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The influence of vehicle on permeation from saturated solutions

Adam C. Watkinson [a,*], Henri Joubin [b], Darren M. Green [a], Keith R. Brain [a,c], Jonathan Hadgraft [c]

[a] *An-eX, Redwood Building, King Edward VII Avenue, Cardiff CF1 3XF, UK*
[b] *Faculte des Sciences Pharmaceutiques et Biologique, 5 Rue Albert Lebrun, 54001, Nancy, France*
[c] *Welsh School of Pharmacy, UWCC, Cardiff CF1 3XF, UK*

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Abstract

The penetration of two compounds from three different vehicles through a silicone membrane has been studied. The permeants under investigation were cyanophenol and cyanonaphthalene and the solvents studied were water, ethanol and polyethylene glycol 400 (PEG 400). Initially, Franz cell experiments were conducted to determine gross vehicular and receptor phase effects. Subsequently, attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy permitted the measurement of both solvent and solute permeability and the deconvolution of the diffusional and partitioning steps involved in the permeation process. It has been shown that the extent to which a vehicle may alter the degree of penetration of a substance can, in the cases studied, be related to the degree of penetration of the vehicle itself. Further, the permeant parameter that is most affected by such vehicular penetration is its solubility in the membrane under study rather than its diffusion coefficient. In situations where the permeant is at very high concentrations in the vehicle (in this case around 50% w/v) the nature of the primary permeant may start to affect the degree of penetration of the vehicle itself.

Keywords: Vehicle; Permeation; ATR-FTIR; Diffusion coefficient; Partition coefficient

1. Introduction

The investigation of membrane permeability is often carried out in vitro by the determination of simple diffusion profiles using Franz type diffusion cells. This approach is extremely useful if all that is required is the determination of permeability coefficients, or the estimation of the efficacy of a transdermal system. Recently (Potts and Guy, 1994), some doubt has been cast on the use of this methodology for the determination of compounds that exhibit slow diffusion and consequently lengthy lag times. The assessment of the onset of steady state (a pre-requisite to the calculation of diffusional parameters) is not always clear-cut in these cases and may not even be attainable in the time frame of an experiment (especially if the membrane under study is for

* Corresponding author.

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example, epidermal, and the experiment duration limited by the natural in vitro degradation of barrier function). Further, one of the problems in understanding the barrier function of skin and synthetic membranes and in assessing the mechanism of action of penetration modifiers is that it is very difficult to separate the diffusional from the solubility and partitioning steps involved in the transfer process.

Attenuated total reflectance Fourier transfer infrared spectroscopy (ATR-FTIR) has been used to examine the diffusion process in both creams (Wurster et al., 1993) and synthetic membranes (Brandt, 1985; Hemmelmann and Brandt, 1986; Brandt and Hemmelmann, 1987; Potts et al., 1994). The technique is particularly useful as it allows the deconvolution of diffusional and partitioning phenomena in synthetic membranes where the diffusional path length is known (Watkinson et al., 1994a,b). Some of the problems associated with the attainment and assessment of steady state are also circumvented. ATR-FTIR also allows the concurrent measurement of the diffusional parameters of both the primary permeant and its formulation solvent(s) if both species diffuse into the membrane under study (this is reliant on the two, or more, species having IR absorbances that are distinct from each other and those of the membrane). The technique is fairly well established and a full discussion of the underlying theory is not appropriate here as it is covered adequately in recent publications (e.g., Wurster et al., 1993). However, for the sake of clarity the basics are covered in section 2 of this paper.

This study describes a series of experiments designed to investigate various phenomena relevant to membrane permeation. The work involved the assessment of how three different vehicles (ethanol, polyethylene glycol 400 (PEG 400) and water) affected the penetration of two permeants of differing lipophilicities (cyanaophenol and cyanonaphthalene) through a single synthetic model membrane. By utilising the two techniques outlined above (Franz diffusion methodology and ATR-FTIR spectroscopy) we hoped to demonstrate some aspects of how vehicles can affect the process of membrane permeation.

2. Materials and methods

2.1. Materials

The permeants, 4-cyanophenol (95%) and 4-cyanonaphthalene (98%), were purchased from Aldrich and used as received. Acetonitrile, ethanol and PEG 400 were obtained from BDH. Silicone membranes (thickness 300 μm) produced by Samco [1] (St. Albans, UK) were used as supplied.

2.2. Solubility studies

The solubilities of cyanophenol and cyanonaphthalene were determined in each of the solvent systems examined by stirring excess quantities of each compound with the relevant solvent for 24 h. After this time period samples were taken and, after filtration, analysed by HPLC for solute content. The chromatographic conditions for the analysis of cyanophenol and cyanonaphthalene in receptor phase samples were as follows, Constametric 3200 pump, SpectroMonitor 3200 detector and a Marathon autosampler. Data collection and integration were conducted using LCtalk [1] software. Separation was conducted on an Apex I ODS 25 cm $\times$ 4.6 mm column (Jones Chromatography); mobile phase, acetonitrile/water 30:70 for cyanophenol (cyanonaphthalene, 60:40); detection, UV at 284 nm; and flow rate, 1.0 ml/min. The retention times of cyanophenol and cyanonaphthalene under these conditions were 4.6 and 6.7 min, respectively. Calibration curves were constructed using four standard solution for each of the compounds studied.

2.3. Franz diffusion studies

Diffusion profiles were determined using Franz-type cells. The donor chambers were filled with 1 ml of saturated solutions of solute in the appropriate solvent (either ethanol, PEG 400 or water) with sufficient excess solid present to maintain saturation throughout the experiment. The receptor phase was distilled water which was

continually stirred and maintained at 25° C during the experiment. The silicone membranes were sandwiched between these two chambers. Six replicates were conducted for each of the systems studied. 200 μl samples were withdrawn from the receptor chamber at 1, 2, 4, 6, 8, and 24 h and replaced with fresh water. Samples were analysed for cyanophenol and cyanonaphthalene content using the HPLC methods outlined above.

2.4. ATR-FTIR studies

ATR-FTIR studies were conducted as follows. Membranes were placed in direct contact with the surface of a ZnSe attenuated total reflectance crystal mounted on a Nicolet 710 FTIR spectrometer. Once the membrane was flat on the crystal a specially constructed PVC trough was placed on top of it. The trough and film were sealed together with petroleum jelly (the join was monitored for leaks throughout the experiment). The solvent/solute system under study was then placed in the PVC trough above the film and the trough was sealed with a plastic cover held in place by a brass weight. The spectrometer was linked to a PC equipped with Nicolet Omnic software to allow the automated collection and subsequent manipulation of IR spectra. The time interval between the collection of spectra was 3 min. Three experiments were run for all solvents for both compounds. As the experiments progress there is an increase in the IR peak areas associated with the penetrating species. After each run the peak areas associated with the CN stretch at 2230 cm^{-1} and the OH stretch at about 3300 cm^{-1} were calculated to give measurements of the permeation of the cyano compound and the solvent, respectively.

Eq. 1 (where C is the permeant concentration at the interface at any time t, D the permeant diffusion coefficient, h the film thickness and C_0 the solubility of penetrant in the membrane) describes the build up of a penetrant at the lower membrane/crystal interface with time (Wurster et al., 1993). There is an initial period where the permeant concentration at the interface increases followed by an exponential rise to a plateau that represents saturation of the membrane with the permeant:

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

It is possible, assuming that the Beer-Lambert law is obeyed, to replace the concentration terms in this equation with experimental absorbance values to give Eq. 2 where A is the area under the curve of the penetrant peak (at time t) of the IR absorbance relating to the permeant and A_0 the area under the curve of the penetrant peak corresponding to the situation where the membrane is saturated (in the plateau region of the curve):

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

Experimental values of penetrant peak areas against time were fitted using Eq. 2 and a non-linear curve fitting package (Ultrafit from Biosoft, Cambridge, UK) on an Apple Macintosh computer. The values of D/h^2 and A_0 were allowed to vary until the best fit was achieved as measured by the minimisation of χ^2. Values of the diffusion coefficient were subsequently calculated using the assumed diffusional path length of 300 μm (the membrane thickness). This process was conducted for both the permeant and the solvent for each of the systems studied.

3. Results and discussion

3.1. Cyanophenol

The solubilities of cyanophenol in the three test solvents are listed in Table 1 together with the fluxes and permeability coefficients of the compound across the silicone membrane studied.

Table 1
Fluxes (J, in $\mu g\ cm^{-2}\ h^{-1}$), solubilities (S, in $mg\ ml^{-1}$) and permeability coefficients (P, in $cm\ h^{-1}$) of cyanophenol in the systems studied

Vehicle	J	S	P ($\times 10^4$)
EtOH	122 ± 2	652	1.87
PEG 400	25 ± 2	520	0.48
H_2O	42 ± 2	15.1	27.8

The associated permeation profiles of cyanophenol from each solvent through silicone membranes gained using the Franz cell technique are shown in Fig. 1.

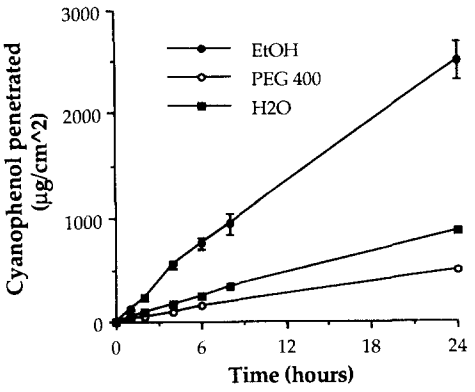

Fig. 1. Permeation of cyanophenol through silicone membranes from different vehicles (± SE).

The vehicles used in these studies were all deliberately saturated with respect to the solute and contained sufficient excess solute to maintain a constant donor concentration during the experimental time frame in all cases. Under stable, (equilibrium) conditions flux will be at a maximum when the outer layer of a membrane is saturated and this will occur when the vehicle is also saturated with solute. The saturated solubility of a solute in a vehicle will be related to that in the membrane by, $C_{vehicle} = C_{membrane}/K$ where K is the partition coefficient. Hence, if $C_{membrane}$ is to remain constant the product $KC_{vehicle}$ must stay constant. Thus, in an ideal situation all saturated solutions of the same permeant in any solvent system should produce an equal flux through a membrane that is independent of solute concentration.

Examination of the values presented in Table 1 shows that for cyanophenol the flux across the silicone membrane used here was not constant. The direct implication of this result is that some or all of the situations investigated were not ideal, i.e., there was some interaction between the vehicle and the membrane that increased either the product $KC_{vehicle}$ or the diffusion coefficient of the solute in the membrane. Such an interaction may involve diffusion of the solvent into the membrane where it may increase or decrease the partition and/or diffusion coefficient of the solute. Alternatively, it is possible the vehicle may act as an extraction solvent and remove something from the membrane, e.g., a plasticizer, thus modifying its resistance to permeation. PEG 400 produced the lowest flux (Table 1) of the three systems investigated with the flux from water being approximately twice this value. By far the greatest flux arose from the system where ethanol was the vehicle.

If the deviations from ideality seen here are explicable in terms of vehicle/membrane interactions it might be expected that the influx of vehicle into the membrane would be related to this deviation, i.e., if, for example, solvent influx into the membrane were responsible for increased solute penetration we would expect this influx to be in the order $EtOH > H_2O >$ PEG 400. It is not possible to say at this stage if any of

Table 2
Diffusion coefficients, D, plateau values, P and pseudo (diffusion × membrane solubility) term, DP for cyanophenol

Vehicle	Data from CN stretch of cyanophenol			Data from OH stretch of solvent		
	D_{CP} ($\times 10^7$) ($cm^2\ s^{-1}$)	P_{CP}	DP_{CP} ($\times 10^7$)	D_{OH} ($\times 10^7$) ($cm^2\ s^{-1}$)	P_{OH}	DP_{CN} ($\times 10^5$)
ETOH	0.61 ± 0.09	51 ± 0.7	31.1	1.0 ± 0.2	667 ± 53	6.67
PEG 400	1.6 ± 0.16	0.67 ± 0.10	1.07	0	0	0
H_20	1.1 ± 0.1	2.3 ± 0.2	2.5	0.77 ± 0.09	151 ± 13	1.16

these systems are behaving ideally. However, on the premise that influx of solvent into a membrane will increase the partition coefficient of the solute it can be said that only the PEG 400 system may be ideal and that the other two represent positive deviations from this. If this were the case we would expect little or no diffusion of PEG 400 into the membrane compared to ethanol or water. This is not an unlikely scenario as the size of a PEG 400 molecule is significantly greater than that of the other two species and on this basis alone we might expect its flux into the membrane to be lower. Of course, the reverse argument also applies in that if solvent influx into the membrane were to reduce solute penetration the order of solvent penetration would be reversed.

Table 2 contains the results of the ATR-FTIR studies conducted using the same cyanophenol solutions as a permeants. In it are contained values that pertain to the diffusional and partitioning behaviour of both the solute and solvent in each of the systems studied. This is made possible by the concurrent monitoring of changes in both the CN stretch peak area (associated with the accumulation of cyanophenol at the interface) and the OH stretch peak area (associated with the accumulation of the solvent, either ethanol, water or PEG 400 at the interface). Comparison of the fitted values for the diffusion coefficient of cyanophenol (D_{CP}) shows that there is little difference between those where water ($1.06 \pm 0.05 \times 10^{-7}$ cm^2 s^{-1}), PEG 400 ($1.61 \pm 0.16 \times 10^{-7}$ cm^2 s^{-1}) and ethanol ($0.61 \pm 0.09 \times 10^{-7}$ cm^2 s^{-1}) were the solvents. All of these diffusion coefficients yield lag times ($t_{lag} = h^2/6D$) of less than 1 h, in keeping with the Franz cell results (the measurement of such small lag times using Franz cells is obviously a problem that ATR-FTIR can circumvent). However, there is a much greater difference in the values of the plateau (P_{CP}) that are reached in these experiments. This parameter, as described in section 1, is related to the solubility of the permeant in the membrane. The largest plateau value for cyanophenol was produced for the ethanolic system (51 ± 0.7) followed by the water system (2.3 ± 0.2) and then the PEG 400 system (0.67 ± 0.10).

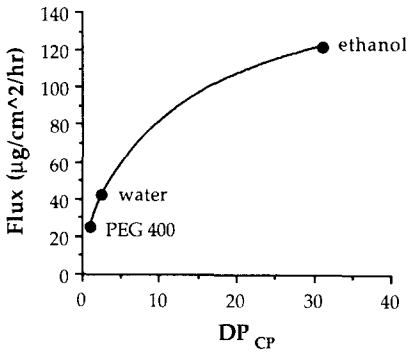

Fig. 2. Relationship between DP_{CP} and measured flux, J, for cyanophenol in silicone membranes.

To gain an overall picture of how each of these solvents affects the permeation process the plateau values were multiplied by the diffusion coefficients to give DP_{CP}, a pseudo value for $DC_{membrane}$ (the product of diffusion coefficient and permeant concentration in the membrane). These results are given in Table 2 (it must be stressed that these figures are not values of $DC_{membrane}$ but are proportional to this composite parameter through the fact that the plateau level is related to $C_{membrane}$). These results clearly reflect those of the Franz cell experiments (in that the rank order of DP_{CP} is the same as that of the fluxes seen in the Franz cell work) and indicate that vehicular induced changes in partitioning into the membrane have the greatest responsibility for the deviations from ideality seen.

A plot of fluxes obtained from the Franz cell work against DP_{CP} values is shown in Fig. 2. It is interesting to note that the relationship between J and DP_{CP} is not, as might be expected, linear. This requires further investigation as it is difficult to draw too many conclusions based on only a small number of systems. However, J (the flux) is a measured parameter which is probably more reliable and hence it appears that the DP value for the ethanolic system is overestimated. This implies that either the diffusion coefficient or the plateau level are too great. The former is very similar to those values measured from the water and PEG 400 vehicles so it is perhaps more likely

that the plateau value is overestimated. In ATR spectroscopy the depth, d_p, to which the IR beam penetrates an object placed on the crystal surface is related to the relative refractive indices of the membrane and crystal by Eq. 3 (Xu and Balik, 1988) below (where n_1 is the refractive index of the crystal, n_2 the refractive index of the membrane, λ the beam wavelength in the denser medium and θ the angle of incidence at the interface.

$$d_p = \frac{\lambda}{2\pi n_1 \left[\sin^2\theta - \left(\frac{n_2}{n_1}\right)^2\right]^{0.5}} \quad (3)$$

The absorption of solvent by the membrane may alter the ratio n_2/n_1 such that the depth of penetration of the IR beam into the membrane is changed. If the beam penetration depth is increased it follows that the beam will pass through a greater number of penetrating molecules and thus be attenuated to a greater degree, i.e., the CN absorption band will increase. This may lead to an increased plateau level relative to the case where the refractive indices of the system are constant. Examination of the degree of solvent penetration as shown by P_{OH} in Table 2 indicates that there is a much greater penetration of ethanol than water or PEG 400 into the membrane. The refractive indices of water and ethanol are extremely similar (about 1.3) so any differential changes in the refractive index of the membrane on solvent uptake must be due to differing interactions of the solvent with the membrane. In the case of ethanol one can envisage that it may have a greater disruptive affect on the optical nature of a polymer than water thus leading to a possibly greater change in the magnitude of n_2/n_1 in Eq. 3.

There is also a wavelength term in Eq. 3, meaning that the depth of penetration of the beam into a membrane placed on the ATR crystal will be dependent on the wavelength of the incident radiation, i.e., the depth of beam penetration will vary with wavenumber and thus position in the IR spectrum. For example, the CN stretch at a frequency of 2230 cm^{-1} has a wavelength of 4.5×10^{-4} cm whilst that of the OH stretch at a frequency of 3300 cm^{-1} has a wavelength of 3.0×10^{-4} cm. The IR beam would therefore be expected to penetrate a membrane above the crystal to a greater depth (approx. 50% greater) when measuring the CN stretch compared to the OH stretch (and thus give an accordingly stronger absorbance). However, in this paper no direct numerical comparisons are made between the relative degrees of penetration of the solvent and the solute. The assessment of trends regarding the relationship between the penetration of different solvents with the same IR absorbance and a solute will not be affected by this argument and are perfectly legitimate. If, for example, this technique were to be used to assess the relative membrane solubilities of compounds that have markedly different IR absorbances this effect may become important.

A second way of approaching a comparison of the two techniques used in this work is to use the results of one to predict those of the other. The first problem in trying to use the results of the ATR experiments to predict those of the Franz cell work is that the values of the plateau levels (P_{CP}) measured in the membrane are only a reflection of the relative membrane solubilities of the permeant in the presence of each solvent. i.e. as pointed out earlier they are not absolute solubility values. This problem can be made simpler by a normalisation procedure. If all the Franz data are normalised with respect to the same reference point and the P_{CP} values are also normalised in the same manner direct comparison of the data from the two methods becomes possible.

Firstly, the Franz diffusion data at each time point in all the systems studied were normalised with respect to the amount of cyanophenol permeated from the PEG 400 vehicle at 24 h (this vehicle was chosen as a baseline because it appears to behave ideally and does not penetrate the membrane itself). Secondly, the ATR derived diffusion coefficients and values of P_{CN} (Table 2) were placed in Eq. 4 (Crank, 1975) to produce simulated profiles for diffusion through an inert slab of thickness 0.03 cm. In this equation the value of C_m (the drug concentration in the mem-

brane) was replaced by P_{CN} as this is proportional to C_m:

$$u(t) = hC_m\left[D\frac{t}{h^2} - \frac{1}{6} - \frac{2}{\pi^2}\sum_{n=1}^{\infty}\frac{(-1)^n}{n^2} \cdot \exp\left(\frac{-Dn^2\pi^2 t}{h^2}\right)\right] \quad (4)$$

The data produced by Eq. 4 were again normalised with respect to the 24 h value for the PEG 400 system that the equation yielded to allow comparison with the Franz cell data. The two data sets for PEG 400 and water are compared in Fig. 3. As would be expected the cyanophenol/PEG 400 data sets are almost perfectly superimposed. However, although the fit to the cyanophenol/water data is good up to about 8 h there is a noticeable drop of the experimental data away from the fitted line in the ensuing time period up to 24 h. This may indicate time-dependent changes in the membrane during the Franz study that are not seen in the ATR work as the experimental time frame is shorter.

Fig. 4 compares the normalised cyanophenol/ethanol Franz data set with that predicted by the ATR data. It is clear that there is a large discrepancy between the two sets of results. This has already been pointed out (Fig. 2) and discussed above in that it implies a gross

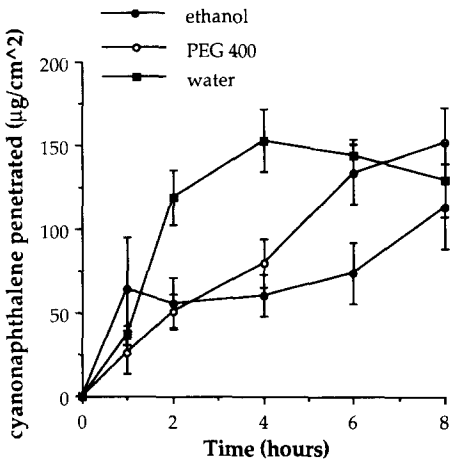

Fig. 3. Permeation of cyanonaphthalene through silicone membranes from different vehicles.

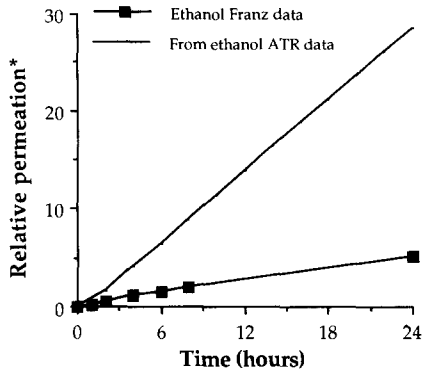

Fig. 4. Comparison of ATR derived simulated diffusion profiles with Franz cell diffusion profiles.

overestimation of the cyanophenol diffusion coefficient or more likely its membrane solubility.

Table 2 also contains the results obtained by integrating the areas under the OH stretches associated with permeating solvents from the systems investigated. It was suggested earlier that the PEG 400 vehicle may represent a near ideal system and that if this were so there may be only negligible diffusion of this solvent into the membrane. This appears to be confirmed by the lack of any OH peak in the ATR experiments conducted with PEG 400 as the vehicle. The hypothesis that the degree of solvent penetration may effect the deviation from ideality is also supported by these data as the DP_{OH} value for ethanol is greater than that for water. This assumes that these two solvents have an equal effect on the membrane per molecule. It may be that the areas under the OH peaks associated with equal amounts of ethanol and water will not be the same (there are two OH stretches in water and only one in ethanol and the contact area of the molecules may differ as may the degree of intensity of the stretch itself). To quantitate these effects two (short) experiments were performed. Mixtures containing equal volumes of D_2O and ethanol, or H_2O and ethyl alcohol-*d*, were made up and their (respective) ATR-FTIR spectra recorded. The ratio of the OH peak area of H_2O to ethanol from these experiments was 3.4. This implies that H_2O will give a larger peak area than ethanol for the same volume of solvent at

Table 3
Solubility (mg ml^{-1}) of cyanonaphthalene in the systems studied

Vehicle	S
EtOH	477
PEG 400	55
H_2O	0.069

the interface. However, because the two substances have different densities (H_2O = 1.0, ethanol = 0.785) there will be a different number of molecules at the interface per unit volume of solvent. Calculation of the molecular ratio per unit volume (H_2O/ethanol) yields a value of 3.3. The extreme proximity of this value to that measured experimentally implies that the 'OH peak area per molecule' is the same for each species. In this respect the equilibrium ratio of ethanol to water OH areas of approx. 4.4 (calculated from the plateau areas in Table 2) is a true reflection of the molecular ratio of ethanol to water in the membrane. This technique could, perhaps, be used to assess the densities of materials relative to a known standard.

A further point to note is that the values of the plateau levels reached by the cyanophenol peaks are not correlated to the solubilities of cyanophenol in the vehicles. This implies that we are indeed monitoring the situation inside the membrane itself with the ATR technique and not just seeing the applied solutions diffusing through and out of it.

3.2. Cyanonaphthalene

Table 3 contains the measured solubilities of cyanonaphthalene in the three systems studied and the greater lipophilicity of this compound (than cyanophenol) is clearly reflected here.

The water solubility is much reduced (approx. 220-fold) and the ethanol and PEG 400 solubilities are also lower than for cyanophenol (1.4- and 9.5-fold, respectively). This is a reflection of the relative lipophilicities of all the compounds involved here.

Fig. 3 shows the results of the Franz diffusion cell experiments with cyanonaphthalene as the permeant from the three solvents studied. Upon examination of this data it is apparent that the diffusion curves gained for the ethanol and water vehicles quickly rise to a plateau value and increase no further. This is most likely due to receptor phase saturation as the water solubility of the diffusant is very low (calculation of the maximum $\mu g\ cm^{-2}$ that the receptor could accommodate yields a value of about 180 $\mu g\ cm^{-2}$ indicating that this plateauing effect occurs in the correct region even if the curves are somewhat erratic in nature). There is no such effect where the solvent is PEG 400. This is possibly due to the slower rate at which this compound may enter the membrane making saturation of the receptor a slower process which does not occur within the time frame of the experiment. Comparison of this cyanonaphthalene flux (from PEG 400 = 28 ± 2 $\mu g\ cm^{-2}\ h^{-1}$) with that of cyanophenol (25 ± 2 $\mu g\ cm^{-2}\ h^{-1}$) from the same solvent shows that there is little difference between them.

The ATR results for the diffusion of cyanonaphthalene through the silicone membranes are shown in Table 4. The rank ordering of the overall diffusion process (DP_{CN}) is the same as seen with cyanophenol, i.e., diffusion from EtOH > H_2O > PEG 400. The success with which these results were achieved shows how the ATR technique has an advantage over the use of Franz cells for the determination of diffusional parameters for lipophilic compounds. The receptor phase

Table 4
Diffusion coefficients, D, plateau values, P and pseudo (diffusion × membrane solubility) term, DP for cyanonaphthalene

Vehicle	Data from CN stretch of cyanonaphthalene			Data from OH stretch of solvent		
	D_{CN} ($\times 10^7$)	P_{CN}	DP_{CN} ($\times 10^7$)	D_{OH} ($\times 10^7$)	P_{OH}	DP_{OH} ($\times 10^5$)
ETOH	1.6 ± 0.19	31 ± 3.6	49.6	3.9 ± 0.75	334 ± 53	13.0
PEG 400	5.4 ± 0.78	0.36 ± 0.15	1.94	0	0	0
H_2O	1.2 ± 0.19	8.8 ± 1.6	10.6	0.88 ± 0.12	142 ± 12	1.25

saturation that occurred with the Franz study is a common problem when determining data for such lipophilic compounds. Often the only way around this is to use a non-aqueous or more hydrophobic receptor to better accommodate permeant. These alternative receptor phases are more likely to interfere with the membrane being studied in a manner that may affect its barrier function (this is particularly the case with biological membranes where processes such as lipid extraction may occur).

The majority of these differences in DP_{CN} can be accounted for by differences in the plateau level, indicating that the solubility of the cyanonaphthalene is increased in the membrane where ethanol and water are the solvents. Again this appears to correlate with the influx of solvent into the membrane indicating that this is an important factor in this diffusion process.

The diffusion coefficient of cyanonaphthalene from the PEG 400 system is significantly higher than the values obtained for diffusion from the other two systems. This is also the case for the cyanophenol/PEG 400 system (Table 2) although to a much lesser degree. It is feasible that the diffusion of ethanol and water into the membrane actually reduce the diffusion coefficient of the solute. The mechanism by which this might occur is not clear but if the small molecules of ethanol and water formed an associated solvent cage around the solute molecule it is possible to envisage a lower diffusion coefficient for this complex than for a lone solute molecule. This situation will not arise when PEG 400 is the solvent as it does not appear to enter the membrane.

Comparison of the diffusion coefficients for cyanophenol and cyanonaphthalene show that they are all of approximately the same order except in the case where PEG 400 is the solvent. Here the value obtained for cyanonaphthalene is about 3.4-times larger than that obtained for cyanophenol. It is conceivable that PEG 400 may complex cyanophenol in the donor phase to a greater extent than cyanonaphthalene (simply because of the difference in molecular size) and therefore slow its diffusion. This process should not affect the partitioning as the absolute amount or solubility of a compound is not a kinetically controlled parameter. In fact, comparison of the plateau levels reached for cyanonaphthalene and cyanophenol show that they are greater for cyanophenol in the case of the ethanol and PEG 400 vehicles but lower for the cyanophenol in the case of the water vehicle. This result is at odds with the rank ordering of the solubilities of these two compounds in the three solvents.

Comparison of the ATR data obtained for the diffusion of the solvents into the membrane in the two cases studied, i.e., for cyanophenol and cyanonaphthalene shows some interesting phenomena. Both the diffusion coefficient and plateau level are statistically the same for the system where water and PEG 400 (here they were both zero) were the solvents. However, in the case where the ethanol was the vehicle the plateau values and diffusion coefficients of the solvent obtained for the cyanophenol system are significantly different than those obtained for cyanonaphthalene system. The diffusion coefficient of ethanol is greater in the cyanonaphthalene system and the plateau level greater in the cyanophenol system. This implies that, in this case, the permeation of the solvent is related to the nature of the permeant. In a system where the concentration of solute in the solvent is so high (as it is in the ethanol, 652 and 477 mg ml^{-1} for cyanonaphthalene and cyanophenol, respectively) that the solution becomes in the region of 50% solute it is not surprising that the nature of the solute may affect the diffusional behaviour of the solvent (in the same way that the reverse is true for more dilute, in terms of solute, systems).

4. Conclusions

The concurrent use of ATR-FTIR spectroscopy and Franz diffusion cell methodologies has demonstrated some interesting aspects of how vehicles may affect the process of permeation. It has been shown that the extent to which a vehicle may alter the degree of penetration of a substance can, in the cases studied, be related to the degree of penetration of the vehicle itself. Fur-

ther, it has been shown that the permeant parameter that is most affected by such vehicular penetration is its solubility in the membrane under study. In situations were the permeant is at very high concentrations in the vehicle (in this case around 50% w/v) the nature of the primary permeant may start to affect the degree of penetration of the vehicle itself.

The use of ATR-FTIR for the measurement of diffusional parameters is of great value, especially in the case of lipophilic compounds where the use of non-aqueous receptor phases in Franz experiments may compromise the barrier function that is being investigated.

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