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Effect of nonionic surfactants on transdermal drug delivery: I. Polysorbates

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Summary

Polysorbates which are a prominently safe class of surfactants may offer means of enhancing drug permeation through the skin. In order to determine this effect, in vitro diffusion experiments using freshly excised full thickness hairless mouse skin as well as inert silicone elastomer membrane were carried out. The data of this study clearly revealed that polysorbates, i.e. polysorbate 20 (Tween 20™), polysorbate 21 (Tween 21™), polysorbate 80 (Tween 80™), polysorbate 81 (Tween 81™), had only a slight influence on the permeability of hydrophilic methanol. The permeability was maximally doubled. Neat polysorbate surfactants, however, demonstrated a significant increase of up to 13-fold when applied to hairless mouse skin. In contrast, the permeability of lipophilic octanol decreased as a function of polysorbate concentration, a trend which was evident in the permeation studies through silicone elastomer sheeting as well as in the separate assessment of the thermodynamic activity. This observed effect was due to a decrease in thermodynamic activity as a result of micellar complexation. It was evident from the second sequential run that the effects of polysorbates on the permeability of methanol and octanol were reversible.

Introduction

Some years ago, evidence has been presented that several surface active agents may cause a penetration enhancing effect. Unequivocally, nonionic surfactants reveal an impact with respect to permeability on various biological membranes, e.g. gastrointestinal mucosa (Whitmore et al., 1979; Touitou et al., 1980; Walters et al., 1981a), goldfish gill epithelium (Florance and Gillan, 1975), and the skin (Hwang and Danti, 1983; Aungst et al., 1986; Walters et al. 1988).

The key to evaluating the effect of the nonionic surfactants on percutaneous absorption is to determine the flux of model compounds such as hydrophilic methanol and lipophilic octanol across (i) an inert silicone rubber membrane and (ii) full thickness hairless mouse skin when the test permeants are applied in surfactant solutions at different concentrations. Using a synthetic and a biological membrane has the distinct advantage to distinguish between effects due to changes of thermodynamic parameters or due to those of biologi-

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cal consequences. A conditio sine qua non for penetration enhancers is to assess the reversibility of the observed effect.

Polysorbates (Tween™) constitute a prominently safe class of surfactants. They are widely accepted by many regulatory agencies for use in pharmaceuticals and in cosmetics. Due to the low toxicity and almost the lack of irritancy of the skin they appear to be promising candidates as skin penetration enhancers.

Materials and Methods

Chemicals

Methanol (E. Merck, Darmstadt, Germany), octan-1-ol (BDH Chemicals Ltd, Poole, U.K.), xylol (E. Merck, Darmstadt, Germany), tritiated methanol (spec. act., 5 mCi/mmol; tot. act. 25 mCi) (Du Pont de Nemours, Biotechnology Systems Division, Paris, France), [1-^{14}C]octanol (spec. act., 1 mCi/mmol; tot. act., 500 μCi) (ICN Biomedicals GmbH, Eschwege, Germany), sterile 0.9% sodium chloride solution for irrigation (Fresenius AG, Bad Homburg, Germany), polysorbate 20 (Tween 20™), polysorbate 21 (Tween 21™), polysorbate 80 (Tween 80™), polysorbate 81 (Tween 81™) were used. Polysorbates were provided by Atlas Chemie, Niederlassung der Deutschen ICI, Essen, Germany. All organic solvents were HPLC grade or of equivalent quality.

Stock solutions

In order to form stock solutions, both [^{3}H]-methanol and [^{14}C]octanol were diluted with saline. The alcohol concentrations in saline were chosen to be less than 10^{-4} mol in order to eliminate any permeation enhancing effect of the alcohols by themselves. Each stock solution contained approx. 50 μCi/ml of the test permeant.

Surfactant solutions

Surfactant solutions were prepared in 0.9% sodium chloride solution for irrigation. Dilution of the latter due to addition of stock solutions of the radiolabelled compounds (0.3 ml stock solution + 1.2 ml surfactant solution) was taken into account.

Membranes

Full thickness hairless mouse skin was obtained from female hairless mice, strain hr/hr-C3H/TifBom (Bommice, Bomholtgård Breeding and Research Centre Ltd, Ry, Denmark). The age of the animals exceeded 100 days. They were killed by CO_2. Both abdominal and dorsal skin was carefully excised with a pair of scissors and subcutaneous fat was scratched off with a scalpel.

High performance medical grade silicone elastomer membrane (Silastic™ sheeting Q7-4840, Dow Corning, Corporation, Midland, MI, U.S.A.) of 175 μm thickness was employed as an inert control membrane in diffusion experiments. After removing the cover sheets, the silicone elastomer sheeting was rinsed thoroughly with distilled water in order to remove residual sodium bicarbonate dusted on the cover sheets to facilitate handling.

Two-compartment diffusion cells

The diffusion cell type used in these studies has been described in detail by Durrheim et al. (1980). Briefly, the cell was made of glass, the two cell halves were separated by the membrane. The donor and receiver compartment respectively had a volume of 1.5 ml. They were equipped with two ports, one for sampling and the other one to hold the shaft of the stirrer. The surface area of the membrane of the diffusion cell was approx. 0.8 cm^2 (the exact half cell volumes and surface areas were taken into account when analyzing permeation data). Propeller, stirring shaft and the clamping fixture were made of Teflon.

Diffusion experiments

After mounting the membrane into the diffusion cell, it was immersed in a constant temperature waterbath of 37 °C. The donor chamber was charged with 1.2 ml of surfactant solution the receiver chamber was filled with 1.2 ml of saline. The contents of the diffusion cell were allowed to equilibrate and were stirred at 150 rpm. The study was designed as a dual-label experiment. 50 μl of [^{3}H]methanol and 250 μl of the [^{14}C]octanol stock solution were pipetted into the donor compartment with a micropipette to initiate the diffusion run. The same amount of saline was added to the receiver compartment. 3 min were allowed to

elapse in order to achieve a homogeneous solution before taking a sample of the donor compartment to determine the initial concentration of the test permeants. At designated time intervals, samples of 50 μl were drawn with a micropipette from the receiver compartment. An equivalent amount of saline was added to maintain a constant volume in the receiver chamber. Dilution of the receiver medium was taken into account when processing the permeation data. All samples were processed for counting by adding 7 ml of scintillation cocktail (Readysolv™, Beckman Instruments, München, Germany) and counted in a liquid scintillation counter (Model LS 1501, Beckman Instruments, München, Germany).

After the first set of permeation experiments was finished, both donor and receiver compartment were evacuated with a syringe and rinsed three times with saline. In order to remove the test permeants and residual surfactant completely triple rinsings were carried out every 30 min for 2 h.

A second set of diffusion experiments was initiated with the same skin patches still mounted in the diffusion cells. The procedure resembled the one outlined above; however, the medium in the donor chamber constituted of neat saline plus the appropriate volume of the stock solutions of each test permeant. Samples were taken at the same time points as in the first diffusion run. This second set of experiments depicted the so-called 'reversibility experiment'.

Assessment of the thermodynamic activity

Surfactant solutions The concentration of each surfactant in saline was 0.5, 5.0 and 10.0% (w/v) respectively. The effect of neat polysorbate 20 and 80 on the thermodynamic activity of the two model compounds was determined as well. Care was taken that the ratio surfactant solution vs methanol and octanol resembled the conditions of the infinite dose diffusion experiments. Each preparation was stored at a constant temperature of 37 °C in a vial which was closed with a Teflon coated cap. 200 μl samples of vapor were taken out of the headspace of each individual vial with a gas-tight syringe and injected into the gaschromatograph. The gas-tight syringe was maintained at 37 °C prior to use.

Reference solutions In order to quantitate the amount of methanol and octanol, standard curves were obtained by dissolving a known amount of methanol in xylol and a known amount of octanol in methanol. Appropriate dilutions were made and 1 μl samples were analyzed by gas chromatography as described below. Peak areas were plotted versus concentration, and linear regression of the data was carried out.

Gas chromatography (Hewlett Packard 5890, Integrator: Hewlett Packard 3393, Hewlett Packard, Boeblingen, Germany), Fused Silica Capillary Column FS-SE-54-CB-1; 25 m, 0.32 mm i.d. (Chrompack GmbH, Frankfurt, Germany) was used in order to determine the concentration of methanol and octanol in the headspace of each container (initial oven temperature, 140 °C; rate, 10 °C/min; final oven temperature, 200 °C; injector temperature, 280 °C; detector temperature, 280 °C; flow rate, 3 ml N_2/min; split, 1 : 10).

Data treatment

The partial vapor pressure of methanol and octanol in saline solution was determined by analyzing the concentration of each alkanol in the gaseous headspace of each solution at equilibrium. Based on the ideal gas law the partial vapor pressure $P_{i,gas}$ of each alkanol can be calculated from the following equation:

$$P_{i,gas} = \frac{gRT}{MV}$$

where $P_{i,gas}$ is the partial vapor pressure of the alkanol, g the amount of alcohol (in g) measured, R the ideal gas constant, T absolute temperature (K), M the molecular weight and V the sample volume.

The activity α of methanol and octanol in saline solution was calculated from:

$$\alpha = \frac{P_{i,gas}}{P^{o}_{i,gas}}$$

where $P^{o}_{i,gas}$ represents the vapor pressure of the pure alcohol as experimentally determined.

The thermodynamic activity is the relative fugacity of a solution compared to a standard

solution. With respect to (trans-)dermal drug delivery it describes the escaping tendency of a drug in a vehicle. The activity coefficients γ can in turn be calculated from:

$$\gamma = \frac{\alpha_i}{X_i}$$

where X_i is the mole fraction of alkanol at a given solvent composition and α_i is the activity of component i.

Results and Discussion

Various experimental findings concerning the permeability properties of the aliphatic alcohols through different heterogeneous barriers have been published. They have been employed to elucidate the specific mechanisms of transport through human skin (Scheuplein, 1965; Blank et al., 1967; Scheuplein et al., 1973; Scheuplein 1976) as well as hairless mouse skin (Durrheim et al., 1980; Flynn et al., 1981a,b), to determine the influence of hydration (Behl et al., 1980a; Lambert et al., 1989), to evaluate the effect of scalding and branding on the permeability characteristics of the integument (Behl et al., 1980a,b, 1981; Flynn et al., 1981b), to characterize the permeability of the human nail (Walters et al., 1981a, 1983), to investigate the impact of surfactants on molecular diffusion across the skin (Garcia et al., 1980; Behl et al., 1980d), and to assess the mode of action of 2-pyrrolidone and dimethylformamide of *n*-alcohols through human skin (Southwell and Barry, 1983).

Methanol

At all 10 concentrations studied, i.e. at 0.05, 0.1, 0.5, 1, 2.5, 5, 7.5, 10, 25 and 100%, polysorbate 20 (Tween 20™) (Fig. 1) and polysorbate 80 (Tween 80™) (Fig. 3) do not modify the permeation of methanol through hairless mouse skin significantly. However, polysorbate 21 (Tween 21™) (Fig. 2) doubles the permeability of methanol through hairless mouse skin even at concentrations as low as 0.1%, whereas polysorbate 81 (Tween 81™) (Fig. 4) reveals a significant impact on the barrier properties of the integument starting at a concentration of 5%. Up to a concentration of 25%, both polysorbate 21 (Tween 21™) and polysorbate 81 (Tween 81™) increase the permeability of methanol by a factor of 2–3. The largest penetration enhancing effect is evident when neat surfactants constitute the medium in the donor compartment. Comparing the hairless mouse skin permeation pattern with that through silicone elastomer sheeting reveals that only neat surfactants affect the penetration of methanol through the synthetic membrane. This result implies that the penetration enhancing effect of neat polysorbates is due to an increase in thermodynamic activity of methanol in its respective polysorbate solution rather than due to biological consequences. This hypothesis is further strengthened by separate assessment of the thermodynamic activity of methanol which increases by 2.5 times when applied in neat polysorbate solutions (Table 1).

Figs 2 and 4 clearly demonstrate that polysorbate 21 (Tween 21™) and polysorbate 81 (Tween 81™) are most effective with respect to penetration enhancement. Probably, polysorbates with shorter polyethylene chain length which are insoluble in water have a more significant effect on the leaving tendency of methanol. Also, the more lipophilic polysorbate 21 (Tween 21™) and polysorbate 81 (Tween 81™) affect the barrier properties of hairless mouse skin to a greater extent than their more hydrophilic counterparts polysorbate 20 (Tween 20™) and polysorbate 80 (Tween 80™). This phenomenon occurs at almost

TABLE 1

Thermodynamic activity of methanol and octanol in the respective surfactant solution

Conc. (%)	Polysorbate 20		Polysorbate 80	
	Therm. Act. MeOH	Therm. Act. OcOH	Therm. Act. MeOH	Therm. Act. OcOH
0.05	0.0381	0.1824	0.0378	0.1904
0.5	0.0380	0.0969	0.0386	0.1378
5	0.0392	0.0135	0.0353	0.0197
10	0.0405	0.0062	0.0413	0.0062
100	0.0972	0	0.0982	0

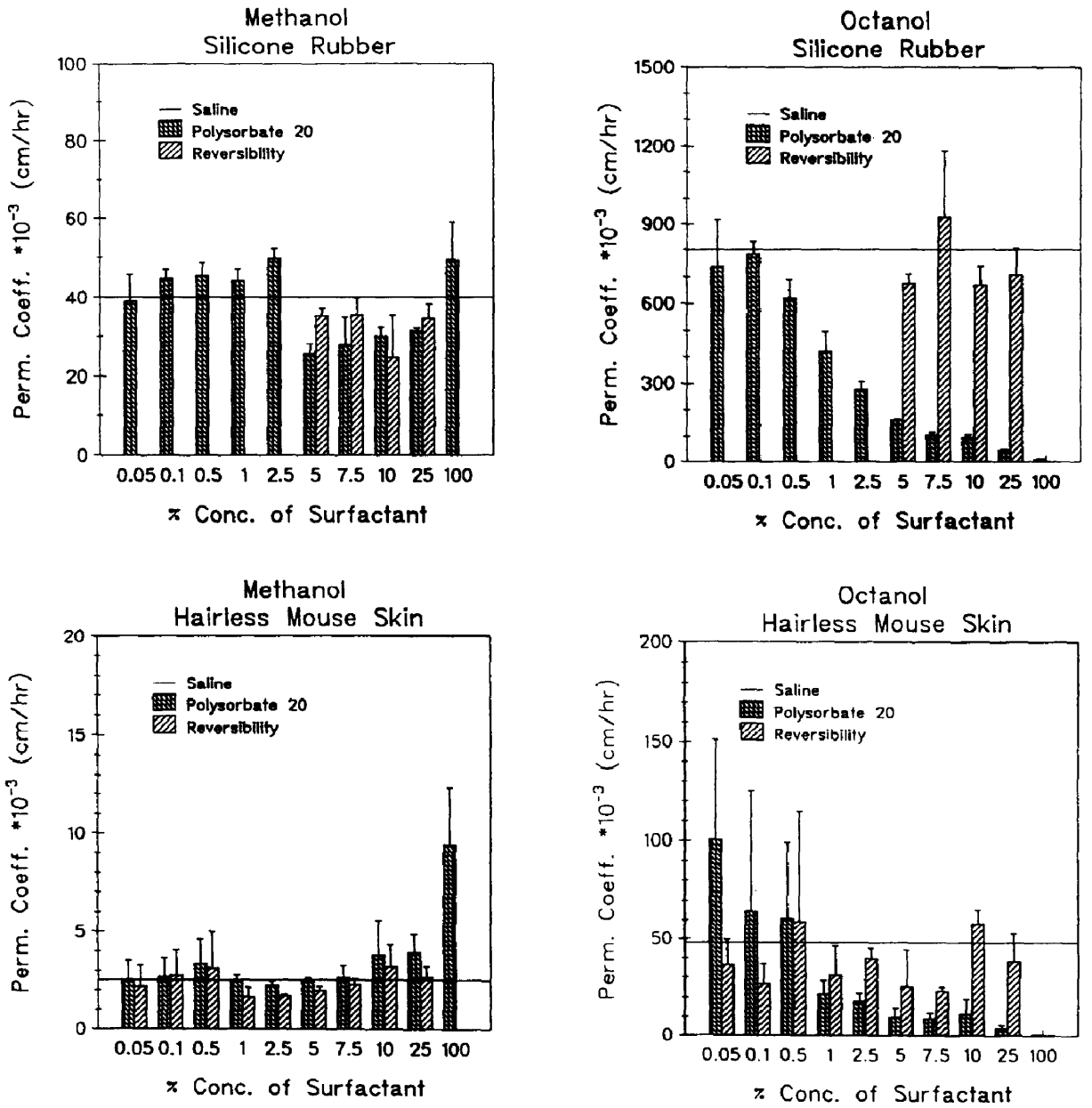

Fig. 1. Effect of different concentrations of polysorbate 20 (Tween 20™) on the permeation of methanol and octanol through either silicone elastomer membrane or full thickness hairless mouse skin. All data represent the mean of three experiments ± S.D. The straight line depicts the permeability coefficient of the test permeants when neat saline constitutes the medium in the donor compartment.

all concentrations of polysorbate 21 (Tween 21™) as well as at 5, 7.5, 10 and 25% concentration of polysorbate 81 (Tween 81™). Due to an increased lipophilicity of polysorbate 21 (Tween 21™) and polysorbate 81 (Tween 81™), changes in the vehicle/ stratum corneum partition coefficient have to be taken into consideration as a possibility for the increase of the permeability of methanol.

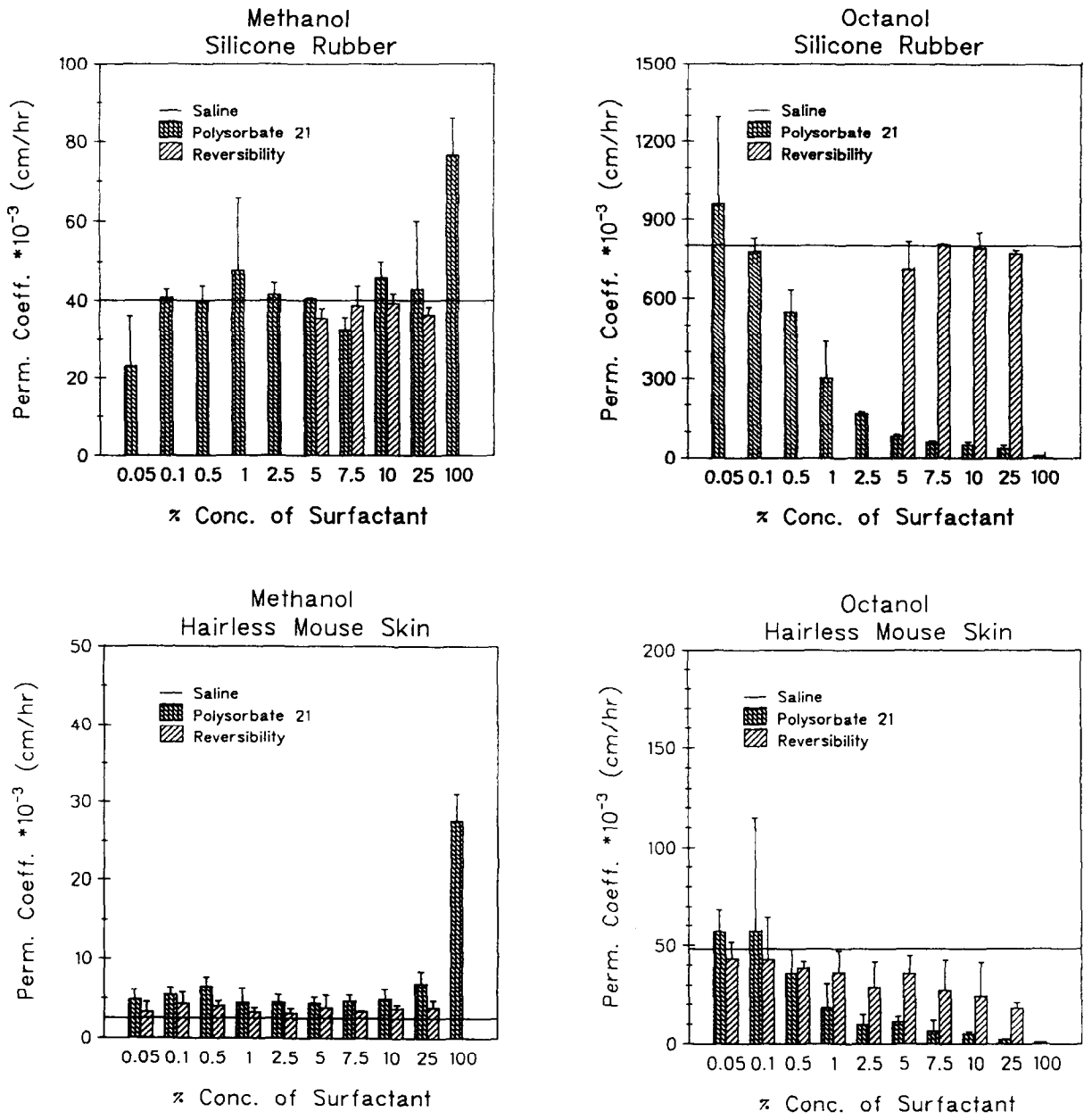

Fig. 2. Effect of different concentrations of polysorbate 21 (Tween 21™) on the permeation of methanol and octanol through either silicone elastomer membrane or full thickness hairless mouse skin. All data represent the mean of three experiments ± S.D. The straight line depicts the permeability coefficient of the test permeants when neat saline constitutes the medium in the donor compartment.

Also, surfactant monomers of polysorbate 21 (Tween 21™) and polysorbate 81 (Tween 81™) might more easily penetrate the skin, thus modifying the membrane's integrity. Significant increases in thermodynamic activity and a certain impact on the integrity of hairless mouse skin have a synergistic effect on the permeability of methanol.

All effects of polysorbate surfactants outlined

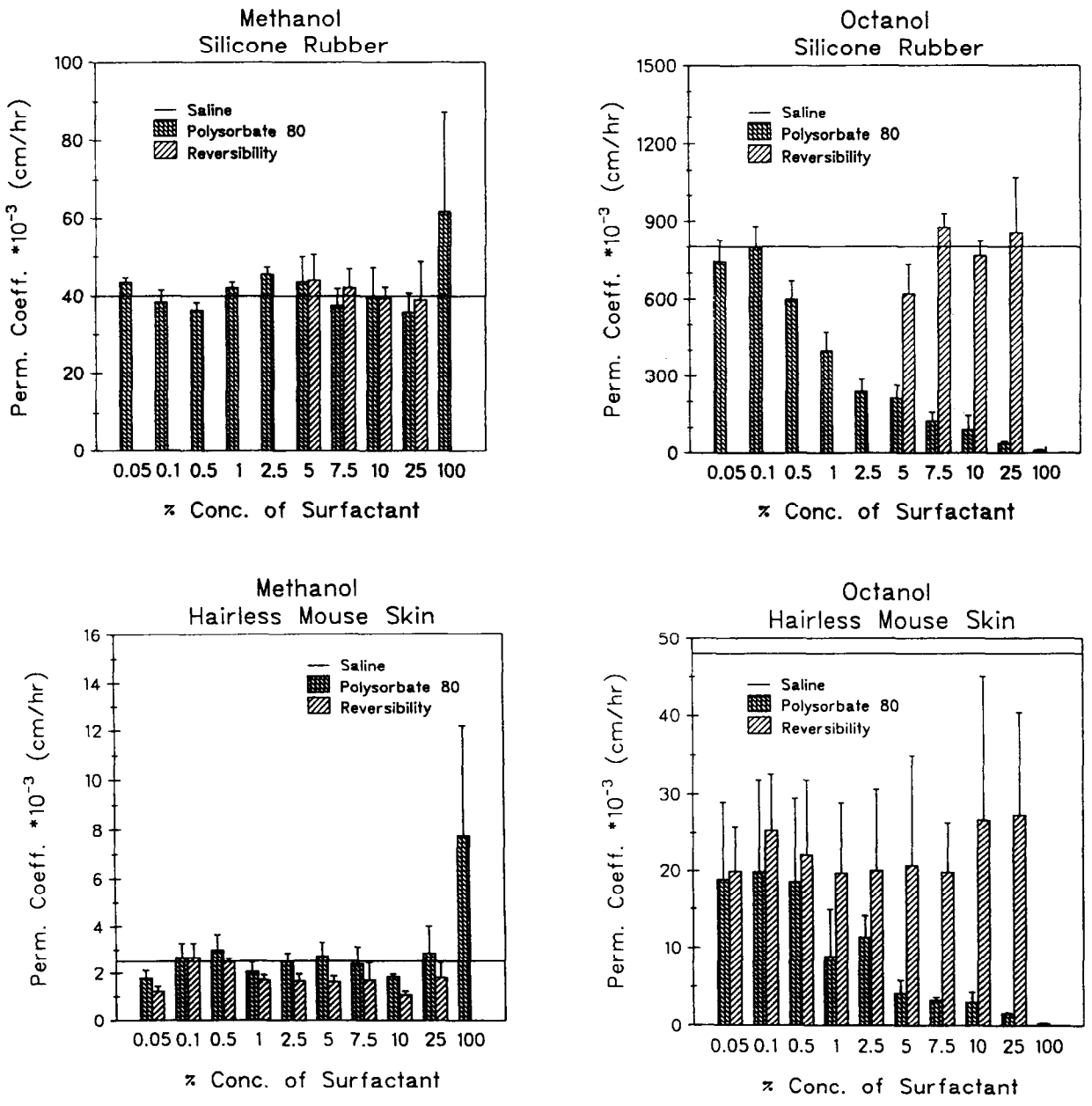

Fig. 3. Effect of different concentrations of polysorbate 80 (Tween 80™) on the permeation of methanol and octanol through either silicone elastomer membrane or full thickness hairless mouse skin. All data represent the mean of three experiments ± S.D. The straight line depicts the permeability coefficient of the test permeants when neat saline constitutes the medium in the donor compartment.

in the previous paragraphs are fully reversible. Due to the high viscosity of neat polysorbate, the extensive rinsing procedure described in Materials and Methods was insufficient to completely remove all surfactant from the donor compartment. Therefore, the reversibility experiment at 100% concentration of polysorbate was omitted.

Octanol

The permeability pattern of lipophilic octanol

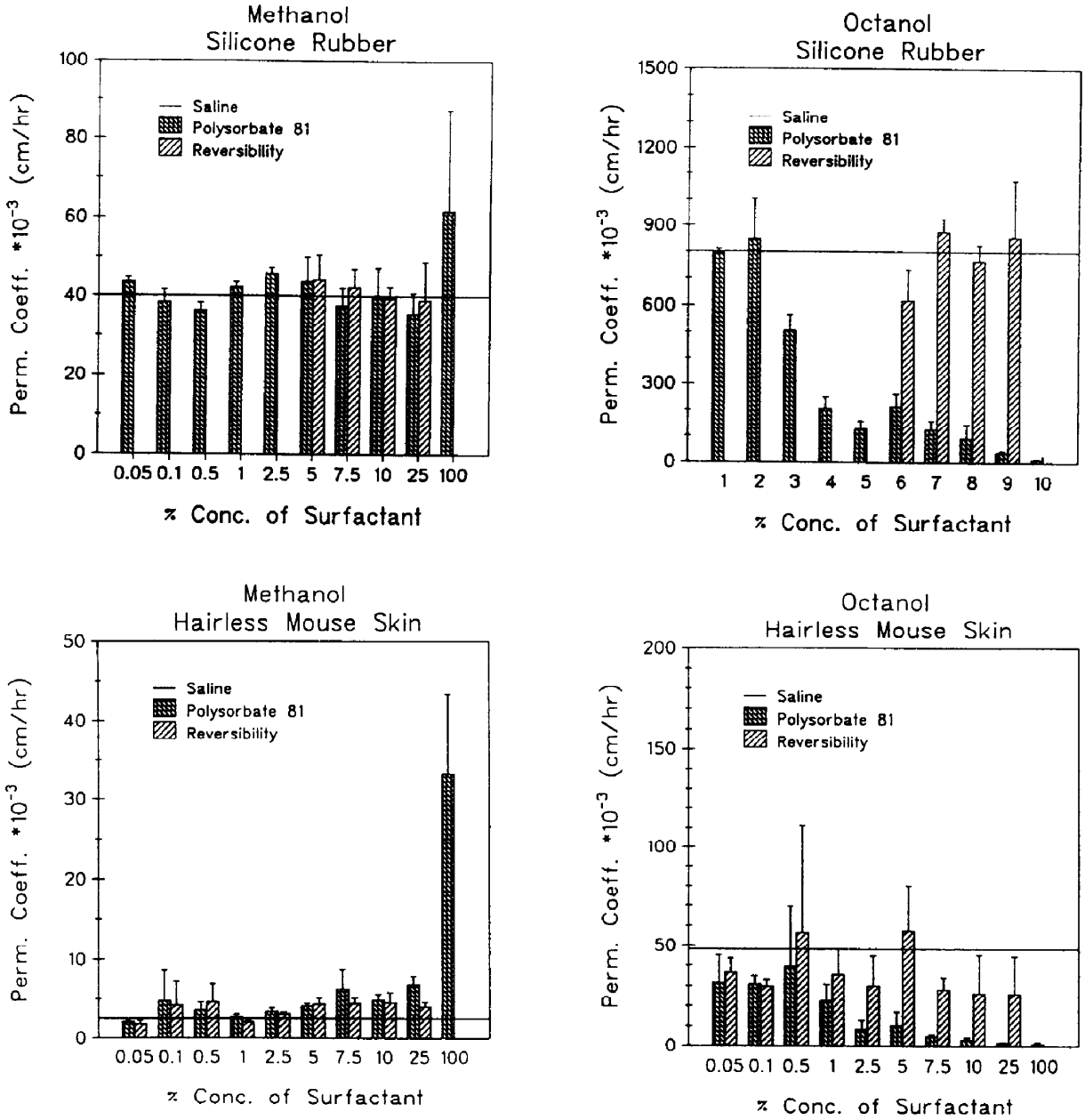

Fig. 4. Effect of different concentrations of polysorbate 81 (Tween 81™) on the permeation of methanol and octanol through either silicone elastomer membrane or full thickness hairless mouse skin. All data represent the mean of three experiments ± S.D. The straight line depicts the permeability coefficient of the test permeants when neat saline constitutes the medium in the donor compartment.

opposes that of methanol when applied in a polysorbate solution. The permeability coefficient through hairless mouse skin decreases at all concentrations of polysorbate 20 (Tween 20™) (Fig. 1), polysorbate 21 (Tween 21™) (Fig. 2), polysorbate 80 (Tween 80™) (Fig. 3), and polysorbate 81 (Tween 81™) (Fig. 4). The only exception is polysorbate 20 (Tween 20™) at a concentration

of 0.05%. The latter surfactant demonstrates an increase in the permeability of octanol of almost 42% at the aforementioned concentration. This surfactant concentration is close to the critical micelle concentration. Doubling the amount of polysorbate 20 (Tween 20™) decreases the permeability coefficient to the same level as seen with the other three polysorbates. At concentrations ranging from 2.5 to 10% all permeability coefficients are of the same order of magnitude ($2-5 \times 10^{-3}$ cm/h). The permeability coefficient further decreases at higher polysorbate concentrations approaching almost zero permeability when octanol is applied in neat surfactant solution. All trends observed with hairless mouse skin are in excellent agreement with both silicone rubber membrane data as well as changes in thermodynamic activity. Table 1 clearly demonstrates that the latter parameter changes as a function of polysorbate concentration.

However, the onset of the decrease of the permeability through silicone elastomer sheeting as a function of surfactant concentration appears to be at a higher surfactant concentration when compared to hairless mouse skin. This applies to all four polysorbates studied, i.e. polysorbate 20 (Tween 20™), polysorbate 21 (Tween 21™), polysorbate 80 (Tween 80™), and polysorbate 81 (Tween 81™). At concentrations of 0.05 and 0.1% of the respective surfactants the permeability of octanol through silicone elastomer sheeting is unaffected. Another difference between the inert synthetic membrane and hairless mouse skin data is a more moderate incremental decrease for the artificial membrane.

Behl et al. (1980d) suggested essentially the same mechanism of micellar solubilization and a corresponding decrease in free drug concentration to explain the effect of polysorbate 80 (Tween 80™) on the permeation of octanol. Levy et al. (1966) studied the effect of polysorbate 80 (Tween 80™) on the absorption of a number of barbiturates across goldfish membranes. An alteration in membrane permeability was observed above a certain bulk concentration but below the CMC of the surfactant. Whitworth and Yatis (1967) found an increase in the absorption of salicylic acid across the external membranes of the frog in the presence of 0.1% polysorbate 80 (Tween 80™).

At concentrations of 0.5 and 1% polysorbate 80 increased the skin penetration of chloramphenicol (Aguiar and Weiner, 1969), polysorbate 60 proved to be effective in enhancing the penetration of naproxen (Chowhan and Pritchard, 1978), but polysorbate 20 had no influence on the permeation of naloxone (Aungst et al., 1986). Shahi and Zatz (1978) observed that polysorbate 80 increased hydrocortisone flux from isopropanol-water mixtures. The authors hypothesized that the nature of the medium could influence the interaction between nonionic surfactants and the skin barrier. Further investigations employing lidocaine solutions in propylene glycol-water vehicles supported this assumption (Sarpotdar and Zatz, 1986a). The effect of polysorbates was a function of propylene glycol concentration. At 80% propylene glycol, steady state flux was increased approx. 3 times by polysorbate 20 or polysorbate 80. It was evident from surface tension studies that the addition of propylene glycol raised the critical micelle concentration of the nonionic surfactants by approximately a factor of 10. The increase in monomer concentration might be an explanation for the observed synergistic effect of propylene glycol and polysorbates.

Sarpotdar and Zatz (1986b) investigated the influence of polysorbates 20, 40, 60 and 80 on hydrocortisone (a more apolar molecule) penetration in vitro. All vehicles tested contained water and varying concentrations of propylene glycol. Skin penetration was significantly enhanced from vehicles containing both propylene glycol and one of the polysorbates mentioned above. The hydrocortisone penetration rate increased as the fatty acid chain length of the surfactant grew from 12 to 18 carbon atoms (polysorbate 20 to 60). There was no effect on hydrocortisone penetration due to unsaturation of the surfactant fatty acid chain (polysorbate 60 vs polysorbate 80).

In our study it is evident that all effects of the four polysorbates, i.e. 20, 21, 80, and 81, on octanol can be clearly attributed to solubilization of the lipophilic test permeant. Even at very high concentrations of polysorbates the barrier properties of the skin are not permanently damaged

since all data demonstrate that the permeability coefficient returns to normal levels when the surfactant is removed in the second sequential diffusion run.

References

Aguiar, A.J. and Weiner, M.A., Percutaneous absorption studies of chloramphenicol solutions. *J. Pharm. Sci.*, 58 (1969) 210–215.

Aungst, B.J., Rogers, N.J. and Shefter, E., Enhancement of naloxone penetration through human skin in vitro using fatty acids, fatty alcohols, surfactants, sulfoxides and amides. *Int. J. Pharm.*, 33 (1986) 225–234.

Behl, C.R., Flynn, G.L., Kurihara, T., Harper, N., Smith, W., Higuchi, W.I., Ho, N.F.H. and Pierson, C.L., Hydration and percutaneous absorption. I: Influence of hydration on alkanol permeation through hairless mouse skin. *J. Invest. Dermatol.*, 75 (1980a) 346–352.

Behl, C.R., Flynn, G.L., Kurihara, T., Smith, W., Gatmaitan, O., Higuchi, W.I., Ho, N.F.H. and Pierson, C.L., Permeability of thermally damaged skin. I: Immediate influences of 60 °C scalding on hairless mouse skin. *J. Invest. Dermatol.*, 75 (1980b) 340–345.

Behl, C.R., Flynn, G.L., Barrett, M., K.A., Linn, E.E. Mohamed, Z., Kurihara, T., Ho, N.F.H., Higuchi, W.I. and Pierson, C.L., Permeability of thermally damaged skin II: immediate influences of branding at 60 °C on hairless mouse skin permeability. *Burns*, 7 (1980c) 389–399.

Behl, C.R., Kreuter, J., Flynn, G.L., Walters, K.A. and Higuchi, W.I., Mechanism of surfactant effects on percutaneous absorption. I: Effects of polysorbate 80 on permeation of methanol and n-octanol through hairless mouse skin. *Am. Pharm. Assoc.*, Abstr 10,1 (1980d) 98.

Behl, C.R., Flynn, G.L., Barrett, M., Linn, E.E., Higuchi, W.I., Ho, N.F.H. and Pierson, C.L., Permeability of thermally damaged skin. IV: influence of branding iron temperature on the mass transfer of water and n-alkanols across hairless mouse skin. *Burns*, 8 (1981) 86–98.

Blank, I.H., Scheuplein, R.J. and MacFarlane, D.J., Mechanism of percutaneous absorption. III. The effect of temperature on the transport of non-electrolytes across the skin. *J. Invest. Dermatol.*, 49 (1967) 582–589.

Chowhan, Z.T. and Pritchard, R., Effect of surfactants on percutaneous absorption of naproxen. I: Comparisons of rabbit, rat, and human excised skin. *J. Pharm. Sci.*, 67 (1978) 1272–1274.

Durrheim, H., Flynn, G.L., Higuchi, W.I. and Behl, C.R., Permeation of hairless mouse skin I: Experimental methods and comparison with human epidermal permeation by alkanols. *J. Pharm. Sci.*, 69 (1980) 781–786.

Florence, A.T. and Gillan, J.M.N., Non-ionic surfactants and membrane transport of thioridazine in goldfish. *Int. J. Pharm.*, 10 (1982) 153–163.

Flynn, G.L., Behl, C.R., Walters, K.A., Gatmaitan, O.G., Wittkowsky, A., Kurihara, T., Ho, N.F.H., Higuchi, W.I. and Pierson, C.L., Permeability of thermally damaged skin III: influence of scalding temperature on mass transfer of water and n-alkanols across hairless mouse skin. *Burns*, 8 (1981a) 47–58.

Flynn, G.L., Durrheim, H. and Higuchi, W.I., Permeation of hairless mouse skin II: Membrane sectioning techniques and influence on alkanol permeabilities. *J. Pharm. Sci.*, 70 (1981b) 52–56.

Garcia, B., Marty, J.P. and Wepierre, J., Etude des facteurs conditionnant l'absorption percutanée des alcools incorporés dans des mélanges eau-monooléate de sorbitanne polyoxyéthylène-myristate d'isopropyle. *Int. J. Pharm.*, 4 (1980) 205–217.

Hwang, C.-C. and Danti, A.G., Percutaneous absorption of flufenamic acid in rabbits: Effect of dimethyl sulfoxide and various nonionic surface-active agents. *J. Pharm. Sci.*, 72 (1983) 857–859.

Lambert, W.J., Higuchi, W.I., Knutson, K. and Krill, S.L., Effects of long-term hydration leading to the development of polar channels in hairless mouse stratum corneum. *J. Pharm. Sci.*, 78 (1989) 925–928.

Levy, G., Miller, K.E. and Reuning, R.H., Effect of complex formation on drug absorption III: Concentration and drug dependent effect of a nonionic surfactant. *J. Pharm. Sci.*, 55 (1966) 394–398.

Sarpotdar, P.P. and Zatz, J.L., Evaluation of penetration enhancement of lidocaine by nonionic surfactants through hairless mouse skin in vitro. *J. Pharm. Sci.*, 75 (1986a) 176–181.

Sarpotdar, P.P. and Zatz, J.L., Percutaneous absorption enhancement by nonionic surfactants. *Drug Dev. Ind. Pharm.*, 12 (1986b) 1625–1647.

Scheuplein, R.J., Mechanism of percutaneous absorption I. Route of penetration and the influence of solubility. *J. Invest. Dermatol.*, 45 (1965) 334–346.

Scheuplein, R.J., Percutaneous absorption after twenty-five years: or "old wine in new wineskins". *J. Invest. Dermatol.*, 67 (1976) 31–38.

Scheuplein, R.J. and Blank, I.H., Mechanism of percutaneous absorption IV. Penetration of nonelectrolytes (alcohols) from aqueous solutions and from pure liquids. *J. Invest. Dermatol.*, 60 (1973) 286–296.

Shahi, V. and Zatz, J.L., Effect of formulation factors on penetration of hydrocortisone through mouse skin. *J. Pharm. Sci.*, 67 (1978) 789–792.

Southwell, D. and Barry, B.W., Penetration enhancers for human skin: Mode of action of 2-pyrrolidone and dimethylformamide on partition and diffusion of model compounds water, *n*-alcohols, and caffeine. *J. Invest. Dermatol.*, 80 (1983) 507–514.

Touitou, E., Donbrow, M. and Rubinstein, A., Effective intestinal absorption of insulin in diabetic rats using a new formulation approach. *J. Pharm. Pharmacol.*, 32 (1980) 108–110.

Walters, K.A., Dugard, P.H. and Florence, A.T., Nonionic surfactants and gastric mucosal transport of paraquat. *J. Pharm. Pharmacol.*, 33 (1981a) 207–213.

Walters, K.A., Flynn, G.L. and Marvel, J.R., Physicochemical characterization of the human nail I. Pressure sealed apparatus for measuring nail plate permeabilities. *J. Invest. Dermatol.*, 76 (1981b) 76–79.

Walters, K.A., Flynn, G.L. and Marvel, J.R., Physicochemical characterization of the human nail: Permeation pattern for water and the homologous alcohols and differences with respect to the stratum corneum. *J. Pharm. Pharmacol.*, 35 (1983) 28–33.

Walters, K.A., Walker, M. and Olejnik, O., Nonionic surfactant effects on skin permeability characteristics. *J. Pharm. Pharmacol.*, 40 (1988) 525–529.

Whitmore, D.A., Brookes, L.G. and Wheeler, K.P., Relative effects of different surfactants on intestinal absorption and release of proteins and phospholipids from the tissue, *J. Pharm. Pharmacol.*, 31 (1979) 277–283.

Whitworth, C.W. and Yantis, L.D., Ability of certain additives to influence the absorption of salicylic acid from solutions in an in vivo study: preliminary report. *J. Pharm. Sci.*, 56 (1967) 1661–1663.