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ATR-FTIR spectroscopic investigations on the effect of solvents on the permeation of benzoic acid and salicylic acid through silicone membranes

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Abstract

The effect of a series of alcohols on the permeation of salicylic acid (SA) and benzoic acid (BA) through silicone membrane was evaluated, using Franz-type diffusion cells. Although permeants were applied at the same thermodynamic activity in all vehicles, the resulting fluxes were found to differ significantly. This was a consequence of the interactions between the vehicles and the membrane. The interactions between the vehicles and the membrane were further investigated using ATR-FTIR spectroscopy. With this technique, it was possible to identify two different diffusion processes when the membrane was pre-treated with buffer, whereas one single diffusion process was observed when the membrane was pre-soaked with the vehicle. The technique was successfully used to deconvolute the relative magnitude of partition and diffusion in the permeation process. It was shown that the permeation of both acids was affected by the effect of the vehicles on the diffusion coefficient and the partition coefficient in the silicone membrane. The solubility of the drug in the impregnated membrane was found to be proportional to the saturated solubility in the vehicle used to treat the membrane. The solubility of BA in the impregnated silicone membrane was twice that of SA. © 2001 Published by Elsevier Science B.V.

Keywords: Benzoic acid; Salicylic acid; Silicone membrane; ATR-FTIR spectroscopy; Permeation

1. Introduction

The main barrier for permeation of most molecules through the skin is the *stratum corneum*. Many strategies have been suggested to overcome the skin impermeability. Penetration enhancement can be achieved by several methods. In order to optimise the formulation, it is essential to understand the mechanism of action of each com-

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ponent of the formulation. The interactions between the components of the formulation and the membrane can alter the diffusion of the drug through or partition into the membrane. Penetration enhancement can, therefore, be a consequence of either an increase in the diffusion

Table 1
Saturated solubilities, steady state fluxes and permeability coefficients of BA in the systems studied[a]

Vehicle	Solubility (mg/ml)	Steady state fluxes (μg/cm^2/h)	Permeability coefficient ($\times 10^{-3}$) (cm/h)
Decanol	123.8 ± 22.2	920 ± 18	7.4
Butanol	239.7 ± 11.7	1366 ± 115	3.7
Octanol	152.6 ± 9.5	1301 ± 129	8.5
Ethanol	340.1 ± 16.1	1546 ± 46	4.3
PG	240.8 ± 9.4	449 ± 42	1.9
Decanol/PG (80:20)	206.8 ± 4.7	664 ± 17	3.2
Decanol/PG (50:50)	225.1 ± 2.2	526 ± 20	2.3

[a] ($\pm$S.D., $n = 3$).

Table 2
Saturated solubilities, steady state fluxes and permeability coefficients obtained for SA in the vehicles selected[a]

Vehicle	Solubility (mg/ml)	Steady state fluxes (μg/cm^2/h)	Permeability coefficient($\times 10^{-3}$) (cm/h)
Decanol	136.9 ± 0.5	523 ± 28	3.8
Butanol	283.7 ± 0.9	1228 ± 109	4.3
Octanol	186.1 ± 2.5	834 ± 66	4.5
Ethanol	368.2 ± 4.6	1333 ± 129	3.6
PG	192.8 ± 19.3	108 ± 14	0.6
Decanol/PG (80:20)	225.1 ± 4.6	295 ± 11	1.3
Decanol/PG (50:50)	267.2 ± 15.4	272 ± 14	1.0

[a] ($\pm$S.D., $n = 3$).

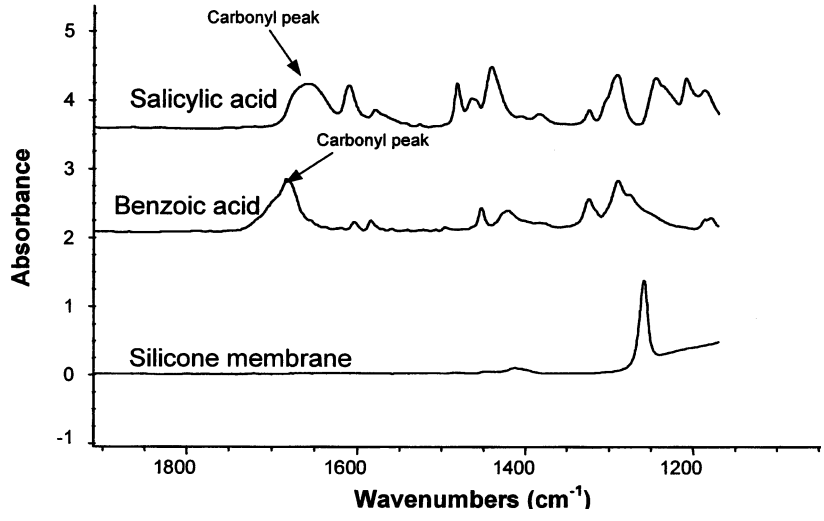

Fig. 1. IR spectra of salicylic acid, benzoic acid with the carbonyl peak around 1700 cm^{-1} and the silicone membrane with absence of any peaks in this region.

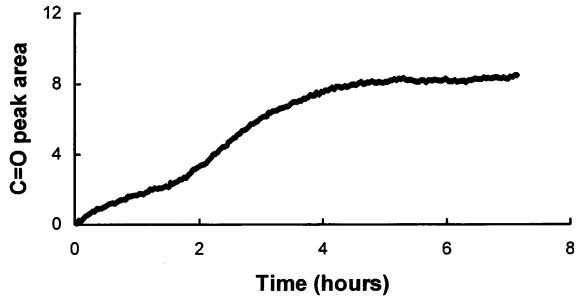

Fig. 2. Diffusion of BA in octanol through silicone membrane pre-treated with phosphate buffer.

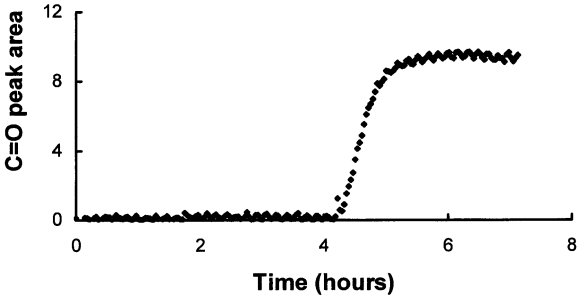

Fig. 3. Diffusion of octanol through silicone membrane (pre-treated with buffer for 4 h) followed by diffusion of saturated solution of benzoic acid in octanol.

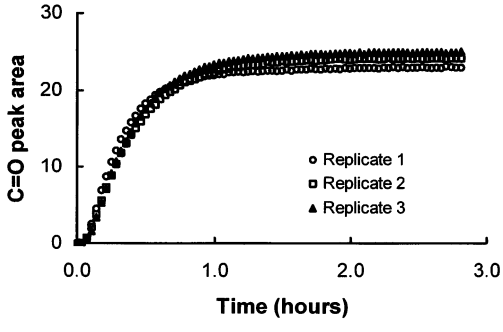

Fig. 4. Diffusion from a saturated solution of benzoic acid in ethanol, through silicone membrane pre-saturated (overnight) with ethanol showing three replicates.

coefficient (D) or the partition coefficient (K) of the drug, or both. If both strategies are possible, within the same formulation, a multiplicative effect on the flux of the permeant can be achieved Wotton et al. (1985).

Permeation experiments using Franz-type diffusion cells are often used to investigate the diffusion properties of a membrane. These experiments can give valuable information concerning the diffusion process, and provide permeability coefficients and steady state fluxes. However, it is often difficult to obtain absolute values of D. This arises as often there are insufficient data produced in the non-steady state region of the permeation profile to provide accurate values. If D cannot be measured it is difficult to separate the vehicle's effect on D and K.

One way to estimate both D and K is to perform a diffusion experiment using an ATR-FTIR technique (Farinas et al., 1994). Attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) has been successfully used to examine the diffusion profile through both silicone membranes and human skin. Harrison et al. (1996) demonstrated that Azone® and Transcutol®, two skin enhancers, exert their effect on human skin 'in vitro' by different mechanisms. Azone reduces the diffusional resistance and Transcutol increases the partitioning of the drug in the skin. On the other hand, Watkinson et al. (1994) showed that the vehicles selected in their study were mostly affecting the partition of the drug in the membrane rather than its diffusion coefficient.

In the present study, the mechanism of action of a series of alcohols on the permeation of two related permeants, benzoic acid and salicylic acid through a silicone membrane was evaluated. Artificial membranes were initially chosen, as they are more homogeneous than skin and therefore data analysis is simplified. It is easier to establish limitations in the experimental technique. The results provide insight into potential difficulties when more complex membranes such as skin are examined.

2. Materials and methods

2.1. Materials

Benzoic acid was purchased from Fisons Scientific Equipment (UK) and salicylic acid, propylene glycol, octanol, ethanol and butanol from Fisher

Table 3
Values of D/h^2 (b value), diffusion coefficient and plateau for BA from ATR-FTIR studies[a]

Vehicle	Thickness h (cm)	D/h^2	$D_{BA} \times 10^{-7}$ (cm^2/s)	Plateau
Decanol	0.0413	0.84 ± 0.10	3.98	12.53 ± 0.40
Butanol	0.0433	1.19 ± 0.03	6.20	19.30 ± 1.13
Octanol	0.0427	0.86 ± 0.06	4.34	14.53 ± 0.57
Ethanol	0.0420	1.27 ± 0.16	6.22	23.97 ± 1.02
PG	0.0423	0.51 ± 0.03	2.53	18.53 ± 0.51
Decanol/PG (80:20)	0.0417	0.52 ± 0.02	2.51	15.83 ± 1.35
Decanol/PG (50:50)	0.0423	0.51 ± 0.08	2.53	19.73 ± 0.98

[a] ($\pm$ S.D., $n = 3$).

Table 4
Values of D/h^2 (b value), diffusion coefficient and plateau for SA from ATR-FTIR studies[a]

Vehicle	Thickness h (cm)	D/h^2	$D_{BA} \times 10^{-7}$ (cm^2/s)	Plateau
Decanol	0.0413	0.70 ± 0.20	3.31	6.25 ± 0.40
Butanol	0.0433	1.23 ± 0.06	6.41	10.04 ± 0.32
Octanol	0.0427	0.78 ± 0.10	3.94	7.54 ± 0.14
Ethanol	0.0420	1.08 ± 0.12	5.31	12.27 ± 0.45
PG	0.0423	0.33 ± 0.10	1.66	7.98 ± 0.79
Decanol/PG (80:20)	0.0417	0.42 ± 0.07	2.03	9.63 ± 0.40
Decanol/PG (50:50)	0.0423	0.63 ± 0.01	3.13	11.0 ± 0.17

[a] ($\pm$ S.D., $n = 3$).

Scientific (UK). Decanol was obtained from Aldrich (UK). Silicone membranes with a thickness of 400 μm were purchased from Samco (St. Albans, UK).

2.2. Methods

2.2.1. Solubility studies

Excess drug was added to each solvent or co-solvent mixture and stirred with a magnetic bar for 48 h in a water bath maintained at 32°C. Solutions were centrifuged for 10 min. The supernatant solution was then diluted and assayed by UV spectroscopy. Experiments were performed in triplicate and mean values with S.D. were calculated.

2.2.2. Silicone membrane swelling

The thickness of the silicone membrane was measured using a micrometer (Mitutoyo, Digimatic Caliper). The membranes were cut to appropriate sizes and the thickness was measured. They were then placed in sample bottles containing the vehicles and soaked overnight. The excess solvent was removed with paper tissue and the thickness remeasured.

2.2.3. Diffusion cell studies

Diffusion studies of a variety of saturated solutions of benzoic acid and salicylic acid across

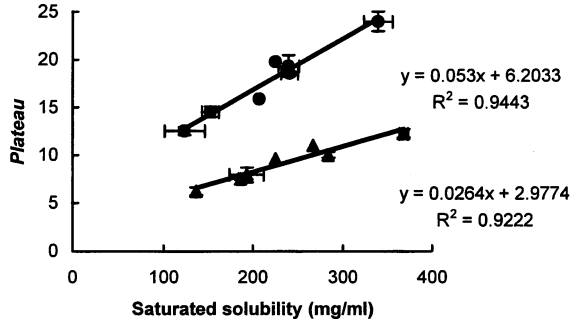

Fig. 5. Benzoic acid (circles) and salicylic acid (triangles) saturated solubility as a function of the P values (ATR-FTIR spectroscopy).

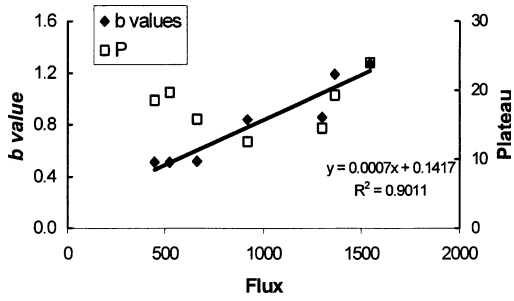

Fig. 6. Relationship between the measured steady-state fluxes obtained using the Franz-type cells and the *b* value (diamonds) and the *P* values (squares) obtained from the ATR-FTIR measurements.

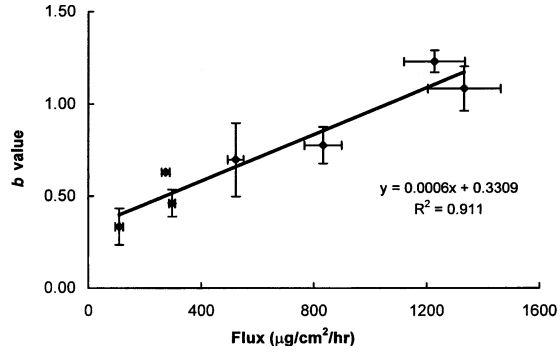

Fig. 8. Relationship between the *b* value (ATR-FTIR spectroscopy) and the measured flux values (conventional diffusion cells) for salicylic acid.

silicone membrane were performed using Franz-type diffusion cells, that have a receptor phase of ~ 2.5ml and a diffusional area of ~ 1cm^2.

Sheets of silicone membrane were soaked overnight in the receptor solution (pH 7.4 phosphate buffer saline (PBS)). The membrane was then placed between the two compartments of the cells.

The receptor compartment was filled with PBS and saturated solutions of the permeants studied were placed in the donor compartment. The cells were placed in a water bath so that the receptor compartment was maintained at 37°C. Under these conditions, which mimic in vivo studies, the membrane surface was 32°C. Excess solute was present to maintain saturation throughout the experiment. Thus, any drug lost from the solution by diffusion is replenished by dissolution of excess drug.

The whole content of the receptor phase was withdrawn at each sampling interval (2 h) for analysis by UV spectroscopy. The receptor phase was then refilled with pre-thermostated PBS.

The diffusion experiments were performed under occluded conditions over a 12-h period. Experiments were performed in quadruplicate.

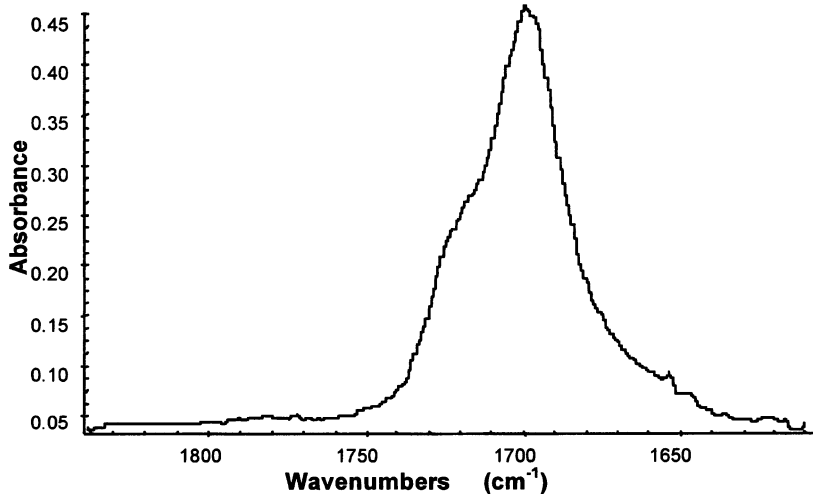

Fig. 7. Spectrum of BA in decanol obtained during the diffusion experiment through silicone membrane. The shoulder on the carbonyl peak of benzoic acid at 1700 cm^{-1} indicates the dimerisation of the drug.

2.2.4. Analysis

Benzoic acid and salicylic acid were analysed by UV spectroscopy using a Uvikon 860. Samples were appropriately diluted for analysis at 225 and 295 nm, respectively. One control for each vehicle was examined where the donor solution consisted of the vehicle without the permeant. This allows for any possible over estimation due to any absorbance of the vehicles which may have co-diffused across the membrane.

2.2.5. ATR-FTIR studies

Attenuated total reflectance infrared spectroscopy (ATR-FTIR) experiments were conducted on a Nicolet 710 FTIR spectrometer.

Briefly, in the ATR-FTIR technique, the membrane is placed in perfect contact with a ZnSe crystal. Perfect contact is observed visually; any air pockets or poor contacts are easily seen. A special trough (forming the donor compartment) is then positioned on the membrane surface and the solution is added. The arrival of both the permeant and the vehicle at the interface between the crystal and the membrane can be evaluated if discrete IR bands can be identified for them. If a saturated solution is used, the diffusion of the permeant into and through the membrane eventually reaches an equilibrium plateau, when the membrane is saturated with the drug. Therefore, the *Plateau* value is proportional to the solubility of the drug in the membrane, and it is related to the partition coefficient (K). The rate at which the *Plateau* is achieved is related to the diffusion coefficient of the permeant in the membrane (D). These parameters can be estimated by fitting an appropriate solution of the Fick's second law of diffusion Eq. (1) to the data.

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

Silicone membranes were soaked overnight either in PBS or in the vehicles and then placed in direct contact with the surface of the ATR crystal. Perfect contact between the membrane and the crystal was ensured as this can influence the degree of penetration of the IR beam. The PVC trough holding the donor solution was sealed with silicone grease and petroleum jelly to avoid possible leakage of the vehicle during the course of the experiment.

Saturated solutions of benzoic acid and salicylic acid were prepared at room temperature and then applied to the membrane. The trough was covered with a brass weight to help seal and prevent evaporation of the volatile solvents used.

Ten scans were taken (2 cm^{-1} resolution) every 2 min and an average spectrum was produced at each time point. The FTIR spectrometer was connected to a computer equipped with Omnic version 3.1 software (Nicolet Instrument Corp.) that allowed the collection and subsequent analysis of the IR spectra. All the experiments were performed in triplicate.

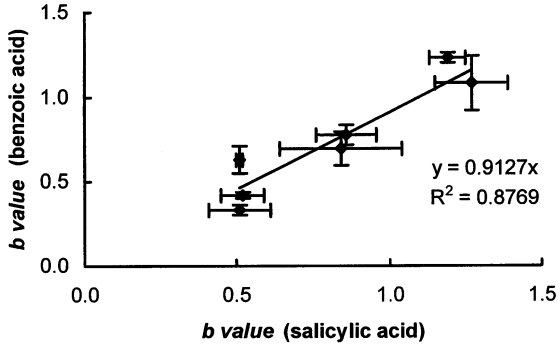

Fig. 9. Relationship between *b* values of salicylic acid and benzoic acid in the vehicles selected.

3. Results and discussion

3.1. Diffusion cell studies

Diffusion of benzoic acid (BA) and salicylic acid (SA) from saturated solutions through impregnated silicone membranes was evaluated. The flux was obtained by monitoring the cumulative amount of drug diffused as a function of time, and measuring the gradient of the graph once steady state was reached. Permeation was rapid and steady state was established within min.

Table 1 and Table 2 list the steady state flux values of SA and BA in the systems studied through silicone membrane, together with the saturated solubilities of both the drugs determined at 32°C.

Provided that there are no interactions between the vehicles and the membrane, all the saturated solutions of the same permeant should produce the same permeation rate. As saturated solutions with excess drug were used in the present studies, the permeation rates were anticipated to be constant for the different vehicles studied. However, the results show that the flux of BA and SA through the silicone membrane was not constant. This implies that interactions between the vehicles and the membrane occurred with a resulting variation in the flux values. These interactions might result from changes of either the diffusion coefficient (D) or the partition coefficient (K). Using a conventional diffusion experiment it can be difficult to deconvolute the diffusion from the partition phenomena. This is specially a problem for experiments where lag times are short and data collection in the non-steady state is not easy. For example, in these experiments, extrapolation of the linear portion of the profile gives an intercept on the time axis, which is variable and not significantly different to zero.

The permeation profiles and the saturated solubilities of the SA and BA in the vehicles selected were found to be similar. This is probably a consequence of the similar molecular structure of the two permeants. However, BA was found to have a higher permeation rate. This was more evident when PG was used as the vehicle, where the permeation rate of BA was four times the value obtained for SA.

3.2. Silicone membrane swelling

The increase in the membrane swelling was evaluated after treatment of the membrane with the vehicles. Butanol and octanol produced the greatest swelling of the membrane, increasing its thickness by 30 μm. Ethanol, PG and decanol/PG mixtures increased the thickness by about 20 μm. Decanol was found to produce the smallest increase in the thickness (10 μm). The membrane swelling was therefore not significant in the context of diffusional pathlength, it being less than 10% of the overall thickness.

3.3. ATR-FTIR studies

The ATR-FTIR technique is of great value in a mechanistic understanding of the effect of the vehicle on permeant diffusion provided both species have distinct IR absorbances. The IR spectra of benzoic acid and salicylic acid show peaks at around 1700 cm^{-1}, which corresponds to the carbonyl group present in both the species Fig. 1. The solvents investigated were decanol, octanol, butanol, ethanol, PG and decanol/PG mixtures. One of the reasons that these solvents were selected was that their IR absorbances have no interfering peaks around 1700 cm^{-1}. The IR spectrum of the silicone membrane also has no peaks around 1700 cm^{-1} (Fig. 1). By determining the carbonyl peak area at each time point and plotting it against time, it was possible to evaluate the build up of the drug at the interface between the membrane and the crystal, throughout the experiment.

3.3.1. Benzoic acid

The pre-treatment of the membrane may affect the permeation of the drug. In fact, Pellett et al. (1997) using the ATR-FTIR technique found that the untreated membrane gave a permeability coefficient for 4-cyanophenol almost twice that of a membrane pre-saturated with water. The permeation of saturated solutions of benzoic acid in octanol through silicone membranes pre-soaked in PBS was evaluated. This pre-treatment procedure provides the same experimental conditions as those in the conventional diffusion experiments.

Fig. 2 shows the diffusion profile obtained. Unexpectedly, two diffusion processes appear to be occurring. It is postulated that the first one corresponds to the permeation of BA through the membrane, unaltered by the octanol, followed by the permeation through the solvent modified membrane. In this case, Eq. (1) will not be valid.

To test the above-given suggestion, the diffusion of octanol through silicone membrane pretreated with buffer was evaluated. The time necessary to achieve saturation of the membrane with the octanol was 4 h. In subsequent experiments pure solvent was initially used as the donor phase and it was replaced, after a 4-h period, with

the corresponding saturated solution. Fig. 3 shows the diffusion profile obtained from this experiment. In this case there was only one apparent transport process unlike in the previous experiment. This confirmed the suggestion that the first process in Fig. 2 was occurring in an unaltered membrane. However, this phenomenon cannot be observed in a conventional diffusion experiment as it takes place during the first 4 h, during which time only two data points were obtained. It also exemplifies the problem of analysing the data in the non-steady state region where there could also be a time dependent change in the barrier properties of the membrane.

Few diffusion processes through silicone membranes have been evaluated using the ATR-FTIR technique. The most studied permeant has been cyanophenol, due to its distinct IR absorbance at 2230 cm^{-1}. The changing diffusion barrier with solvent was never observed probably because the vehicles selected in previous studies (water, polyethylene glycol and propylene glycol) were not altering the properties of the membrane significantly. It would have been, therefore, difficult to observe the two diffusion processes (Pellett et al., 1997; Watkinson et al., 1994).

All the subsequent diffusion experiments were performed through silicone membranes pre-saturated with the vehicle. Because different vehicles have different times to change the membrane properties, the silicone was saturated overnight with vehicle. Fig. 4 shows a typical plot using this protocol. It corresponds to the build up of the C=O peak versus time for BA in ethanol. Similar diffusion profiles were obtained for the other vehicles. The plateau (P) and the b ($=D/h^2$) values were estimated by fitting Eq. (1) to the diffusion profiles obtained for BA in the selected solvents and are listed in Table 3. The results show that both the b values and P vary for the different vehicles studied. Since the b value and P are proportional to the diffusion coefficient and the partition coefficient, respectively, the variation in these values implies that the vehicles affected both the diffusion and the partition coefficient.

The diffusion coefficient (see Table 3) was determined using the diffusion pathlength that corresponds to the thickness of the membrane, taking into account the swelling due to the solvent. The diffusion coefficients of BA were found to be higher in the silicone membrane treated with butanol and ethanol compared with the other vehicles (Table 4).

The values of P were plotted against the saturated solubility of the permeant in the vehicle and a linear relationship was obtained Fig. 5. This implies that the saturated solubility of the drug in the treated membrane is proportional to that in the vehicle. The vehicle enters the membrane, interacts and saturates it, thereby determining the partition of the drug into the membrane.

The measured steady-state fluxes obtained with the Franz cells were plotted against the P and the b values (see Fig. 6) obtained from the ATR-FTIR technique. The flux was found to be proportional to the b value but no such relation was observed for P. The linear dependence suggests that measurements of the diffusion coefficients can be used as a tool to understand the flux behaviour, or vice versa, in formulation design.

3.3.2. Salicylic acid

The permeation of salicylic acid through treated silicone membranes was investigated as described above for BA. The results obtained for SA were essentially similar to those of BA. This is a consequence of similar molecular structures of the two drugs. However, comparison of Fig. 5 shows that the gradient for BA is twice that of SA, even though the solubilities of SA and BA in the different solvents are very similar. The difference between the two may be the result of difference in hydrogen bonding. According to Beerbower et al. (1984), BA tends to form dimers depending on the surrounding environment. Fig. 7 shows an IR spectrum obtained from the diffusion experiment of BA in decanol through the membrane. The carbonyl peak at 1700 cm^{-1} from BA shows a shoulder, indicating dimerisation of the drug. The same behaviour was not observed for SA. It is possible that SA, in the vehicles chosen forms intra-molecular hydrogen bonding rather than dimerising. However, the free hydroxyl group will confer some polarity to the overall structure. Therefore, the less polar characteristics of the BA

dimer will interact more favourably with the membrane matrix and the solubility will be increased.

Again, the b values obtained from the ATR-FTIR experiment of SA were found to be proportional to the measured flux values Fig. 8. It is likely that the permeation of SA through silicone membrane is predominantly affected by the interactions between the vehicle and the membrane, and their subsequent influence on D.

Comparison of the values of b obtained from the diffusion process for both permeants indicate a similar effect. This is evident when considering the gradient (~ 1) of the line shown in Fig. 9, where b values for SA and BA are compared.

4. Conclusions

The ATR-FTIR technique was successfully used to deconvolute the diffusion and partition phenomena involved in permeation across impregnated silicone membranes. The measured steady state flux from conventional diffusion cells was influenced by the effect of the vehicles on the diffusion coefficient as well as the partition coefficient of the permeants. The saturated solubility of the drugs in the impregnated membranes was found to be proportional to the saturated solubility of the drugs in the vehicles. The ATR-FTIR technique provided a clear mechanistic understanding of the effects of the vehicles on the permeation of the selected drugs. It was possible to infer that BA solubility in the silicone membrane was twice that of SA, which is probably a consequence of dimerisation of BA.

Acknowledgements

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