International Journal of Cosmetic Science 3, 271–278 (1981)

Aspects of skin permeability

A. DYER*, G. G. HAYES, J. G. WILSON and R. CATTERALL, Department of Pure and Applied Chemistry, University of Salford, Salford, M5 4WT

Received 6 November 1980

Key words: self-diffusion, salicylic acid, skin membranes, zeolite/polystyrene.

Synopsis

The self-diffusion of salicylic acid has been monitored radiochemically through pig-skins (whole and dermal) and human skins (whole, dermal and epidermal).

The effects of pH, temperature and changing dimethyl formamide (DMF) concentration on the penetration of salicylic acid through the various skin membranes have been evaluated.

Conclusions have been drawn as to the mechanisms operating and some comparisons to zeolite/polystyrene membranes have been made.

Quelques aspects de la perméabilite cutanée

Résumé

Un précédent article a été consacré à la description d'une nouvelle méthode pour l'examen de la migration des molécules simples au travers de la peau et de ses modèles. Il s'agissait d'une technique radiochimique utilisant comme cellule un simple tube en U et les données concernant le taux de pénétration étaient analysées par un traitement mathématique sophistiqué conduisant à la définition d'un coefficient de diffusion. Ces études ont été élargies à une série d'expériences pour mesurer la migration de l'acide carboxy-salicylique -^{14}C au travers de:

(a) la totalité et le derme de la peau du cochon;
(b) la totalité, le derme et l'épiderme de la peau humaine.

Cette étude fur menée par des mesures à diverses températures telles qu'il soit possible d'évaluer les barrières d'énergie s'opposant à la pénétration de la substance. Des calculs ultérieurs donnèrent les paramètres d'entropie et de variation d'énergie libre associés au processus de pénétration.

Lorsque l'influence du pH a été étudiée, on a noté qu'aucune pénétration de l'acide salicylique ne se produisait sur la peau humaine pour les pH>3.5. Ceci confirme les travaux déjà publiés par ailleurs.

Lorsque l'on ajoute de 35 à 75% de DMF au système il n'y a pas de migration de l'acide salicylique au travers de la peau humaine. On n'a pas observé d'influence de l'age de la peau qui provenait de l'abdomen et qui était donc vraisemblablement moins affectée par les variations d'âge.

Le derme du porc offre une moindre résistance à la migration (solution aqueuse d'acide salicylique) que la totalité de sa peau.

*To whom correspondence should be directed.

Le derme humain se comporte de la même façon que celui du porc. Mais les résultats obtenus pour la totalité de la peau du porc diffèrent de façon notable de ceux obtenus avec la totalité de la peau humaine. Ceci indique que certaines précautions doivent être prises lorsque l'on utilise la peau du porc comme modèle dans les études de perméabilité cutanée.

Certains résultats sont interprétés en termes de l'état de désordre des molécules d'eau dans les membranes par référence à des études parallèles dans des membranes zéolite/polystyrène.

INTRODUCTION

A previous paper (1) described a new approach to the investigation of self-diffusion through skin based upon a sophisticated mathematical treatment of diffusion profiles and their variations with temperature. It sought to establish the usefullness of this approach by comparison to well characterized porous membranes (zeolite/polystyrene compacts).

The work considered here continues this approach, measuring carboxyl-^{14}C salicylic acid migration through (a) whole and dermal pigskins and (b) whole, dermal and epidermal human skins.

Effects of human skin ageing are considered as are the variation of pH and accelerant concentration on different skins. Attempts to rationalize the results on the basis of thermodynamic parameters calculated from experiments based upon temperature variation have been made. The parameters estimated were the energies, entropies and free energies for the activated self-diffusion step (i.e. E_a, ΔS^* and ΔG^* respectively).

EXPERIMENTAL

Details of cell design, membrane preparations, self-diffusion procedure and radiochemical methods have been described previously (1). There was no chemical pretreatment of the skin.

pH studies were attempted on the self-diffusion of labelled salicylic acid through the full-thickness human and epidermal pig skin membranes in the range pH 3.5-11. Lower pH values were achieved with sodium salicylate, intermediate values with standard phosphate buffers (BDH) and the high pH values with a sodium carbonate/bicarbonate system. When necessary a dilute solution of sodium azide was added to inhibit fungal growth.

To study the effect of age, samples from fifteen different human cadavers were used to measure a self-diffusion coefficient (D*) at 293°K for salicylic acid.

In some experiments dimethyl formamide (DMF) was added to both acceptor and donor arms of the cell (10-100% DMF). The cell was then allowed to stand overnight (with membrane in place) before additions of carboxyl ^{14}C salicylic acid were made to the donor arm to start self-diffusion measurements. (N.B. unlabelled salicylic acid was added to the acceptor arm at the same time – see (1) for full details.)

The membrane studied in this case was full thickness human skin.

Self-diffusion runs were performed on human dermis in the range 298-323°K. Skin samples were from three cadavers and ten experimental runs were made. Carefully removed intact epidermal sheets from four cadavers were used to study epidermal transport in fourteen experiments in the same temperature range. For full thickness human skin eleven runs between 298-313°K were carried out. All samples were abdominal skin except in one case a thigh sample was taken. The concentration of salicylic acid was 3×10^{-3}M in all experiments.

RESULTS

pH studies

Pig epidermis ruptured easily and was subject to a high evidence of leakage ascribed to the penetration of the epidermis by bristles. It was rejected as a suitable membrane.

Experiments on full thickness human skin at 293°K showed very slow penetration in the range pH 3.5–5.0 even after 80 h. Above pH 5.0 no penetration was observed and increasing the temperature to 303 and 313°K did not increase penetration rates at any pH.

Age studies

The results were as shown in Fig. 1 which compares the measured D* at 293°K to age.

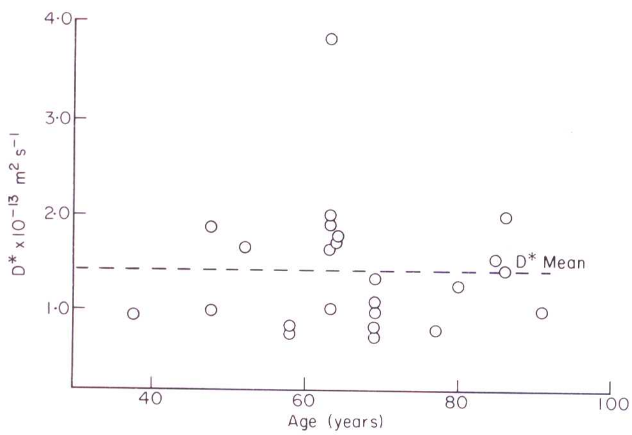

Figure 1. Variation of self-diffusion coefficient (D*), measured at 293°K for salicylic acid penetration through whole human skin, with age.

DMF concentration variation

Table I records the pH of DMF/water mixtures calculated from the Henderson-Hasselbach equation:

$$pK\text{-}pH = \log_{10} Cu/Cd \tag{i}$$

where: Cu = concentration of undissociated species; Cd = concentration of dissociated species: pK = 2.97 for salicylic acid.

Variations of thermodynamic parameters calculated from Arrhenius plots of the variation of D* with temperature (2) for salicylic acid moving through human skin in the presence of various DMF concentrations were as tabulated in Table II (see also Fig. 3).

Runs in the range 40–70% DMF were discounted as insufficient tracer penetrated the membrane in this range.

Variation in membrane type

Parameters observed were as listed in Table III.

Table I. Properties of salicylic acid in various solutions

Solution % DMF	pH	% Dissociation of salicylic acid
0	3.0	52
10	3.15	60
20	3.22	64
30	3.38	72
40	3.57	80
50	3.81	87
60	4.09	93
70	4.42	97
80	4.81	99
90	5.22	99
100	7.20	100

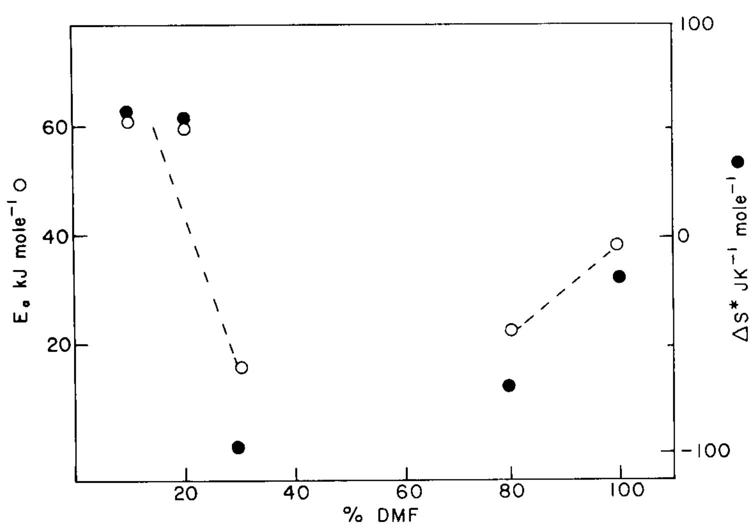

Figure 2. Variation of thermodynamic parameters, with DMF concentration, for the self-diffusion of salicylic acid through whole human skin.

Table II. Variation of self-diffusion parameters for salicylic acid penetration in whole human skin

Vehicle % DMF	E_a (kJ mole^{-1})	ΔS^* (JK^{-1} mole^{-1})	ΔG^* (kJ mole^{-1})
10	61	56	43
20	61	53	43
30	15	–96	39
80	22	–70	39
100	36	–24	40

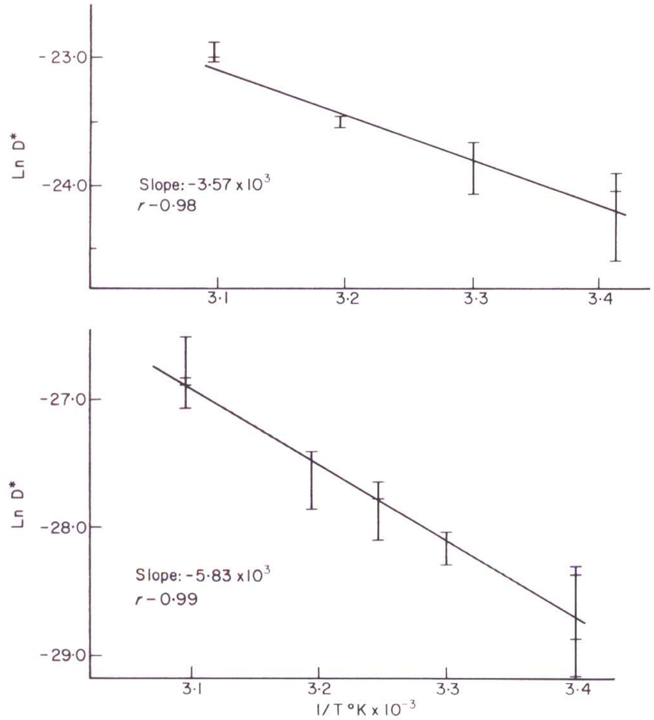

Figure 3. Arrhenius plots for salicylic acid self-diffusion in (a) human dermis and (b) whole pigskin.

Table III. Variation of thermodynamic parameters with membrane type for salicylic acid self-diffusion

Membrane	E_a (kJ mole^{-1})	ΔS^* (JK^{-1} mole^{-1})	ΔG^* (kJ mole^{-1})	D*† (m^{2}s^{-1})	T (°K)
Zeolite X/ polystyrene	35	+17	28	$1.5 \pm 0.3 \times 10^{-11}$	293
Zeolite 2.62 Y/ polystyrene (1)	17	−40‡	26‡	$3.3 \pm 0.2 \times 10^{-11}$	298
Pig dermis	26	−10	27	$2.1 \pm 0.5 \times 10^{-11}$	298
				$3.6 \pm 0.5 \times 10^{-11}$	303
Full thickness pig skin	49	+30	38	$3.8 \pm 1.0 \times 10^{-13}$	294
				$6.0 \pm 0.7 \times 10^{-13}$	303
Human dermis	30	+4	26	$3.4 \pm 1.0 \times 10^{-11}$	293
				$4.5 \pm 0.9 \times 10^{-11}$	303
Human epidermis	48	+24	39	$2.9 \pm 0.5 \times 10^{-13}$	298
Full thickness human skin	65	+78	42	$1.8 \pm 0.1 \times 10^{-13}$	298
				$2.4 \pm 0.3 \times 10^{-13}$	303

†Average of three determinations
‡corrected values from (1)

DISCUSSION

The effect of pH

The results indicated that the ionized form of salicylic acid was unable to penetrate human skin to any significant extent when pH >3.5. This was in agreement with the findings of Arita *et al.* (3) who observed that the transport of salicylic acid through guinea-pig abdominal skin reduced to zero at pH 5.0.

Effect of age

When the results shown in Fig. 1 were averaged a value of $|D^*| = 1.4 \times 10^{-13} m^2 s^{-1}$ was obtained close to that observed for full thickness human skin in the temperature variation experiments (see Table III). No change in salicylic acid permeation rate could be inferred with age.

Chemical and physical changes have been observed in ageing skin but, like the result quoted herein, no relationship to permeability has yet been established (4).

In these studies it might be that an effect of age would be masked by the use of abdominal skin which, in man, is less prone to trauma and so may not show age effects.

Variation in DMF concentration.

DMF was chosen for these studies because it resembled dimethyl sulphoxide (DMSO) in that its aprotic nature has been shown to encourage percutaneous transport (i.e. it acts as a 'vehicle') but without the side effects noted with DMSO, whilst not necessarily being non-toxic (5).

Table II showed that, as the amount of DMF was increased from 0 to 30%, the energy barrier to salicylic acid migration through human skin decreased. At the same time the amount carried through *also* was reduced to such an extent that between about 35 and 75% DMF no sensible rates could be recorded. This did not mean that the *mobility* was decreased but that most of the salicylic acid was not available for migration. When the percentage DMF was >80% the energy barrier rose but more labelled molecules were transported.

In a practical context it has been noted that concentrations of DMSO >70% have been reported as necessary to accelerate penetration whilst some reports record low concentrations (<20%) as enhancing permeation (6), i.e. DMF thus was behaving in a similar manner.

Considering earlier comments it can be conjectured that it seemed unlikely that the vehicle effect was pH determined entirely. Other mechanisms have been suggested for the mode of action of DMSO, viz its abiltiy to increase tissue hydration (7) or to promote protein swelling/unfolding by its substitution for protein bound water (8,9).

Bearing these views in mind, some logic ensued if the results recorded here were considered in three stages, noting that the overall driving force (ΔG^*) changed very little with DMF concentration and recalling that $\Delta G^* = \Delta H^* - T\Delta S^*$ and $E_a = \Delta H^* - RT$.

Initially (DMF 0-30%) the process resembled that occuring in aqueous solution in the temperature variation experiments (see Table III) with the free energy controlled by enthalpic considerations due either to (a) the removal of a surface lipid barrier (10) or (b) by the initial influx of DMF causing a loosening and alteration of protein configuration. It can be noted that the entropy of the self-diffusion step changed little so the second explanation which implied a change in order seemed less likely.

In the range 35–65% DMF the process observed seemed to be characterized by a low energy of activation but a high change in order (ΔS^* -ve) implying that the driving force was controlled by changes in order, perhaps the substitution of DMF for protein bound water. A similar entropy controlled process has been observed for hydroquinone self-diffusion through the human erythrocyte ghost membrane (11). Of course the pH value was unfavourable to salicylic acid movement as well but this did not apply apparently when the DMF concentration rose to >80% and penetration occurred with higher E_a and less negative ΔS^* values.

Perhaps in this final range a saturation condition was reached where order changes were smaller (i.e. most of the bound water had been replaced by DMF) and the energy barrier had risen due to protein denaturing and general membrane damage.

Variation in membrane type

Zeolite/polystyrene membranes

The sole structural and chemical difference between the zeolites X and 2.62 Y lay in the relative amounts of aluminium substituted for silicon in their porous aluminosilicate frameworks (12). This meant that the 2.62 Y structure carried a smaller charge which was reflected in the lower energy barrier observed to salicylic acid migration. The more negative ΔS^* value observed could be taken as a reflection of the less ordered nature of the water molecules confined in the zeolitic cavities of 2.62 Y which meant that the diffusing species exerted more randomization during their diffusional step.

Pig skins

It was shown that pig dermis presented a lower barrier to migration than full thickness pig skin as anticipated. The ΔG^* value observed indicated that, perhaps, the penetration routes followed were not similar.

Human skins

The figures in Table III demonstrate that, in absence of a vehicle, the main barrier to salicylic acid movement occurred in the human epidermis and its movement entailed relatively little disruption of bound water. However, the dermis did present a barrier of a somewhat different nature very like that observed in pig dermis.

The use of pig skin to mimic human skin in the study of percutaneous transport

Pig skin frequently has been suggested as a model for human skin. The results shown here would confirm the validity of pig dermis as a model for human dermis and note that, at physiological temperatures, the rates of penetration; of salicylic acid were very similar (Table III D^*_{303}). Consideration of the full thickness skins showed that, whilst ΔG^* values were similar, the barrier in human skin was appreciably higher which meant that as temperatures increased so penetration rates would inevitably diverge so that comparisons should only be made in the full knowledge of these facts.

ACKNOWLEDGMENTS

The work described herein is taken from the Ph.D studies of G. G. Hayes, supported by Nicholas International, and J. G. Wilson supported by SRC. We are greatly appreciative for the supply of skin samples by Dr D. Wimbourne (Salford Royal Hospital) and from Hope Hospital, Salford.

REFERENCES

1. Dyer, A., Hayes, G. G., Wilson, J. G. and Catterall, R. Diffusion through skin and model systems. *Int. J. of Cosmetic Science* **1** 91 (1979).
2. Dyer, A., Phillips, G. O. and Townsend, R. P. Self-diffusion of zinc in cross-linked crystalline carboxy peptidase A. *J. Chem Soc. Faraday Trans. I* **71** 803 (1975).
3. Arita, T., Hori, R., Anmo, T., Washitake, M., Akatsu, M. and Yajima, T. Studies on percutaneous absorption of drugs I. *Chem Pharm. Bull.* **18** 1045 (1970).
4. Yates, J. R. in Elden, H. R. *Biophysical Properties of Skin*, p.485. Wiley Interscience, NY (1971).
5. Bainova, A. Assessment of the skin lesions in the production of Bulana polyacrylonitrile fibres. *Dermatol. Venerol (Sofia)* **14** 92 (1975).
6. Idson, B. Biophysical factors in skin penetration. *J. Soc. Cosmet. Chem.* **22** 615 (1971).
7. Allenby, A. C., Creasey, N. H., Edginton, J. A. G., Fletcher, J. A. and Schock, C. Mechanism of action of accelerants on skin penetration. *Brit. J. Dermatol.* **81** Suppl 47. (1969).
8. Elfbaum, S. G. and Laden, K. Effect of DMSO on percutaneous absorption, mechanistic study III. *J. Soc. Cosmet. Chem.* **19** 841 (1968).
9. Rammler, D. A. and Zaffaroni, A. Biological implications of DMSO based on a review of its chemical properties. *Ann. N.Y. Acad. Sci.* **141** 13 (1967).
10. Embery, G. and Dugard, P.H. Isolation of DMSO soluble components from human epidermal preparations: a possible mechanism of action of DMSO in effecting percutaneous migration phenomena. *J. Invest. Dermatol.* **57** 308 (1971).
11. Cundall, R. B., Dyer, A. and McHugh, J. O. The diffusion of drugs from resealed human erythrocyte membrane. *J. Chem. Soc. Faraday Trans. I* **77** 1039 (1981).
12. Breck, D. W. *Zeolite Molecular Sieves* p. 176. Wiley Interscience, NY (1974).