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Vapour and liquid diffusion of model penetrants through human skin; correlation with thermodynamic activity

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This work investigates vapour and liquid permeation through human skin of model penetrants benzyl alcohol, benzaldehyde, aniline, anisole and 2-phenylethanol applied in model vehicles butanol, butyl acetate, isophorone, isopropyl myristate, propylene carbonate, toluene, n-heptane and water. Vapour permeation was a linear function of thermodynamic activity as measured by headspace gas chromatography, except when the vehicle was n-heptane. Liquid permeation did not always follow simple thermodynamic predictions, e.g. for the penetrant, benzyl alcohol, when the vehicle damaged the skin (toluene, n-heptane) or when propylene carbonate produced low fluxes and isopropyl myristate, high values. At comparable thermodynamic activities, liquid fluxes were often ten-fold higher than vapour fluxes, and these differences were reflected by the partition coefficients and the amount of penetrant entering the stratum corneum membrane. The conclusion was that liquid fluxes were membrane controlled, whereas an interfacial effect probably contributed to low vapour permeation.

It is necessary to know the factors which control molecular diffusion through human skin if topical formulations are to be developed and toxic hazards assessed. We have examined the diffusion of penetrants from vapours, organic liquid mixtures and aqueous solutions and its relation to measured thermodynamic activities.

We assume that percutaneous absorption occurs by passive diffusion as defined by Fick's law so that under ideal conditions the steady state rate of diffusion (or flux) through the skin is proportional to the applied concentration of penetrant (for a general review, see Barry, 1983). However, the chemical potential determines the apparent or effective concentration of a solute under non-ideal conditions e.g. when molecules interact in the liquid state or in a topical product. Since most preparations (such as the liquids herein considered) behave non-ideally, the chemical potential gradient of a penetrant, rather than its concentration gradient, provides the driving force for the diffusion process and therefore the potential should replace the concentration term in Fick's law. The thermodynamic activity (a) relates to the chemical potential (μ) by

$$\mu = \mu^\circ + RT\ln a \qquad (1)$$

where μ° is the standard potential, R is the gas constant and T is the temperature in K. For a particular substance μ° is a constant and the relative activities may be used instead of chemical potentials to arrive at the equation derived by Higuchi (1960)

$$J = \frac{Da}{\gamma h} \qquad (2)$$

where J is the steady state flux per unit area of membrane, D is the diffusion coefficient for a substance permeating through stratum corneum, h is the thickness of the stratum corneum and γ is the activity coefficient of the substance in the horny layer.

By definition a liquid and its equilibrium vapour have the same chemical potential and thus should ideally diffuse through a membrane at the same rate. Pure liquids and their equilibrium vapours and saturated solutions and their equilibrium vapours all have maximal thermodynamic activities equal to one. Thus, assuming that the vehicle does not alter the diffusional properties of the skin, and that the stratum corneum is the rate limiting step to passive diffusion, all topical preparations containing a drug at maximal thermodynamic activity should produce maximum absorption. We examine this concept for these four types of donor system and shows that, in practice, significant deviations from simple theory arise.

Initial work used benzyl alcohol as a model,

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non-ionic, volatile, hydrogen-bonding molecule; further investigations extended the comparison to the analogues, benzaldehyde, aniline, anisole and 2-phenylethanol. We have previously shown that steady state vapour fluxes of benzyl alcohol from 0·5 mole fraction binary mixtures with butanol, butyl acetate, isophorone, isopropyl myristate, propylene carbonate and toluene were directly proportional to thermodynamic activity as measured by headspace gas chromatography (Barry et al 1985). The same mixtures applied directly to the skin in the liquid state have the same thermodynamic activity and thus the same driving force for diffusion. In theory, benzyl alcohol from these liquid mixtures should diffuse through the skin at the same rate as from their equilibrium vapours—this proved not to be true in practice.

We have therefore examined fundamental skin diffusion theory and the validity of the assumptions made when vapour and liquid fluxes are compared. The main assumptions appropriate to the comparisons made in this paper are:

(i) The area, thickness, and nature of the stratum corneum are identical during vapour and liquid experiments.
(ii) Hydrostatic pressure differences do not significantly modify vapour fluxes compared to liquid values.
(iii) The vehicle does not change the permeability of the skin.
(iv) Penetrant molecules are removed rapidly from the receptor side of the stratum corneum.
(v) Vapour equilibrates rapidly throughout the donor compartment of the diffusion cell.
(vi) Molecules reaching the stratum corneum surface dissolve instantaneously in the barrier and diffuse passively and independently through it.
(vii) The stratum corneum is the rate-limiting barrier for diffusion through the skin—the effects of stationary layers in the donor or receptor are negligible, as are interfacial effects. (Assumptions applicable to skin diffusion work in general are discussed in detail by Barry 1983).

MATERIALS AND METHODS

Materials

Benzyl alcohol, butanol, butyl acetate, isophorone, isopropyl myristate, propylene carbonate and toluene were as used previously (Barry et al 1985). Benzaldehyde, anisole, aniline, 2-phenylethanol, ethanol, and n-heptane (BDH Chemicals Ltd.) were laboratory grade >99%. ^3H_2O, activity 5mCi cm^{-3} was from The Radiochemical Centre, Amersham, Fisofluor-1, from Fisons Scientific Apparatus and Trypsin (Bovine Pancreas, type 11, activity 1290 BAEE units mg^{-1} protein) came from Sigma Chemical Co.

Diffusion experiments

Dermatomed human abdominal cadaver skin (~0·4mm thick) was prepared and mounted in glass diffusion cells (4–6 replicates) as described previously for vapour diffusion experiments (Barry et al 1985). Liquid diffusion measurements used the same arrangement except that the donor compartment was filled with liquid instead of vapour (Harrison et al 1983). For direct comparison the vapour and liquid fluxes for a penetrant were determined successively through the same skin sample.

The diffusion cells were equilibrated at 30 ± 0·1 °C for 24 h with 0·9% NaCl (saline) on each each side of the skin and then four days of diffusion experiments followed. The general experimental design was:

Day 1: Tritiated water diffusion was measured to check the permeability of each skin specimen. Highly permeable specimens (flux > 3 mg cm^{-2} h^{-1}) were rejected on the assumption that they were damaged e.g. by microscopic tears or pin holes. Scheuplein & Ross (1970) used this criterion for eliminating damaged skin membranes.
Day 2: Measurement of benzyl alcohol vapour diffusion.
Day 3: Measurement of benzyl alcohol liquid diffusion.
Day 4: Water diffusion was measured again to account for any change in skin permeability since Day 1 due to further hydration or physical or solvent damage.

For Day 1 and 4 experiments, tritiated water (activity ~4 μCi cm^{-3}) was placed in the donor compartment and saline in the receptor compartment. Samples (70 μl) were removed from the receptor (Hamilton syringe) and replaced with fresh saline at 3, 4, 5 and 6 h, added to 10 cm^3 FisoFluor-1 scintillation fluid and counted in a Packard Tri-Carb 460 scintillation counter; donor activity was similarly measured at 0 and 6 h.

For Day 2 and 3 measurements, benzyl alcohol vapour and liquid diffusion were determined from pure benzyl alcohol, n-heptane saturated with benzyl alcohol (mole fraction of alcohol = 0·076) and from 0·5 mole fraction binary mixtures with vehicles, butanol, butyl acetate, isophorone, isopropyl myristate, propylene carbonate and toluene; the receptor

was 50% v/v aqueous ethanol. For toluene, butanol and isopropyl myristate, experiments were also done for other mole fractions. Vapour and liquid absorption through human skin of the benzyl alcohol analogues was determined using the same experimental design except that pure benzaldehyde, aniline, anisole or 2-phenylethanol replaced the benzyl alcohol. Vapour and liquid absorption from saturated aqueous solutions of the analogues was also measured. To maintain a saturated solution in the donor, excess pure analogue was added to the donor solution. Samples (0·75 cm^3) were removed from the receptor for GC analysis (see Barry et al 1985) at hourly intervals between 5 and 9 h and were replaced by fresh aqueous ethanol solution.

For each experiment, the cumulative amount of penetrant reaching the receptor was plotted as a function of time; linear regression analysis provided correlation coefficients >0·99 i.e. pseudo steady state was established. The slopes gave the steady state flux values J ($\mu g\ cm^{-2}\ h^{-1}$) and these divided by the donor concentration provided the permeability coefficients k_p ($cm\ h^{-1}$). Diffusion coefficients in the stratum corneum (D) were calculated from the intercept on the time axis i.e. they were found from the lag time (L) using the equation

$$D = \frac{h^2}{6L} \quad (3)$$

The barrier thickness, h, was taken as 32 μm to correspond with the hydrated thickness of stratum corneum after 25 h immersion, as measured by Scheuplein & Morgan (1967).

Tritiated water receptor counts were similarly used to find the total amount penetrated and hence to calculate fluxes and permeability coefficients. The ratio of water permeabilities defined by

$$\frac{\text{Water } k_p, \text{ Day 4}}{\text{Water } k_p, \text{ Day 1}} = \text{Damage Ratio} \quad (4)$$

provided the 'water damage ratio' which was a measure of the change in skin permeability during the experiment. The idea of such a ratio was used by Matoltsy et al (1968).

Benzyl alcohol vapour and liquid diffusion was also measured through silastic membrane 0·508 and 0·127 mm thickness (Dow Corning Corporation, USA).

Partition experiments

The amounts of benzyl alcohol entering the stratum corneum from the vapour and liquid phases were measured for pure benzyl alcohol and for the binary mixtures with each of the vehicles used in the diffusion experiments. Stratum corneum was prepared by the heat separation method of Kligman & Christophers (1963). Whole skin with the fat removed was dipped in water at 60 °C for 45 s. The epidermis was then gently pushed away from the dermis; it was straightened by floating it on water and then kept flat on aluminium foil. Epidermal tissue was digested away from the stratum corneum overnight at 37 °C by floating the membrane on 0·0001% trypsin in 0·5% $NaHCO_3$ buffer, pH 8·0–8·6. Discs (2·0 cm^2) were punched with a cork borer and dried in a desiccator over silica gel for at least 72 h. For each partition measurement discs from two skin specimens were weighed (5-place balance) and placed with 2 cm^3 test liquid in a sorption tube sealed with a ground glass stopper. Preliminary tests measured the effect of various sorption and desorption times on benzyl alcohol concentration within the stratum corneum; 48 h was satisfactory for full sorption and desorption (Harrison 1984). Therefore, the sorption tubes were maintained at 30·0 ± 0·1 °C for 48 h. Discs were removed and excess benzyl alcohol wiped from the tissue surface with Whatman filter paper No. 1. (An alternative method for eliminating excess benzyl alcohol by rinsing in ethanol introduced errors into partition coefficient determinations). The benzyl alcohol held in the stratum corneum was then desorbed into 3 cm^3 ethanol over 48 h at 30·0 ± 0·1 °C and its concentration measured by GC. Vapour sorption was determined by suspending two tissue discs above the liquid in a sorption tube for 48 h; desorption was as above. In all desorption experiments the concentration of benzyl alcohol in solution was less than 1% of the ethanol with which it was totally miscible. Experiments confirmed that the desorption procedure removed all the benzyl alcohol and that none remained bound to the stratum corneum.

Liquid benzyl alcohol (b.a.) partition coefficients (K_m) between a vehicle and the stratum corneum (s.c.) were calculated from

$$K_m = \frac{\text{b.a. desorption conc.} \times \text{volume (3 cm}^3\text{)}}{\text{s.c. initial dry weight} \times \text{b.a. sorption conc.}} \quad (5)$$

Vapour partition coefficients were calculated using the same equation and vapour concentrations found by headspace gas chromatography (Barry et al 1985).

Benzyl alcohol concentration in stratum corneum during diffusion experiments

Stratum corneum membranes prepared as above were set up in 11 diffusion cells. On Day 1, tritiated water diffusion was measured to ensure that each membrane was intact. On day 2, pure benzyl alcohol penetration was determined for liquid (5 cells) or vapour (6 cells). At the end of the experiment i.e. after 9 h benzyl alcohol diffusion, the stratum corneum available for permeation was cut out, wiped and placed in ethanol in desorption tubes for 48 h. The benzyl desorption concentration was measured by GC and the stratum corneum was washed, dried to constant weight over silica gel and its weight recorded.

To compare vapour and liquid solubilities in the stratum corneum, the concentration achieved in the first layer of the barrier membrane during a diffusion experiment (C_m) was calculated. To determine this we used

$$C_m = \frac{\text{b.a. desorption conc.} \times \text{volume (3 cm}^3) \times 2}{\text{s.c. final dry weight}} \quad (6)$$

The average membrane concentration was multiplied by 2 to deduce C_m because the concentration gradient across the barrier was assumed to fall linearly to zero at the receptor side of the membrane (steady state Fickian conditions). The stratum corneum final dry weight was used because the weight could not be determined before the diffusion experiment (in eqns 5 and 6 *weight* of tissue is used as is common in skin diffusion work).

Analogue aqueous solubility

Benzyl alcohol, benzaldehyde, aniline, anisole and 2-phenylethanol aqueous solubilities were measured by equilibrating excess analogue with water in a shaking bath at 30·0 ± 0·1 °C for 72 h. After phase separation, 2 cm³ of the aqueous layer was removed with a warmed pipette, diluted to a half with distilled water, and the analogue concentration determined by GC. All analogues could be detected using the same column as for benzyl alcohol (Barry et al 1985). Benzaldehyde and aniline were also measured under the same chromatograph conditions. Oven temperature was lowered to 150 °C for anisole and increased to 190 °C for 2-phenylethanol. The GC was calibrated using standards prepared in 50% v/v aqueous ethanol for each of the analogues.

Headspace analysis of the donor equilibrium

The time taken for benzyl alcohol vapour to reach equilibrium in the donor section of the diffusion cell was tested. The donor compartment was isolated from the receptor by a layer of aluminium foil and the cell was made watertight with two rings of parafilm between the cell halves. Pure benzyl alcohol (0·5 cm³) was added to the donor reservoir of a series of such cells which were equilibrated at 30·0 ± 0·1 °C. At various times vapour samples were removed using a gas tight syringe and analysed by GC.

RESULTS AND DISCUSSION

We previously showed that when the steady state vapour fluxes for benzyl alcohol in 0·5 mole fraction mixtures were plotted as a function of thermodynamic activity, the relation was linear (Barry et al 1985). However, our present results for liquid phase fluxes through the same skin specimens showed no such clear linear dependence. Also the liquid fluxes were all much greater than the corresponding vapour flux e.g. pure benzyl alcohol liquid flux was approximately ten times greater than its vapour value (Table 1).

One of our fundamental assumptions was that the vehicle did not affect the permeability of the skin i.e. permeability coefficients from one phase should remain constant with changing concentration of the vehicle and penetrant mixtures under Fickian conditions. Of our miscible test vehicles (isophorone, isopropyl myristate, propylene carbonate, toluene, butanol and butyl acetate), toluene produced the greatest benzyl alcohol liquid flux and butanol yielded an average-to low benzyl alcohol liquid flux. Taking these two vehicles as examples, further liquid and vapour experiments were done with benzyl alcohol at a range of mole fractions to find out if k_p did remain constant with changing concentration. (Similar experiments were done with isopropyl myristate—see later). For benzyl alcohol vapour diffusion through skin from vehicles butanol and toluene, the permeability coefficients were similar and they remained fairly constant with changing concentration. Their overall mean k_p values were 48 ± 7 cm h^{-1} (n = 25) for butanol and 41 ± 5 cm h^{-1} (n = 25) for toluene. Thus, vapour diffusion conformed approximately to Fickian behaviour. For benzyl alcohol liquid phase diffusion from butanol, k_p's were still fairly constant but at lower values—overall mean, 59 ± 16 × 10^{-5} cm h^{-1} (n = 25). However, with toluene the permeability coefficients increased dramatically with increasing toluene concentration (decreasing benzyl alcohol mole fraction). The liquid fluxes were also much higher from the toluene mixtures than for pure benzyl alcohol. These facts show that liquid toluene can considerably increase the permeability of the skin, even to the extent that the process overrides the effects on the flux of

Table 1. Permeation of benzyl alcohol through human skin—relationship between mole fraction in vehicles and vapour flux (V), vapour permeability coefficient (k_p), liquid flux (L), liquid permeability coefficient, L/V and damage ratios (means ± s.d., n = 4–6).

Benzyl alcohol mole fraction	Vehicle	Vapour flux V ($\mu g\ cm^{-2}\ h^{-1}$)	Vapour k_p ($cm\ h^{-1}$)	Liquid flux L ($\mu g\ cm^{-2}\ h^{-1}$)	Liquid k_p ($cm\ h^{-1} \times 10^6$)	L/V	Damage ratio
0·500	Butyl acetate	27 ± 6	46 ± 10	240 ± 51	460 ± 98	8·9	12 ± 8
0·500	Isophorone	21 ± 2	57 ± 6	170 ± 87	360 ± 180	8·1	4 ± 3
0·500	Propylene carbonate	32 ± 5	43 ± 6	150 ± 32	310 ± 67	4·7	1 ± 0.5
1·000	—	52 ± 12	48 ± 11	540 ± 240	520 ± 230	10	3 ± 3
0·256	Butanol	24 ± 4	60 ± 11	220 ± 150	550 ± 370	9·2	2 (n = 2)
0·313	Butanol	19 ± 6	41 ± 12	300 ± 40	650 ± 84	16	3 ± 2
0·500	Butanol	32 ± 8	47 ± 12	300 ± 62	470 ± 95	9·4	3 ± 1
0·596	Butanol	40 ± 10	51 ± 13	640 ± 420	870 ± 560	16	3 ± 2
0·824	Butanol	39 ± 5	42 ± 5	410 ± 250	460 ± 280	11	2 ± 1
0·118	Isopropyl myristate	22 ± 5	52 ± 11	380 ± 140	3830 ± 1430	17	1 ± 0·5
0·269	Isopropyl myristate	29 ± 8	53 ± 14	670 ± 250	4460 ± 1670	23	2 ± 0·5
0·500	Isopropyl myristate	40 ± 10	51 ± 13	530 ± 180	1610 ± 530	13	5 ± 3
0·699	Isopropyl myristate	33 ± 8	38 ± 9	1050 ± 690	1980 ± 1300	32	3 ± 2
0·836	Isopropyl myristate	50 ± 10	53 ± 10	1970 ± 660	2770 ± 920	39	5 ± 0·5
0·205	Toluene	24 ± 4	39 ± 7	2840 ± 1460	10500 ± 5400	118	21 ± 18
0·385	Toluene	31 ± 7	41 ± 9	2120 ± 830	4510 ± 1770	68	21 ± 17
0·500	Toluene	37 ± 3	44 ± 4	1840 ± 660	3110 ± 1120	50	6 ± 4
0·713	Toluene	36 ± 7	38 ± 8	1710 ± 590	2200 ± 760	48	5 ± 4
0·806	Toluene	32 ± 7	33 ± 7	1190 ± 1150	1210 ± 1170	37	6 ± 5
0·076[a]	n-Heptane	26 ± 3	25 ± 3	6890 ± 1290	57400 ± 10700	265	36 ± 11

[a] Saturated solution of benzyl alcohol in n-heptane.

increasing benzyl alcohol concentration and thermodynamic activity.

To probe further the mechanism of this action, tritiated water permeabilities derived before and after vapour and liquid diffusion were used to find water damage ratios (eqn 4) for toluene–benzyl alcohol and butanol–benzyl alcohol mixtures (Table 1). These results should be compared with the change in water permeability arising during the normal four day experiment as measured for four standard cells in which no benzyl alcohol or vehicle came into contact with the skin (damage ratio 1·9 ± 1·1). The butanol had relatively little effect on the subsequent skin permeability to water, whereas the toluene data showed that this solvent increased skin permeability, particularly at high vehicle concentrations (low benzyl alcohol concentrations). This effect suggests one reason for the high permeability coefficients for liquid phase benzyl alcohol diffusion from the toluene vehicle is a direct damaging effect on the skin.

It appeared that vapour contact did not increase skin permeability; to confirm this we incorporated a further tritiated water run between vapour and liquid runs during diffusion experiments for the 0·5 mole fraction benzyl alcohol binary mixture with toluene. Two standard cells were also run in which no liquid was in contact with the stratum corneum on Day 2 and only 50% v/v aqueous ethanol on Day 4. Vapour damage was measured as the ratio of water permeabilities on Day 3 and Day 1, and the liquid damage on Days 5 and 3. Vapour and liquid damage ratios for toluene were 0·66 ± 0·1 and 23 ± 4 respectively, compared to the mean standard cell ratios of 0·77 and 1·2. These results clearly show that toluene vapour contact did not increase the skin's permeability to water but liquid contact did. Additional experiments (see Harrison 1984) suggested that liquid toluene damage was probably irreversible and arose from chemical alteration of the stratum corneum or removal of skin lipids. As toluene only exerted its damaging action in the liquid state, it probably functions by extracting lipids out of the tissue and into the bulk liquid; it cannot do this in the vapour state.

Despite the fact that butanol liquid had little effect on skin permeability, the benzyl alcohol liquid flux from this vehicle was still approximately ten times

greater than the vapour flux at all concentrations. Similar differences were observed for the vehicles butyl acetate, isophorone, isopropyl myristate and propylene carbonate. In particular, the somewhat high benzyl alcohol liquid fluxes from isopropyl myristate suggested that this solvent can reduce the barrier properties of the stratum corneum. Isopropyl myristate is used in topical preparations and as a model compound for skin lipids (Ostrenga et al 1971; Albery & Hadgraft 1979; Barry 1983). This similarity to skin lipids may allow it to open up pathways for diffusion once it enters the stratum corneum. Whatever its action, skin permeability in the presence of this material appeared to return to near normal on removal of the isopropyl myristate as demonstrated by the relatively low damage ratios. In this aspect, isopropyl myristate differed in its mechanism for increasing permeability compared with the damaging solvent, toluene.

Benzyl alcohol is not miscible in all properties with n-heptane so we examined only a saturated solution. The vapour flux was anomalous as it was only half the expected rate (from thermodynamic considerations, a saturated solution of benzyl alcohol in n-heptane should have behaved like neat benzyl alcohol). In contrast, very high rates of diffusion were measured for the same mixture applied as a liquid and this correlated with very high damage ratios (36 ± 11). We determined additional vapour and liquid damage ratios as for toluene (see above); values were 0·88 ± 0·18 and 26 ± 12 respectively, showing that liquid hydrocarbon contact caused the damage.

Since a vapour and liquid in equilibrium have the same chemical potential and should generate the same flux, analogues of benzyl alcohol and their saturated aqueous solutions were investigated to see if the above divergence from simple thermodynamic theory was unique to benzyl alcohol. Results are shown in Table 2 along with analogue water solubilities. Because part of the experimental design required saturated aqueous solutions, we took the opportunity to test a theoretical correlation between partition coefficients and aqueous solubilities.

Yalkowsky (1981) described how molar water solubilities (X_w) of liquid solutes may be predicted from octanol–water partition coefficients (PC) from

$$\log X_w = \log PC - 0{\cdot}94 \qquad (7)$$

Since octanol–water partition coefficients for our analogues are published or may be calculated using fragmentation methods (Hansch & Leo 1979), estimates of X_w could be used to check the fit of theory for these materials. Table 3 shows that observed

Table 2. Permeation through human skin—analogue vapour (V) and liquid (L) fluxes ± s.d. for pure liquids (p) and saturated aqueous solutions (s), boiling points (B.P. °C), water damage ratios from diffusion experiments and water solubilities (μl cm^{-3}).

Analogue/B.P.		Flux (μg cm^{-2} h^{-1}) L_p	L_s	V_p	V_s	Flux ratios L_p/L_s	V_p/V_s	Water damage ratios p	s	Water Solubility
Anisole	154	990 ± 300	140 ± 50	420 ± 100	340 ± 55	7·1	1·2	2·9 ± 0·7	2·5 ± 0·7	1·9
Benzaldehyde	180	1970 ± 720	450 ± 70	410 ± 70	300 ± 60	4·3	1·4	7·3 ± 3·6	2·0 ± 0·6	7·4
Aniline	184	1870 ± 1260	760 ± 460	260 ± 50	250 ± 50	2·5	1·0	3·3 ± 1·2	2·9 ± 1·6	34
Benzyl alcohol	204	540 ± 240	610 ± 530	52 ± 12	40 ± 5	0·89	1·3	3·1 ± 2·6	2·1 ± 0·5	36
2-Phenylethanol	218	650 ± 60	260 ± 60	27 ± 8	21 ± 1	2·5	1·3	8·0 ± 7·6	2·2 ± 0·7	20

Table 3. Log analogue water solubilities (X_w) observed and predicted from published log octanol-water partition coefficients (PC). The table also includes PC's calculated by a fragmentation method.

Analogue	Published PC	Fragment evaluation PC[a]	log X_w Predicted	Observed	Difference
Benzyl alcohol	1·10[b]	1·10	−0·98	−0·46	−0·52
Benzaldehyde	1·45[c]	1·48	−1·11	−1·14	0·03
Aniline	0·90[b]	0·90	−0·89	−0·43	−0·46
Anisole	2·11[b]	2·06	−1·26	−1·86	0·60
2-Phenylethanol	1·36[d]	1·34	−1·04	−0·78	−0·29

[a] Hansch & Leo (1979) [b] Fujita et al (1979) [c] Umeyama et al (1971) [d] Iwasa et al (1965).

molar water solubilities at 30 °C were all within a factor of four of the predicted solubility calculated from published octanol–water partition coefficients measured at 25 °C. Yalkowsky & Valvani (1980) demonstrated a linear relation between predicted and observed water solubilities over a span of nine orders of magnitude; they encountered errors of up to a factor of ten. Thus, our analogue water solubilities were reasonably well predicted, even with the temperature difference. Observed water solubilities reported by Moriguchi (1975) for benzyl alcohol and aniline were the same as presented here.

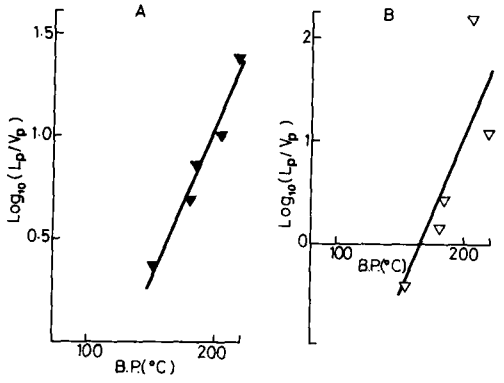

FIG. 1. (A) The logarithm of the ratio of the fluxes of pure analogue liquid to vapour (L_p/V_p) plotted against analogue boiling point. (B) Similar plot for saturated aqueous solutions.

All the pure analogues showed differences between vapour (V_p) and liquid (L_p) fluxes similar to benzyl alcohol but to different extents (Table 2). When $\log_{10}$ (L_p/V_p) for each analogue was plotted against its boiling point, a linear correlation (coefficient 0·985) was obtained (Fig. 1A). Thus, the difference between vapour and liquid flux increased with the boiling point of the pure liquid. One explanation could be that the more slowly evaporating substances did not allow vapour concentrations to reach equilibrium in the diffusion cell donor compartment; this is unlikely—see later. Alternatively, dissolution into the surface layer of the stratum corneum may have been limited by some effect related to volatility. When the same graph was drawn for the analogue saturated aqueous solutions (Fig. 1B) the same trend occurred although the ratios between saturated aqueous solution fluxes (L_s and V_s) were lower than for the pure homologues except for benzyl alcohol.

Liquid phase fluxes for the analogues were generally lower from the saturated aqueous solutions than from the pure liquids, but vapour fluxes were almost the same from either system in accordance with simple thermodynamic theory. The overall mean ratio of vapour fluxes i.e. pure liquid vapour flux/saturated aqueous solution—V_p/V_s—was 1·25 ± 0·13, so that the penetrant diffused, at the most, only slightly more readily through human skin from the pure liquid vapour than from saturated aqueous solution vapour. Thus the presence of water molecules in the donor liquid did not greatly affect vapour permeation either by reducing evaporation of the analogue or dissolution of vapour molecules into the stratum corneum.

Water damage ratios for the analogue diffusion experiments were 4.8 ± 4·1 (n = 22) for pure liquids and 2·3 ± 0·8 (n = 17) for the saturated aqueous solutions. If we presume that liquid phase contact damaged the stratum corneum as for benzyl alcohol, then the higher penetration of the analogues from the pure liquid than from the aqueous solution may have arisen from skin damage, but the evidence is not conclusive.

One assumption made about diffusion through the skin from liquids and their equilibrium vapours is that molecules which reach the skin surface should dissolve or partition into the surface layer to the same extent so as to produce the same surface concentration i.e. once dissolved, molecules from a liquid or a vapour should be indistinguishable by their source. To investigate whether vapour and liquid benzyl alcohol molecules were dissolving in the stratum corneum to the same extent, partition coefficients were determined from each phase. Vapour and liquid K_m's for pure benzyl alcohol and for 50% v/v solutions with toluene and butanol were 5·1, 5·3 and 4·1 respectively for the liquid phase and $3{\cdot}1 \times 10^5$, $2{\cdot}5 \times 10^5$ and $4{\cdot}0 \times 10^5$ for the vapour phase. Thus the vapour K_m values were about 10^5 times larger than the liquid values. Similar differences were found by Scheuplein (1967) for water and the long chain alcohols (C_1–C_{12}). These differences arise from the much smaller vapour concentration applied to the tissue compared with the liquid concentration. Scheuplein's results showed that the C_m values (the stratum corneum surface concentrations) for vapour and liquid phases were approximately equal. To a large extent this was true for our results for benzyl alcohol which had a 10^6 fold difference between equilibrium vapour and liquid concentrations (vapour = 1·07 μg cm^{-3}; liquid = 1·04 g cm^{-3}). However there was still more than a

ten-fold difference between the C_m's for benzyl alcohol vapour (0·33 mg mg^{-1}) and liquid (5·3 mg ml^{-1}) which meant that more benzyl alcohol entered the stratum corneum from the liquid than from the vapour during the 48 h equilibrium period. This difference was comparable with the difference observed between vapour and liquid diffusion fluxes. These results strongly suggest that the mechanism which allows the liquid to dissolve more readily in the stratum corneum is present in both types of experiment (diffusion and partition) and that the amount of benzyl alcohol entering the stratum corneum from each phase was responsibile for the differences in the rates of diffusion.

To determine if the benzyl alcohol membrane concentrations found during partition experiments truly reflected what happened during a diffusion experiment, we measured the benzyl alcohol vapour and liquid uptake into stratum corneum immediately after a diffusion experiment. Benzyl alcohol diffusion had to be measured through isolated stratum corneum rather than full thickness skin. This minor experimental change did not affect our results, as shown by the fact that vapour and liquid fluxes through hydrated stratum corneum and hydrated dermatomed skin were similar: stratum corneum, vapour flux ($\mu g\ cm^{-2}\ h^{-1}$) = 45 ± 13 (n = 6), liquid flux = 420 ± 90 (n = 5); dermatomed skin, vapour flux = 52 ± 12 (n = 6), liquid flux = 540 ± 240 (n = 6). The difference between the membrane concentrations (C_m) more than accounts for the difference between the fluxes (vapour C_m (mg mg^{-1}) = 0·11, liquid C_m = 6·4).

There still remained a further possible explanation for the differences between vapour and liquid fluxes by way of experimental artifacts. Was it possible that the vapour did not reach equilibrium within the donor compartment of the diffusion cell i.e. was the benzyl alcohol vapour evaporating slower from the reservoir than it was lost through the skin? To check this possibility we determined the rate at which benzyl alcohol reached its equilibrium vapour concentration within a diffusion cell. The results of a headspace gas chromatography analysis are shown in Fig. 2 as a plot of peak height versus equilibration time. When more than two replicates were made standard deviations are represented. After only five minutes the vapour concentration was near to the final equilbrium value. Therefore during a diffusion experiment the donor vapour was rapidly generated and maintained. The 5 h initial equilibration period at the beginning of each diffusion experiment should have provided ample time for donor equilibration and for steady state diffusion to be established. Additional experiments showed that there were no significant effects arising from changes in reservoir volume and surface area, donor compartment volume and supplementation of vapour supply by means of a flow through donor system (Harrison 1984).

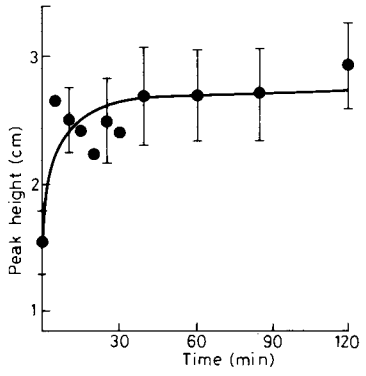

FIG. 2. Effect of donor compartment equilibration time on benzyl alcohol vapour concentration as represented by gas chromatograph peak height response ± one standard deviation for multiple tests.

CONCLUSIONS

This work aimed to investigate if absorption through human skin from vapour and liquid phases depended simply on the thermodynamic activity of the penetrant or were other effects significant. However, while vapour results showed a linear relationship between flux and thermodynamic activity (except for benzyl alcohol in n-heptane), liquid phase data were less consistent. In particular, when the vehicle was toluene or n-heptane, benzyl alcohol fluxes were high; water damage ratios showed that these hydrocarbon solvents irreversibly reduced the skin's barrier property. When permeability coefficients were measured over a 0–1 mole fraction range, results confirmed the non-Fickian nature of benzyl alcohol permeation from toluene (Table 1). Fig. 3 shows the benzyl alcohol liquid fluxes from the other solvents, which did not show such dramatic vehicle effects. These results more reasonably followed the predicted linear relationship between flux and thermodynamic activity, suggesting that thermodynamic control of benzyl alcohol penetration may operate for non-damaging solvents (which are not penetration enhancers) when we compare like-with-like i.e. liquid flux with liquid flux, vapour with vapour. However, even within the liquid series there were

anomalies. Propylene carbonate provided significantly lower fluxes than those predicted by thermodynamic activity considerations; the behaviour of this solvent warrants further investigation. Additionally, the flux for isopropyl myristate was somewhat high, as discussed previously.

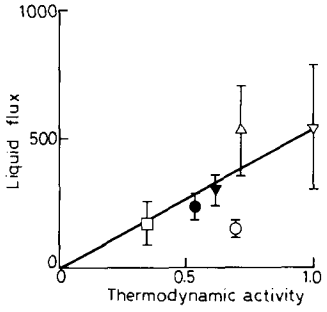

FIG. 3. Mean ± s.d. benzyl alcohol liquid fluxes (μg cm^{-2} h^{-1}) from pure benzyl alcohol (-▽-) and from 0·5 mole fraction bindary mixtures with isophorone (-□-), butyl acetate (-●-), butanol (-▼-), propylene carbonate (-○-), and isopropyl myristate (-△-) plotted against thermodynamic activity found by headspace gas chromatography. The diagonal represents the predicted linear relationship between flux and activity.

When we compared different phases, the situation was more complex. Simple theory asserts that a vapour and a liquid in equilibrium should diffuse through a membrane at the same rate. However, even after allowing for skin damage, liquid phases (pure compounds or aqueous solutions) usually provided higher fluxes than did vapours for the materials tested (benzyl alcohol, benzaldehyde, aniline, anisole and 2-phenylethanol). To account for this discrepancy we need to re-examine the assumptions made in the introduction.

(i) The same skin specimens were used for the vapour and liquid experiments to ensure that the area and thickness of the stratum corneum were comparable. They may have changed slightly during experimental procedures but not by factors of ten or more, as did some of the fluxes.

(ii) Hydrostatic pressure on the skin differed between vapour and liquid diffusion experiments because of the absence of a donor liquid phase during a vapour run. The mean hydrostatic pressure calculated to be produced by donor liquid was about 1·4 × 10^{-3} atm which was assumed negligible in comparison with atmospheric pressure. The effects on skin area and thickness due to distortion of the membrane during vapour runs was also assumed to be negligible.

(iii) The assumption that the vehicle had no effect on skin permeability was shown to be obviously false for liquid toluene and n-heptane. The other vehicles did not have as dramatic effects, but vehicle effects are involved in skin permeation work, as shown further by high flux values for isopropyl myristate and low ones for propylene carbonate.

(iv) The receptor compartment was maintained at less than 10% benzyl alcohol saturation to ensure that sink conditions prevailed.

(v) The vapour flux measured during diffusion experiments was the result of several possible rate limiting processes including evaporation of the liquid to form the donor phase and diffusion through the stratum corneum. Headspace analysis confirmed that the liquid would rapidly evaporate to reach equilibrium vapour concentrations within the donor compartment (Fig. 2). Simple theoretical calculations confirmed this experimental result. The diffusion coefficient for benzyl alcohol in air at 30 °C was approximately 0·074 cm^2 s^{-1} (Harrison 1984); diffusion coefficients for gases in air are usually between 0·05 and 1 cm^2 s^{-1}. From our results we calculated that the diffusion coefficient of benzyl alcohol permeating stratum corneum was 2·8 × 10^{-10} cm^2 s^{-1}. Thus, benzyl alcohol molecules diffuse about 10^8 times more rapidly through air than through skin. The rate of evaporation of benzyl alcohol in still air (V) from a circular plane (radius r) was calculated from the diffusion coefficient in air D′ using the equation given by Egerton (1929)

$$V = 4rD' \log_e \frac{p - p^o}{p - p^s} \qquad (8)$$

where p is the total pressure, p^o is the pressure of the vapour in air at a distance from the liquid surface and p^s is the saturation vapour pressure of benzyl alcohol at the surface (2·5 × 10^{-4} atm). The rate, V, was thus 4·3 × 10^{-5} gs^{-1}. As the equilibrium benzyl alcohol concentration found by headspace analysis was 1·07 μg ml^{-1} with a donor compartment volume of ~ 8 cm^3, the vapour in the donor should take less than a second to equilibrate. In practice it takes longer because the cell has to reach 30 °C and some benzyl alcohol partitions into the skin. Even so, the rapid evaporation of benzyl alcohol into air was sufficient to maintain donor vapour saturation.

(vi) We made the assumption that molecules reaching the stratum corneum would dissolve instantaneously and diffuse passively and independently through the barrier. At high concentrations it is less likely that molecules will diffuse independently.

However, vapour and liquid permeation coefficients remained relatively constant for the 'non-damaging solvent' butanol between benzyl alcohol mole fractions 0·2–1, so that Fickian behaviour apparently occurred.

(vii) Finally, we assumed that the stratum corneum provided the rate-limiting step to diffusion through skin. Certainly benzyl alcohol diffused similarly through dermatomed whole skin and through stratum corneum alone. The only other step not considered so far is an interfacial or surface phenomenon which may account for a lower vapour flux than a liquid flux. To investigate this further, we measured benzyl alcohol diffusion through silastic membrane, an implant material used as a model membrane (see e.g. Flynn & Roseman 1971; Most 1970). We found that benzyl alcohol liquid diffusion through silastic membrane was directly proportional to membrane thickness so that k_p decreased by the same factor as membrane thickness (for 0·508 mm thickness, k_p was 0·0017 cmh^{-1}; for 0·127 mm, k_p = 0·0069). Although vapour diffusion showed a typical Fickian relationship with time, fluxes were much lower than for the liquid and appeared to be independent of membrane thickness, with k_p virtually a constant (0·508 mm, k_p = 68; 0·127 mm, k_p = 84). These results were compatible with some interfacial or boundary effect. Vapour lag times were also about 10 times longer than liquid lag times, confirming the enhanced barrier effect. The silastic membranes were thicker than stratum corneum but were more permeable e.g. liquid diffusion through the 0·127 mm silastic membrane was about 13 times faster than through skin. However, the differences between vapour fluxes for the same membranes were only about 1·7 fold, illustrating that effective vapour barriers were similar. Thus liquid diffusion appeared to be membrane-controlled but something other than the membrane resistance seemed to limit vapour diffusion for stratum corneum and silastic membranes. It would be worthwhile to compare vapour and liquid diffusion for other types of membrane to determine how often non-membrane rate limiting characteristics operate.

Our most important finding was that vapour and liquid fluxes varied for all the analogues for systems of the same thermodynamic activity. The greatest difference was observed for 2-phenylethanol; its vapour flux from pure liquid was 24 times lower than its liquid flux. In contrast, Scheuplein (1967) found that the permeability of stratum corneum to water was about 0·2 mg cm^{-2} h^{-1} at 25 °C whether the water was presented to the skin as a vapour or a liquid. His report also included K_m and k_p values for the aliphatic alcohols exposed to the skin as vapours and dilute solutions. Differences between vapour and liquid diffusion were explained entirely in terms of partitioning into the skin. Similarly Blank & Scheuplein (1969) compared the penetration of hexanol and octanol from pure liquids and aqueous solutions. The k_p and K_m values reported contained concentration terms which caused very different results for vapour and liquid phases. However, had the fluxes of the alcohols been compared, they would have been approximately the same for both phases. The differences between vapour and liquid fluxes of the type which we have obtained for benzyl alcohol and its analogues would have been smaller for water and the more volatile aliphatic alcohols so that these differences would probably not have been detected above normal skin permeability variations. A final example of vapour/liquid differences comes from the work of Blank et al (1957). They found that the rate of penetration of the anticholinesterase agent, sarin, into excised human skin was 3–4 times greater from liquid application than from vapour, but they did not discuss this result further.

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