusion cells.

The aims of this present study were to attempt absolute quantitative calibration of the ATR-FTIR system, and to examine the effect of the membranes being in an effectively identical state of hydration at the beginning of both the ATR-FTIR spectroscopy and diffusion cell experiments. Permeability co-efficients within a silicone membrane for the model permeant, 4-cyanophenol, formulated as saturated solutions in water (15.1 mg/ml), were determined using the two different methods.

2. Materials and methods

2.1. Materials

4-cyanophenol (95%) was purchased from Aldrich (Dorset, UK), methanol (HPLC grade) from Fisher Scientific (Loughborough, UK), and acetonitrile (HPLC grade) from Rathburn Chemicals (Walkerburn, UK). Silicone membranes were purchased from Samco (St. Albans, UK) and had a specified thickness of 300 μm. However, measurement of the membranes with a micrometer (Moore and Wright, UK) demonstrated the thickness to be 275 μm; this value was used in all calculations. All materials were used as received unless indicated.

2.2. Diffusion cell experiments

Saturated solutions of cyanophenol in water were prepared by adding excess compound to the solvent and mixing at room temperature overnight. Silicone membranes which had been soaked in water for at least 48 h (pre-saturated) or, for the control experiment, membranes that were used as received (untreated), were mounted in Franz-type diffusion cells with a diffusional surface area of about 1 cm^2 and a receptor volume of approximately 2.5 ml (these values were accurately measured for each cell and all results normalised accordingly). The cells were allowed to equilibrate for 1 h prior to application of the donor solution. The diffusion from saturated solutions of cyanophenol, in the presence of excess solid, across these membranes was monitored by removing 400 μl samples from the receptor phase at pre-determined time points and replacing it with an equal volume of receptor fluid. Water which had been degassed by vacuum filtration through a 0.45 μm cellulose nitrate membrane filter prior to use was used as the receptor medium. A magnetic bar was added to each receptor compartment and the diffusion cells were placed on a magnetic stirring bed at room temperature. The control experiment reproduced the conditions used in a previous publication (Watkinson et al., 1995). Permeability co-efficients were determined from these data by the use of Eq. (4).

2.3. ATR-FTIR spectroscopy

A zinc selenide ATR crystal (Spectra-Tech) with an angle of incidence of 45° was positioned in a Nicolet 710 FTIR spectrometer. This was linked to a Viglen 486 PC computer and, using Omnic version 1.2 software (Nicolet Instrument Corp.) which allowed automated collection of spectra, ten scans were taken at each time point to produce an averaged spectrum. Silicone membranes which had been soaked in water for at least 48 h (pre-saturated) or, for the control experiment, membranes that were used as received (untreated) where placed flat on the crystal surface. A donor compartment was then placed on top of the membrane and a combination of silicone grease (Ambersil, Bridgwater, UK) and white soft paraffin (The Boots Company PLC, Nottingham, UK) was used to produce a leak-proof seal between the donor compartment and the membrane. Silicone membranes were cut large enough to cover the crystal surface entirely thus preventing any of the sealants from coming into contact with the crystal. A brass weight (430 g)

completely covered the top opening of the donor compartment to inhibit evaporation of the donor phase and reinforce the seal.

An FTIR spectrum of cyanophenol shows a strong absorption band at 2230 cm^{-1} corresponding to the cyano (CN) stretch frequency. The IR spectrum of the silicone membranes and water were featureless in this region. Plots of CN peak area against time were generated and Eq. (5) was applied (with $n = 10$) using a non-linear curve fitting program (Ultrafit v1.03, Biosoft®, Cambridge, UK) to obtain diffusion co-efficients and plateau levels. In this manner, the diffusion of cyanophenol from saturated aqueous solutions across pre-saturated (with water) and untreated membranes were investigated.

2.4. Calibration of the ATR crystal

Pre-weighed silicone membranes were soaked overnight (at least 16 h) in known volumes of aqueous solutions containing different concentrations of cyanophenol. After careful blotting of the surface with tissue, the membranes were placed on the ATR crystal, and the CN peak area was measured. The cyanophenol was then extracted from the membranes using four 2 ml volumes of methanol. After 1 h equilibration periods, 1.5 ml was recovered (i.e. 75%) from each extraction. A final fifth 2 ml volume of methanol was allowed to equilibrate for at least 48 h. The first four methanol volumes (combined together) and the fifth volume were evaporated to dryness and the residues were redissolved in 1 ml of mobile phase. These were analysed for cyanophenol content by HPLC.

2.5. HPLC analysis

Cyanophenol was assayed by HPLC using a Milton Roy LDC Constametric IIIG pump set at a flow rate of 1 ml/min, with a Spectromonitor III variable wavelength UV detector set at 284 nm and a CI4100 computing integrator. An Apex ODS 5 μm (25 cm × 4.6 mm) column (Jones Chromatography, Hengoed, UK) and a mobile phase of 30% acetonitrile, 70% water adjusted to pH 3.0 with acetic acid was used. Samples were injected via a 50 μl loop using a Marathon (Spark Holland) autosampler. The retention time of cyanophenol was ~9 min and calibration curves were constructed on the basis of integrated peak area.

3. Results and discussion

3.1. Diffusion cell studies

Diffusion profiles of cyanophenol from saturated solutions in water (with excess solid present) across silicone membranes stored under ambient conditions prior to the experiment (untreated), and membranes that had been soaked in water for at least 48 h before the experiment (pre-saturated) are compared in Fig. 1. On close examination it appeared that, although the permeability of the two membranes at 1 h was similar, there was a slight divergence of the profiles thereafter. The membranes were in different states of hydration at the beginning of the experiment (untreated and pre-saturated), and therefore, it would have been expected that any difference in diffusion through them would be manifested early in the process and then be minimised as water moved into the

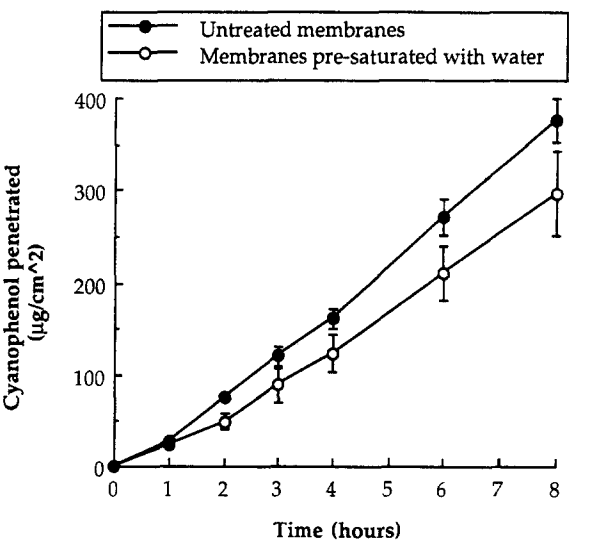

Fig. 1. Diffusion of saturated aqueous solutions of cyanophenol in water across silicone membranes (mean ± S.E., $n = 4$).

Table 1
Parameters calculated from diffusion cell data using the P_1P_2 method (mean ± S.E., $n = 4$)

Membrane	P_1P_2 ($\times 10^{-4}$) cm h^{-1}
Untreated	33.2 ± 2.2
Pre-saturated	27.6 ± 3.6

$P_1P_2 = Kh \times D/h^2 = KD/h$ = permeabiltiy coefficient (k_p).

untreated membrane. The data in Fig. 1 appeared to demonstrate an opposite effect to this, and therefore the significance of the differences between the data sets were tested at each time point. Analysis of variance showed no significance difference ($P > 0.05$) between the data sets at all time points. Permeability co-efficients for the systems in Fig. 1 were determined using the P_1P_2 method (Eq. (4)) as 33.2 ± 2.2 × 10^{-4} cm h^{-1} and 27.6 ± 3.6 × 10^{-4} cm h^{-1} for the untreated and pre-saturated membranes respectively (Table 1). A statistical comparison indicated the significance of the difference between these values was low (oneway anova, $P = 0.22$). The values found in this work compare favourably with those found in an earlier study (27.8 × 10^{-4} cm h^{-1}; Watkinson et al., 1995) for permeation through an untreated membrane. Hence, in the case of an aqueous vehicle, although the pre-saturation of the membranes with water affected the shape of the permeation profiles to a small extent (Fig. 1), it made little difference to the values of the calculated permeability co-efficients (Table 1).

3.2. ATR-FTIR studies

ATR-FTIR spectroscopy was also used to monitor the diffusion of 4-cyanophenol from saturated aqueous solutions in the presence of excess solid across pre-saturated and untreated silicone membranes. Fig. 2 shows a typical set of data and the fit to Eq. (5) using Ultrafit software.

Table 2 contains the results of these ATR-FTIR studies, in terms of fitted diffusion co-efficients and plateau levels, together with pseudo flux values (J_{pseudo}) which are the products of the diffusion co-efficient (D) and the plateau level (A_0). These pseudo flux values are not equal to any measured flux but are, in theory, proportional to it, by virtue of the same diffusional distance (h) and the fact that the plateau level (A_0) is related to the concentration (C_m) of cyanophenol in the outer layers of the membrane that drives the diffusion process (i.e. from Ficks first law at steady-state we have $J = C_mD/h$). We have made the reasonable assumption that the level at which the ATR-FTIR spectroscopic data plateaus corresponds to the saturation of the lower portion of the membrane (that part in contact with the crystal) with cyanophenol. Furthermore, we suggest that this equates to the concentration in the outer layers that drive the diffusion process (because when a saturated solution is in contact with the membrane, it is assumed that the outer layers of the membrane are saturated with penetrant). Hence, if the plateau level (A_0) is proportional to C_m such that $A_0 = \alpha C_m$ then we get $J = (A_0D)/(\alpha h)$. Thus, the flux is proportional to, but not equal to, the product of D and A_0, both of which can be derived from the ATR-FTIR spectroscopy experiments.

The pseudo flux values for the two membrane systems were different by a factor of about two (Table 2). This difference was manifested by a doubling of the plateau level whilst the diffusion co-efficient remained approximately constant. It is interesting to note that, although the plateau level

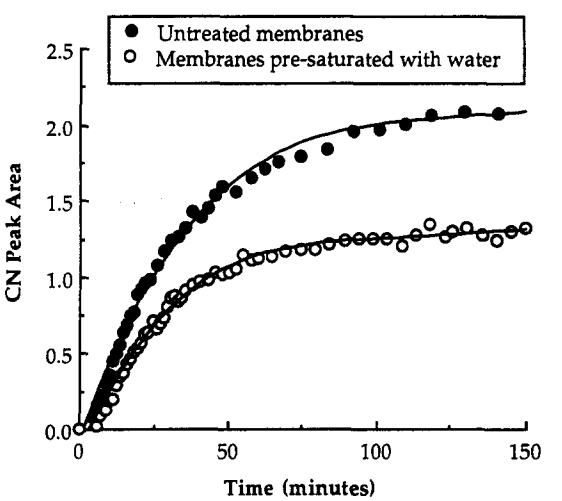

Fig. 2. Fitting of the data generated by ATR-FTIR spectroscopy using Ultrafit software.

Table 2
Diffusion coefficients, plateau levels and pseudo J values for the permeation of cyanophenol in silicone membranes from a water vehicle using pre-saturated and untreated membranes (mean ± S.E.)

Condition of membrane	Diffusion coefficient (D) ($\times 10^{-4}$) ($cm^2\ h^{-1}$)	Plateau (A_0)	Pseudo J ($D \times A_0$)
Untreated ($n = 4$)	6.83 ± 0.27	2.27 ± 0.29	15.5
Pre-saturated with water	6.34 ± 0.57	1.31 ± 0.04	8.31

found for the untreated membrane in this work was equal to that found by Watkinson et al., 1995 (which was also for an untreated membrane), there was an approximately two-fold difference between the values of the diffusion co-efficients. This was probably caused by batch-to-batch variations in the silicone membranes. It should be stressed that all of the membranes used in the present study were from the same batch.

The flux of cyanophenol across the pre-saturated and untreated membranes, as determined by diffusion cell experiments, was the same (Fig. 1). Nevertheless, it is clear from Table 2 that the pseudo flux values, as measured by ATR-FTIR spectroscopy, were different for the pre-saturated and untreated membranes. The pre-saturated membranes, analysed by both ATR-FTIR spectroscopy and using diffusion cells, were deliberately loaded with water prior to use. However, it is also likely that the untreated membranes used in the diffusion cell study would have been loaded with water before application of the donor solution, due to the inevitable uptake of the aqueous receptor medium during the 1 h equilibration period. Most receptor phases are aqueous based and it has been shown that water diffuses into and saturates a silicone membrane of 275 μm thickness within approximately 3 h (Pellett et al., 1995). In this respect, it is clear that the untreated membrane used in the ATR-FTIR spectroscopy study was the only one that was not loaded with water before the donor phase was applied. This may be the reason why there was a difference between the two ATR-FTIR spectroscopically derived values for pseudo flux. Furthermore, on calibration of the ATR-FTIR spectrometer, one might expect the pre-saturated membranes to yield permeability co-efficients identical to those found with the diffusion cells (as, in this case, all of the membranes were loaded with water prior to the experiment). In contrast, the pseudo flux value for the untreated membrane was higher than that for the pre-saturated membrane. Therefore, calculation of a permeability co-efficient from the ATR-FTIR spectroscopy data for the untreated membrane would yield a value greater than those obtained from both the diffusion cells and the ATR-FTIR spectrometer with the pre-saturated membranes. This issue is addressed in the following section.

3.3. Calculation of the permeability co-efficient as determined by ATR-FTIR spectroscopy

The use of ATR-FTIR spectroscopy for the determination of diffusional parameters in membranes is a relatively unexploited technique. Thus far, all ATR-FTIR spectroscopic data of this type has been quoted in terms of plateau levels and pseudo flux values. This is primarily because the issue of calibration of the ATR-FTIR system has not been addressed. Once this has been achieved it would be theoretically possible to compare permeability co-efficients measured using the ATR-FTIR system with those from diffusion cells.

In order to determine permeability co-efficients using ATR-FTIR spectroscopy, it was first of all necessary to determine the apparent partition co-efficient of cyanophenol between silicone and water. The apparent partition co-efficient is, in effect, a distribution co-efficient, which simply reflects the ratio of cyanophenol in the membrane to that in the vehicle. The plateau level of the cyanophenol in the membrane is a function (Eq. (6)) of the partition co-efficient (K), which is a ratio of the concentration of the compound in the outer layers of the membrane (C_m) to the concentration in the vehicle (C_v):

$$K = \frac{C_m}{C_v} \quad (6)$$

A calibration curve was constructed by first soaking silicone membranes in solutions of cyanophenol of different concentrations, then placing them on the surface of the ATR crystal and measuring the resultant CN peak areas. After this measurement the cyanophenol in the membranes was extracted with methanol five times (the success of this procedure being demonstrated by its absence in the fifth volume). Fig. 3 shows the calibration curve obtained in this manner. A good correlation ($R^2 = 0.931$) was obtained and the calibration curve was used together with the plateau levels in Table 2 to obtain the concentrations of cyanophenol in the membrane at the end of the diffusion experiments. This, together with the known vehicle concentration, was then used to determine the effective partition co-efficients as described below.

The plateau level for cyanophenol in the pre-saturated silicone membranes was 1.31 (Table 2). Using the calibration curve (Fig. 3), this equated to a concentration of cyanophenol present in the membrane at the end of the experiment of 1989 $\mu g\ ml^{-1}$. Therefore, assuming a negligible change in the vehicle concentration (15 100 $\mu g\ ml^{-1}$), a partition co-efficient of 0.132 was calculated (1989/15 100). A mean value for the permeability co-efficient was calculated by substituting into Eq. (2): $K = 0.132$; $D = 6.34 \times 10^{-4}\ cm^2\ h^{-1}$ (Table 2) and $h = 0.0275$ cm. This process gave a figure

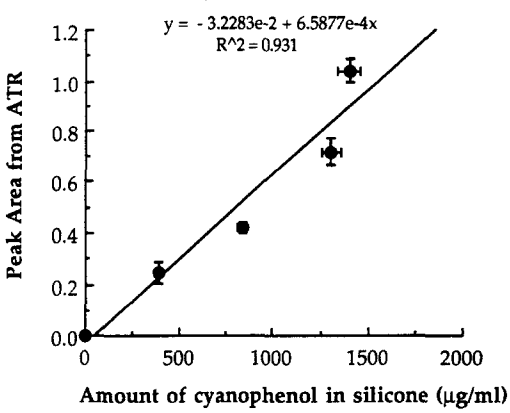

Fig. 3. Calibration of the ATR crystal for aqueous solutions of cyanophenol in silicone membranes.

Table 3
Summary of permeability coefficients determined by ATR-FTIR spectroscopy and diffusion cells within silicone membranes

Membrane	Measured permeability coefficients (cm h^{-1})		
	Diffusion cells	ATR-FTIR	Ratio
Untreated membrane	33.2×10^{-4}	56.5×10^{-4}	1.70
Pre-saturated membrane	27.6×10^{-4}	30.3×10^{-4}	1.10

of 30.3×10^{-4} cm h^{-1}, which compares favourably with the value of $27.6 \pm 3.6 \times 10^{-4}$ cm h^{-1} (Table 1) obtained from the diffusion cell data. A similar calculation for the untreated membrane, where the plateau level was found to be 2.27 (which equates to a membrane concentration of 3540 $\mu g\ ml^{-1}$, and hence a partition co-efficient of 0.234) yielded a value for the permeability co-efficient of 56.5×10^{-4} cm h^{-1}. As predicted above, this was greater than the values obtained from both the diffusion cells and the ATR-FTIR spectrometer for the pre-saturated membranes. A summary of these results is presented in Table 3. Therefore, there was a clear difference between the ATR-FTIR results gained for the untreated and pre-saturated membranes and this difference was manifested mainly in the plateau levels (and hence the partition co-efficients of cyanophenol within those membranes).

It should be noted that the procedure used to calibrate the ATR crystal involved placing membranes containing different amounts of cyanophenol on the crystal and measuring the CN peak area which was then correlated with the amount of cyanophenol subsequently extracted. These membranes were soaked in aqueous solutions of cyanophenol for lengthy periods. Therefore, they are more similar in nature to the pre-saturated membranes used in the diffusion experiments performed using ATR-FTIR spectroscopy and diffusion cells. In the above calculations, it was necessary to assume that a Beer-Lambert calibration plot (Fig. 3) remained linear up to a CN peak area of 2.27 (for the untreated membrane, Table (2).

This assumption was necessary because, after soaking the membranes in saturated aqueous solutions of cyanophenol, the maximum achievable CN peak area was 1.1.

There are at least two possible reasons for the difference between the plateau levels found for the pre-saturated and untreated membranes with ATR-FTIR spectroscopy. Firstly, this phenomenon may have been due to the use of an invalid calibration for the untreated membrane. If the calibration was incorrect, then it can be assumed that the presence of water in the membrane altered, in some way, the signal associated with the CN peak. This is feasible, as the influx of water into the membrane may change its refractive index, which is a determinant of the depth of penetration of the IR beam and hence the signal strength from the CN group of cyanophenol. If the refractive index of the membrane changed, one would expect a difference in signal strength from equal amounts of cyanophenol in pre-saturated and untreated membranes. However, although water probably has a lower refractive index than silicone (and a decrease in membrane refractive index will result in a drop in IR beam penetration), the difference is unlikely to be great enough to explain the observed two-fold variance in plateau levels. An alternative possibility that may have induced changes in refractive index of the pre-saturated membrane is the removal of leechable membrane materials (for example fillers or plasticisers) by water. Furthermore, if the influx of water into the membrane induced swelling, then this may also have changed the signal from the cyanophenol. If either, or all, of these effects were significant then the application of the calibration derived from pre-saturated membranes to the data derived for the untreated membrane may be questionable. This is the first possible reason why the permeability co-efficient derived using ATR-FTIR spectroscopy was higher for the untreated membrane than the pre-saturated membranes.

Secondly, it is possible that the use of the calibration derived from membranes saturated with water for the untreated membrane was valid i.e. that the influx of water and possible concurrent efflux of membrane components did not change the refractive index of the silicone. This argument is backed by the fact that, after the untreated membrane on the ATR crystal became saturated with cyanophenol and the signal plateaued at a peak area of 2.27, there was no subsequent reduction in this plateau level (Fig. 2), i.e. if changes in refractive index were responsible for the differences observed in plateau levels in untreated and pre-saturated membranes then one might have expected the saturation plateau level (2.27) in the untreated membrane to drop as water entered it. This was clearly not the case (Fig. 2) which implied that the presence of water in the membrane did not affect it's refractive index. Therefore, if we now assume that the calibration was linear up to the region where the CN peak area was 2.27, then this was a true reflection of the amount of cyanophenol in the untreated membrane at equilibrium in the ATR experiment. The implication of this was that partitioning of cyanophenol into the membrane was dependent on whether the silicone was soaked in water or not prior to the ATR experiment. Consequently, it appeared that the presence of water in the membrane reduced the partitioning of cyanophenol into it which was curious as the value of $K < 1$. It was more likely that the presence of water in the membrane may have reduced its capacity to hold cyanophenol, by the extraction of removable constituents such as plasticisers and fillers, but that this did not alter the refractive index of the silicone.

3.4. An assessment of the effect of using air or water as a background spectrum in ATR-FTIR spectroscopic measurements

For the ATR-FTIR spectroscopy experiments, air was routinely used as the background reference spectrum although, during a diffusion run, water diffused into the membrane. The effect of water on the measurement of the CN peak area was investigated by using either air or water as a background spectrum and placing different known concentrations of aqueous solutions of cyanophenol directly on the crystal surface. CN peak areas were measured for the different background spectra and are compared in Fig. 4.

Since the gradient of the straight line was approximately unity, it was shown that, in this case, the type of background spectrum did not interfere with the measurement of the CN peak area.

4. Conclusions

It has been demonstrated that, as long as all initial conditions are kept effectively the same, there was a close correlation between permeability co-efficients measured by the use of regular diffusion cells and ATR-FTIR spectroscopy. Furthermore, it could be suggested that the deconvolution of diffusional and partitioning effects is more reliable when performed using the ATR-FTIR spectroscopy technique. This argument is largely based around the ease and reproducibility of the measurement of plateau levels determined using ATR-FTIR spectroscopy. The levels at which these curves plateau are a true reflection of the solubility of a permeant in a membrane and represent a definite end-point to the experiment. As already stated, the interpretation of diffusion cell data is complicated by the difficulty of assessing when steady-state diffusion is achieved (Potts and Guy, 1994). Although the P_1P_2 method of fitting, as used in this paper, makes this approach less subjective, it is still reliant upon the attainment of steady-state diffusion within the experimental time frame. Furthermore, the variation associated with the measurement of relatively small lag times is large and the number of data points on a diffusion profile limited by practical constraints. These observations raise questions regarding the appropriateness of the use of diffusion cells for the assessment of subtle mechanistic analyses of permeation processes. However, they in no way invalidate the use of such techniques for the measurement of gross formulation effects.

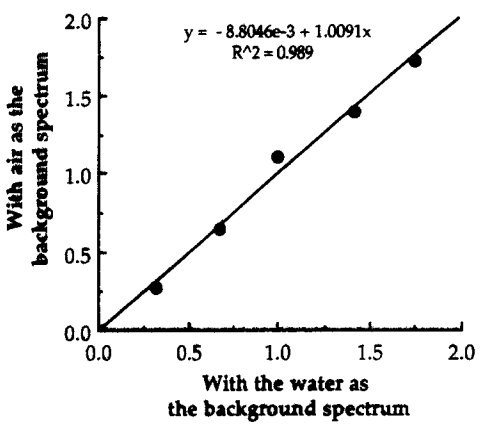

Fig. 4. The effect of different background spectra on the measurement of the CN peak area.

5. Nomenclature

a	factor relating A_0 to C_m
A_0	level at which the absorbance of the CN group plateaus in an FTIR-ATR experiment (plateau level)
C	concentration of a permeant at the ATR crystal/membrane
C_m	saturation concentration of a permeant at the crystal membrane interface
C_v	concentration of permeant in the vehicle
D	diffusion co-efficient
h	diffusional pathlength
J	steady-state flux
k_p	permeability co-efficient
K	partition co-efficient
P_1	Kh
P_2	D/h^2
t	time
t_{lag}	lag time
$u(t)$	flux across a membrane

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