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Correlation of thermodynamic activity and vapour diffusion through human skin for the model compound, benzyl alcohol

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This work tested the potential for predicting percutaneous absorption rates of a volatile penetrant from any vehicle by using thermodynamic activity measurements. Benzyl alcohol was chosen as a non-ideal, hydrogen bonding, volatile model penetrant. A manual headspace gas chromatography method measured benzyl alcohol vapour concentrations and thermodynamic activities above binary mixtures with vehicles: butanol, butyl acetate, isopropyl myristate, isophorone, toluene and propylene carbonate. Benzyl alcohol vapour diffusion through human, abdominal skin was also measured in-vitro for these mixtures. The benzyl alcohol vapour flux was linearly related to the activity, suggesting that percutaneous absorption is controlled by thermodynamic activity when the vehicle has no effect on the stratum corneum barrier.

Many varied chemicals reach our skin, whether applied as pharmaceutical or cosmetic products, hazardous waste materials from spillages or by vapour contact. For toxicity testing and for therapeutic control, we need to know the rate and extent of this absorption. The work reported here has two main aims. First, to assess the potential for predicting percutaneous absorption from the measured thermodynamic activity, so reducing the lengthy procedures involved in permeability measurements in-vitro with limited supplies of human skin. Second, to develop techniques for measuring vapour permeation through human skin, a factor of importance with toxic materials such as nerve gases (Schaefer et al 1982).

For most substances penetrating human skin, the stratum corneum provides the main barrier to passive diffusion (Scheuplein 1967). Under ideal, sink conditions, the quasi-steady state flux of molecules through unit area of skin (J) may be represented from Fick's law by

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

where D is the diffusion coefficient of the penetrant in the horny layer, K_m is the partition coefficient of the penetrant between the stratum corneum and the vehicle, C is the concentration of penetrant in the vehicle and h is the thickness of the stratum corneum (for further discussion of the assumptions involved in the derivation of eqn 1, see Barry 1983). Higuchi (1960) developed the treatment for non-ideal mixtures to yield

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

where a is the thermodynamic activity of the penetrant in its vehicle and γ is its effective activity coefficient in the stratum corneum (assumed constant for our work). Thus, the rate of percutaneous absorption should be directly proportional to thermodynamic activity; we have tested this relationship.

We chose headspace gas chromatography (hsgc) as the method for measuring thermodynamic activity as it can be used for many volatile compounds, alone or mixed, and automatic headspace analysers are available. The method samples equilibrium vapours above a test material (usually liquid) in a closed system and analyses them by gc (Kolb 1980; Hachenberg & Schmidt 1977). Raoult's law states that, for a liquid sample consisting of an ideal mixture, the partial vapour pressure of substance i (p_i) is proportional to the vapour pressure of pure component i (p_{oi}) and the concentration of i in the liquid phase expressed in mole fractions (x_i)

$$p_i = p_{oi}x_i \quad (3)$$

For a non-ideal (real) mixture, molecular interactions in the liquid phase frequently alter this relationship, which may be corrected by inserting the activity coefficient of the component i, γ_i

$$p_i = p_{oi}x_i\gamma_i \quad (4)$$

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The activity coefficient is the ratio between the actual partial pressure above a non-ideal liquid and the partial pressure predicted by Raoult's law for the same temperature and pressure. Since the thermodynamic activity of i (a_i) is equal to $\gamma_i x_i$, equation (4) becomes

$$p_i = p_{oi} a_i \quad (5)$$

During hsgc, we can compare the peak response for the vapour above a pure liquid in a closed system, F_{oi} (which is proportional to p_{oi}) with the response for a vapour above any liquid mixture, F_i (which is proportional to p_i). The thermodynamic activity and activity coefficient can be found directly using

$$a_i = \frac{F_i}{F_{oi}} \quad (6a)$$

$$\gamma_i = \frac{F_i}{F_{oi}} \cdot \frac{1}{x_i} \quad (6b)$$

These relationships assume that no specific interactions exist between components of the mixture in the vapour phase.

Although hsgc is frequently used to analyse trace volatile substances, only a few workers have employed it in physicochemical applications (see e.g. Kolb 1980; Hachenberg & Schmidt 1977; Harrison et al 1982). Al-Khamis et al (1982) showed a relationship between release rates of drugs from topical preparations and thermodynamic activity.

Benzyl alcohol was chosen as a model skin penetrant to investigate vapour absorption through skin and to test the relationship between absorption and thermodynamic activity. It is a non ionic volatile molecule which hydrogen bonds in the liquid state and should therefore behave non-ideally in mixtures. It is used pharmaceutically and it partitions from 0·9% NaCl (saline) about 100 times more readily into the epidermis than the dermis and it does not bind to the dermis (Menczel & Maibach 1970, 1972). Therefore benzyl alcohol should diffuse through stratum corneum and be readily removed, so maintaining sink conditions.

MATERIALS AND METHODS

Materials

Benzyl alcohol (BDH Chemicals Ltd.) complied with manufacturer's specifications: minimum assay (glc) 99%, refractive index n^{20} 1·539–1·541, boiling range 95% 202–206 °C, density at 20 °C 1·043–1·048 g cm^{-3}. Maximum limits of impurities: benzaldehyde 0·2%, chlorine compounds 0·1%, non-volatile matter 0·05%. Butan-1-ol, butyl acetate and isophorone (Cambrian Chemicals) were ≥99%, isopropyl myristate (Fluochem Ltd.) was ≥95%, toluene (BDH Chemicals Ltd.) was laboratory grade as was propylene carbonate (Aldrich Chemical Co. Ltd.)—all were used as received.

Headspace gas chromatography—vapour analysis

The concentration of benzyl alcohol vapour above a series of binary mixtures (0–1 mol fraction) was measured for toluene, butanol, butyl acetate, isopropyl myristate, isophorone and propylene carbonate (Fig. 1). Closed systems were 25 cm^3 flasks sealed with tin foil (to avoid sorption effects into septa) containing ~ 3 cm^3 of test mixture, equilibrated in a water bath at 30 °C ± 0·05 °C for 2h. Vapour samples (0·5 cm^3) were removed slowly to ensure filling of the 1 cm^3 gas-tight syringe (Scientific Glass Engineering P.T.Y. Ltd.) previously warmed to 35–40 °C to prevent condensation; the first 0·1 cm^3 was ejected back into the flask to purge the inner space of the syringe (Drozd & Novak 1977). The sample was immediately introduced into the gc column and the syringe was flushed several times with air to reduce memory effects (Ramsay & Flanagan 1982). Measurements for a series of benzyl alcohol mixtures in one vehicle were taken on one occasion to minimise changes in gc response. Response linearity was checked daily by calibration with a series of 2·0 µl benzyl alcohol liquid standards in 50% v/v aqueous ethanol.

Column and conditions

A Pye-Unicam 103 gas chromatograph with 1·6 m glass column, o.d. 6mm, i.d. 4mm was packed with 10% Carbowax 20M on Chromosorb W, AW,

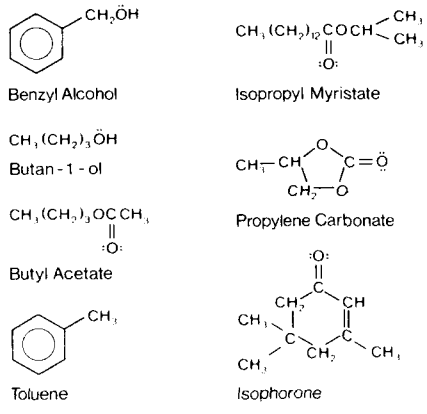

FIG. 1. Structural formulae of benzyl alcohol and the vehicles.

DMCS, 60–80 mesh support (Phase Separation Ltd.). Conditions were: Temperature–oven 175 °C, detector 200 °C, injection port 200 °C; Gas flow rates ($cm^3\ min^{-1}$)–nitrogen 30, hydrogen 35, air 450; Chart speed–5 mm min^{-1}. A Teflon coated septum (Scientific Glass Engineering Ltd.) prevented loss of sample into rubber.

Flask temperature and the headspace method

The temperature inside the closed system as measured with a probe (Digiton Instrumental Ltd., Model 3750K) ranged from 27·6 °C just inside the inner seal, to 30·0 °C at the liquid-vapour interface, when the water bath was at 30·0 °C; at the sampling point it was 29·9–30·0 °C. The effect of water bath temperature (25·2, 30·2, 35·3 and 40·4 °C) on pure benzyl alcohol vapour concentration was determined.

Vapour calibration of gas chromatograph

To determine benzyl alcohol vapour concentrations the chromatograph was calibrated with vapour produced when known amounts of liquid were totally vapourized in 25 cm^3 flasks (volume was determined by weighing the flasks empty, then filled with water). Because of the tiny liquid volumes necessary, dilutions of benzyl alcohol in acetone were used (2–25μl alcohol/25 cm^3 acetone). Leggett (1977) used a similar method with trinitrotoluene in benzene.

Diffusion experiments

Dermatomed whole human skin was prepared from mid line abdominal autopsy specimens (male or female, mean age 71 ± 11 years) by a method similar to that of Coldman et al (1969). Skin strips were flattened in a press and fat removed so that they were of uniform thickness. Strips were removed and, with stratum corneum uppermost, each strip was covered with polythene, then a glass or Perspex plate, and pressed between two metal plates. After several hours at −20 °C, the upper plates were removed, the skin surface warmed until just mobile to touch, the polythene was lifted and the upper ~0·4 mm of skin was removed (Davies Dermatome 7; Duplex Electro Dermatome). Skin membranes were clamped between the two halves of diffusion cells (Fig. 2) adapted from a design of Scheuplein (1965). The donor part (~7·5 cm^3) provided a liquid reservoir (0·5–1·0 cm^3, surface area ~ 1 cm^2) to generate vapour. A side arm could be filled with drierite to minimise stationary water layers building up on the stratum corneum surface due to diffusion from the receptor cell. The membrane was supported by a disc

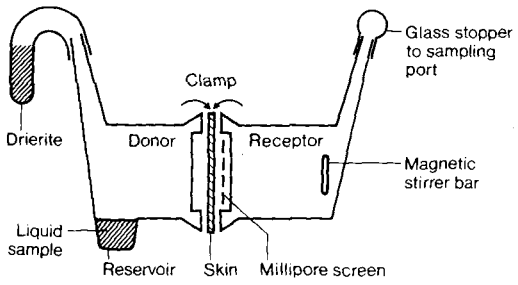

FIG. 2. Diffusion cell.

(diameter 1·6 cm) cut from perforated stainless steel Millipore screen (YY/22/142/64) (pinch clamps supplied by Arthur H. Thomas Co., USA). The effective diffusion area was 2·01 cm^2. The receptor contained 4·5–5·0 cm^3 50% v/v aqueous ethanol (to ensure dissolution of benzyl alcohol) and was stirred with a Teflon coated magnetic spin bar (Bel-Air Products, USA) driven by an immersed mechanical rotor unit (Rank Brothers, Bottisham, Cambridge) around which three cells could be set.

For two days before diffusion measurements, cells were assembled with saline on either side of the skin in a water bath at 30 °C ± 0·05 °C to allow the stratum corneum to hydrate. Benzyl alcohol vapour diffusion was then measured from pure liquid and for 0·5 mol fraction mixtures with each of the vehicles measured for headspace analysis. Test mixture (0·5 cm^3) was placed in the reservoir and benzyl alcohol concentration in the receptor was maintained below 10% saturation (sink condition). Receptor samples (0·75 cm^3) were removed for gc analysis at hourly intervals between 5 and 9 h and were replaced by fresh aqueous ethanol. Four replicate experiments were performed for each binary mixture and six for pure benzyl alcohol. It was confirmed that evaporation rate and vapour stationary layers did not contribute to the measured flux (Harrison 1984) i.e. passage across the stratum corneum provided the rate limiting step in absorption.

Headspace analysis/Treatment of data

Triplicate injections were made for each headspace test and pure benzyl alcohol vapour samples were injected at regular intervals so that thermodynamic activities could be calculated from equation 6a. Where the second solvent in the binary mixture gave a response, triplicate peak heights were again measured and related to the response for pure second solvent. Thus, pure liquid, either benzyl alcohol or second solvent, was given the standard

activity of one. Mean plus standard deviations were calculated for each set of data.

Vapour diffusion experiments

The benzyl alcohol concentration in receptor samples was determined by gc analysis; the chromatograph was calibrated with liquid benzyl alcohol standard solutions in 50% v/v aqueous ethanol and one standard was injected at regular intervals between samples to check for any change in response.

The total amount of benzyl alcohol penetrating through the skin and into the receptor was calculated and plotted as a function of time; graph linearity was checked by linear least squares regression analysis. The benzyl alcohol flux was calculated from the slope of the graph i.e. flux equals slope/skin area.

The benzyl alcohol vapour donor concentration was calculated by multiplying the equilibrium vapour concentration for pure benzyl alcohol by the activity found by hsgc.

RESULTS AND DISCUSSION

The temperature probe showed that the minimum temperature of the vapour sample taken from within the closed system was 29·7 °C when the vapour was totally mixed during sampling. At the depth which the sample was normally removed the vapour temperature was 29·9–30·0 °C. Altering the water bath temperature, and thus the flask equilibrium temperature, showed that the vapour concentration increased almost exponentially with temperature. The same effect was found for caproic acid by Larsson et al (1978). Between 30·0 and 31·0 °C the peak height increased by up to 10%. This compares with the 7% change per degree for ethanol between 23·8 and 50·0 °C found by Davis & Chace (1969). Thus the maximum error introduced into headspace results by fluctuations in the closed system temperature was 3·5%.

The linear increase of peak height response with increasing vapour concentrations (correlation coefficient 0·990) is shown in Fig. 3. Extrapolation of the data to that obtained for neat benzyl alcohol vapour provides the standard benzyl alcohol vapour concentration at 30·0 °C as 1·07 μg cm^{-3}. Vapour pressure can then be calculated from vapour concentration using the ideal gas law, PV = nRT; it was 0·19 mm Hg for pure benzyl alcohol at 30·0 °C

Knowing the vapour pressure and peak heights for pure benzyl alcohol and assuming linear chromatographic response, the partial vapour pressures were calculated as a function of temperature and plotted

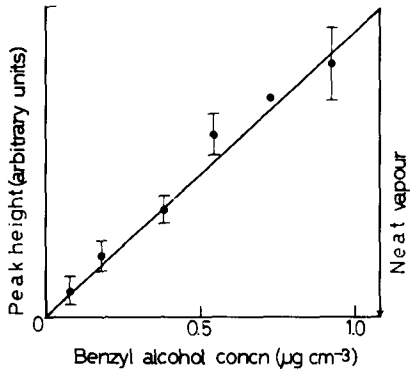

FIG. 3. Gas chromatograph calibration for benzyl alcohol vapour. Peak height is plotted against known benzyl alcohol concentration and the error bars represent ± one standard deviation.

in Fig. 4 as log p versus 1/T along with published results (Stull 1947); the correlation coefficient was 0·999. Leggett (1977) demonstrated the same relationship for trinitrotoluene by using a headspace method. This linear relationship is derived from the Clausius-Clapeyron equation, which may be written in the form

$$\frac{d(\log p)}{d(1/T)} = \frac{\Delta H_v}{2{\cdot}303R} \qquad (7)$$

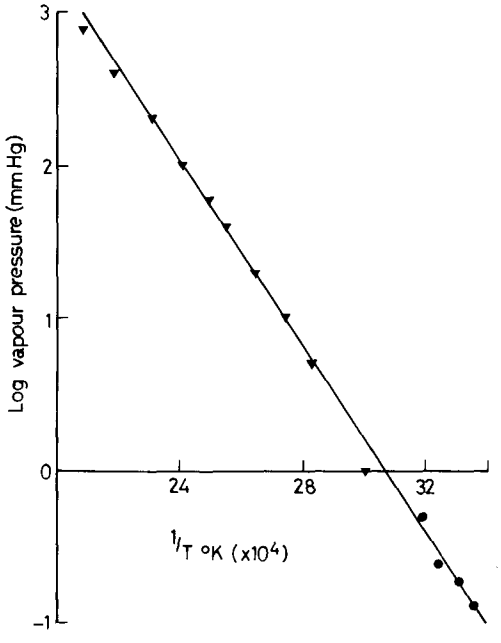

FIG. 4. Log benzyl alcohol vapour pressures plotted against the reciprocal of absolute temperature. (- ▼ -) published results (Stull 1947) (- ● -) headspace results.

where ΔH_v is the change in heat content per mole of benzyl alcohol during vaporization; the vapour is assumed ideal and the system is well below the critical temperature. The integrated form of equation 7 provides

$$\log p = \frac{-\Delta H_v}{2{\cdot}303RT} + \text{constant} \qquad (8)$$

which defines the linear relationship in Fig. 4 assuming a constant value of ΔH_v over the temperature range considered. The heat of vaporization of benzyl alcohol was calculated as 57900 J mol^{-1}. This compares well with the published value of 58970 J mol^{-1} (Weast 1978–1979), particularly as there are errors in the approximations used to calculate ΔH_v as well as in the headspace analysis.

If the entropy of vaporization (δS_v) for benzyl alcohol is calculated from the heat of vaporization according to

$$\Delta S_v = \frac{\Delta H_v}{T_{bp}} \qquad (9)$$

where T_{bp} is the boiling point, it is 123 J deg^{-1} mol^{-1}. Trouton's rule states that the entropy of vaporization is about 88 J deg^{-1} mol^{-1} for most liquids. The high value for benzyl alcohol is typical of hydrogen bonding molecules e.g. ethanol, 110 and water, 109 J deg^{-1} mol^{-1}. Thus benzyl alcohol behaves as a normal hydrogen bonding volatile liquid.

The thermodynamic activities and vapour concentrations for benzyl alcohol mixed with vehicles toluene, butanol, butyl acetate, isopropyl myristate, isophorone and propylene carbonate were plotted as functions of benzyl alcohol mole fraction. Where the vehicles also provided a gc response, their activities were included on the plot (toluene, butanol, butyl acetate, isophorone). These vehicles were selected primarily as simple pure model compounds which would interact to various degrees with the model penetrant, benzyl alcohol—isopropyl myristate and propylene carbonate are also pharmaceutical adjuvants. However, at this stage of our investigations we did not wish to complicate further the development of our technique by using complex multiphase systems such as creams. Examples of headspace results for vehicles in which benzyl alcohol behaves non-ideally are given in Fig. 5a (toluene—positive deviation from Raoult's law) and in Fig. 5b (isophorone—negative deviation from Raoult's law). The diagonals represent Raoult law behaviour for ideal binary mixtures. Incidentally, the standard deviations illustrated in Fig. 5 show why we

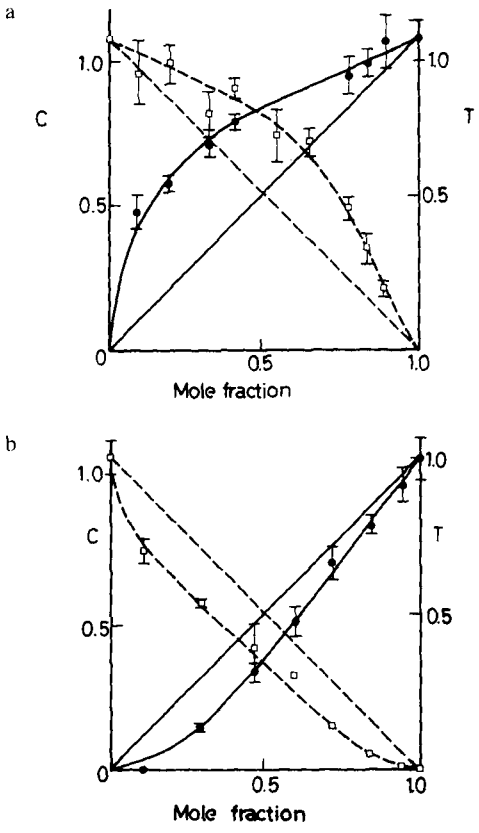

FIG. 5. (a) Headspace analysis results for benzyl alcohol binary mixture with toluene plotted against mole fraction of benzyl alcohol; benzyl alcohol vapour concentration and thermodynamic activity(- ● -), toluene thermodynamic activity (- □ -). Error bars represent ± one standard deviation. C is concentration in μg cm^{-3}, T is thermodynamic activity. (b) Headspace analysis results for benzyl alcohol binary mixture with isophorone plotted against mole fraction of benzyl alcohol; benzyl alcohol vapour concentration and thermodynamic activity (- ● -), isophorone thermodynamic activity (-□-). Error bars represent ± one standard deviation. C is concentration in μg cm^{-3}, T is thermodynamic activity.

have not calculated further thermodynamic parameters from our data e.g. activity coefficients and partial/total molar excess free energies of mixing (Kolb 1980). A manual hsgc method is accurate enough when the intention is to correlate data with drug absorption through human skin, as the biological variation in permeability of this tissue is great (Southwell et al 1984); a precise automatic method is necessary for an extensive thermodynamic analysis of the vapour state alone.

We can explain the non-ideal effects of vehicles on intermolecular hydrogen bonding, and thus on the equilibrium vapour concentration, of benzyl alcohol in terms of the hydrogen bonding capacity of the

vehicle compared with that of the alcohol. For the binary mixtures to behave ideally, the vehicle must bond with benzyl alcohol to the same extent as the alcohol self-associates, and this depends on molecular structure (Fig. 1). Since hydrogen bonds are generally stronger than other molecular interactions between non-ionic species, the extent to which the vehicles form hydrogen bonds with benzyl alcohol determines if the mixture appears ideal. Butanol and butyl acetate gave only small deviations from ideal behaviour, suggesting that hydrogen bonding in their mixtures with benzyl alcohol was similar in extent to that in pure alcohol. Isopropyl myristate, propylene carbonate and toluene (Fig. 5a) deviated positively from Raoult's law i.e. these vehicles reduced the amount of intermolecular hydrogen bonding in the liquid phase and thus increased the availability of vapour phase molecules, so raising vapour concentration. When isophorone was added to benzyl alcohol (Fig. 5b) the extent of bonding increased and therefore the vapour concentration decreased (negative deviation). These diagrams also illustrate that the vapour of both components in a binary mixture show the same deviation from ideal behaviour, as they arise from the same molecular interactions. For most mixtures these deviations are virtually identical.

The final stage of the investigation was to attempt to correlate penetrant flux through human skin with thermodynamic activity i.e. to test eqn. 2. Diffusion experiments were run with neat benzyl alcohol and with 0·5 mole fraction binary mixtures, using the vehicles tested by headspace analysis; the relevant thermodynamic activity was derived from graphs such as Fig. 5a, b. Vapour flux diffusion measurements were made between 5 and 9 h after the start of the experiment to ensure that pseudo steady state had been established. The lag time for pure benzyl alcohol vapour diffusion was 1·7 ± 1·2 h, which was less than one third the first sample time of 5 h. (Steady state is only reached when $Dt/h^2 \simeq 0{\cdot}45$, which corresponds to 2·7 times the lag time (Crank 1975)). Vapour fluxes decreased slightly at longer times (about 24–30 h) probably because of the stratum corneum drying out somewhat after its initial hydration.

Mean vapour fluxes ± one standard deviation are plotted against benzyl alcohol thermodynamic activity in Fig. 6. The results show a good correlation between vapour flux and thermodynamic activity (correlation coefficient 0·969). The vehicles tested appeared to make no contribution to the benzyl alcohol flux in the vapour state. We can conclude

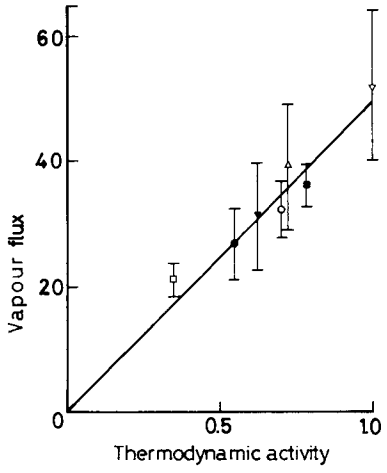

FIG. 6. Mean benzyl alcohol vapour flux through human skin from neat benzyl alcohol and from 0·5 mole fraction binary mixtures ± one standard deviation plotted against thermodynamic activity found by headspace gas chromatography. Vapour flux is in $\mu g\ cm^{-2}\ h^{-1}$; (- □ -) isophorone, (- ● -) butyl acetate, (- ▼ -) butanol, (- ○ -) propylene carbonate, (- △ -) isopropyl myristate, (- ■ -) toluene, (- ▽ -) neat benzyl alcohol.

that, for the materials and conditions used in these experiments, when vehicle components have no effect on the barrier properties of the skin, the rate of vapour absorption through human skin is proportional to the thermodynamic activity as determined by headspace analysis. Thus, a suitable method has been validated for determining the thermodynamic activity of a model volatile skin penetrant (benzyl alcohol). In theory, we could combine a single measurement of the thermodynamic activity of *any* pure volatile penetrant with its measured vapour flux through skin to construct a straight line plot passing through the origin as shown in Fig. 6. Then, for any topical vehicle however complex, we could simply measure the thermodynamic activity of the penetrant via headspace analysis and read-off the appropriate skin flux from the graph. This procedure would thus minimize the number of permeability measurements needed in, for example, the development of a topical formulation, thus fulfilling the first aim of our work as specified in the Introduction. However, we need to be cautious in that in all the work reported here, the topical vehicle does not directly contact the skin but only via the vapour state. It remains to be seen if direct association between vehicles and skin alters the simple ideal behaviour reported in this paper.

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REFERENCES

Al-Khamis, K., Davis, S. S., Hadgraft, J., Mills, S. (1982) Int. J. Pharm. 10: 25–28

Barry, B. W. (1983) Dermatological Formulations: Percutaneous Absorption. Dekker, New York and Basel

Coldman, M. F., Poulsen, B. J., Higuchi, T. (1969) J. Pharm. Sci. 58: 1098–1102

Crank, J. (1975) The Mathematics of Diffusion, 2nd edn Clarendon Press, Oxford

Davis, P. L., Chace, W. G. (1969) Hort. Sci. 4: 117–118

Drozd, J., Novak, J. (1977) J. Chromatogr. 136: 37–44

Larsson, L., Mardh, P. A., Odham, G. (1978) J. Clin. Micro. 7: 23–27

Leggett, D. C. (1977) J. Chromatogr. 133: 83–90

Hachenberg, H., Schmidt, A. P. (1977) Gas Chromatographic Headspace Analysis. Heyden, London

Harrison, S. M. (1984) Ph.D. Thesis, Bradford University

Harrison, S. M., Barry, B. W., Dugard, P. H. (1982) J. Pharm. Pharmacol. 34: Suppl. 36P

Higuchi, T. (1960) J. Soc. Cosmet. Chem. 11: 85–97

Kolb, B. (1980) Applied Headspace Gas Chromatography, Heyden, London

Menczel, E., Maibach, H. I. (1970) J. Invest. Derm. 54: 386–394

Menczel, E., Maibach, H. I. (1972) Acta Derm. Venereol. 52: 38–42

Ramsay, J. D., Flanagan, A. J. (1982) J. Chromatogr. 240: 423–444

Schaefer, H., Zesch, A., Stuttgen, G. (1982) Skin Permeability, Springer-Verlag, Berlin, pp. 755–758

Scheuplein, R. J. (1965) J. Invest. Derm. 45: 344–345

Scheuplein, R. J. (1967) Ibid. 48: 79–88

Southwell, D., Barry, B. W., Woodford, R. (1984) Int. J. Pharm, 18: 299–309

Stull, D. R. (1947) Ind. Eng. Chem. 39: 517–523

Weast, R. C. (1978–1979) CRC Handbook of Chemistry and Physics, 59th Ed., CRC Press, Boca Raton, C-732