ELSEVIER

International Journal of Pharmaceutics 225 (2001) 75–82

international journal of pharmaceutics

www.elsevier.com/locate/ijpharm

Lipid extraction and transport of hydrophilic solutes through porcine epidermis

Sumeet K. Rastogi, Jagdish Singh *

Department of Pharmaceutical Sciences, College of Pharmacy, North Dakota State University, Fargo, ND 58105, USA

Received 8 December 2000; received in revised form 24 April 2001; accepted 29 May 2001

Abstract

The purpose of this study was to investigate the effect of delipidization of the stratum corneum (SC) on the in vitro percutaneous absorption of hydrophilic solutes (i.e. water, urea, and inulin). Fourier transform infrared (FT-IR) spectroscopy was employed to study the extent of delipidization of porcine SC due to chloroform:methanol (2:1) (C:M (2:1)) treatments for various time periods. In vitro percutaneous absorption of [^{3}H] water, [^{14}C] urea, and [^{3}H] inulin were studied through C:M (2:1) treated epidermis in Franz diffusion cells. There was a greater decrease in peak areas of the asymmetric and symmetric C–H stretching absorbances (i.e. increase in lipid extraction) with increasing exposure times of the SC with C:M (2:1). After 40-min treatment, asymmetric and symmetric C–H stretching peak area showed a decrease of 75.9 and 89.9%, respectively. The permeability coefficient of water, urea, and inulin increased with increasing lipid extraction. Enhancement in the permeability coefficient, through 40 min C:M (2:1) treated epidermis in comparison to the control, for water, urea, and inulin was 48.72, 215.65, and 3.90, respectively. Log (permeability coefficient) and log (mol. wt.) for test solutes and leuprolide acetate were found to be inversely related ($R^2 = 0.9974$). In conclusion, this study implies that penetration enhancers that are safe and extract the SC lipids can be selected in order to enhance the percutaneous absorption of polar solutes through the skin.

Keywords: Percutaneous absorption; Fourier transform infrared; Stratum corneum; Lipid extraction; Hydrophilic solutes

1. Introduction

The primary barrier to transdermal diffusion is the stratum corneum (SC), the thin outermost layer of the skin, which is comprised of a regular array of protein rich cells (i.e. corneocytes) that are embedded in a multilamellar lipid domain. The lamellar packing of the SC intercellular lipids is established and several experiments have implicated these lipoidal domains as the integral components of the transport barrier, which must be breached if drugs are to be administered at an appropriate rate (Sweeny and Downing, 1970; Bronaugh and Maibach, 1999).

Dry SC is composed of (by weight) ~75% protein, 25% lipid, and a small percentage of low

* Corresponding author. Tel.: +1-701-231-7943; fax: +1-701-231-7606.

E-mail address: jagdish_singh@ndsu.nodak.edu (J. Singh).

0378-5173/01/$ - see front matter
PII: S0378-5173(01)00766-9

molecular weight material (Mershon, 1975). The corneocytes are almost entirely composed of proteinaceous material, whereas the extracellular spaces contain at least 15 different lipids, with the primary constituents being cholesterol (25 wt.%), ceramides (40 wt.%), and free fatty acids (12 wt.%) (Wertz et al., 1992). A large body of evidence indicates that the barrier lipids are derived from membrane coating granules, small intercellular organelles containing stacks of membranous disks, which extrude their lipid contents into the intercellular space between the uppermost layer of the viable epidermis and the SC (Elias and Friend, 1975; Frithiof and Wersall, 1965; Lavker, 1976; Hayward, 1978).

The SC has a very unique morphology, by which lipids form multi-lamellar arrays in the extracellular spaces surrounding the corneocytes (Williams and Elias, 1987; Micheals et al., 1975). This morphology is analogous to highly impermeable barriers formed by the inclusion of flakes in a homogenous matrix (Micheals et al., 1975; Cussler et al., 1988). Lipids form an extracellular continuum that is highly tortuous. Thus the exceedingly low permeability of the SC may be due, in large part, to its unique morphology (Potts and Francoeur, 1991). Transport of hydrophilic solutes takes place through transfollicular (Cornwell and Barry, 1993) and polar routes (highly tortuous pathway of polar regions of the intercellular lipids) (Li et al., 1998; Hatanaka et al., 1994). SC lipids extraction may form additional pathways and thereby increase the transport of polar solutes (Peck et al., 1994). High concentrations of ethanol ($>50\%$) have been reported to form pores in the SC (Ghanem et al., 1987). This effect has been attributed to the lipid extraction properties of *n*-alkanols (Kai et al., 1988).

Diffusion barrier reduction may be achieved by the use of chemical (Smith and Maibach, 1995; Williams and Barry, 1992; Friend et al., 1988; Bhatia and Singh, 1998) and physical (Singh and Bhatia, 1996; Bhatia et al., 1997a; Ganga et al., 1996; Pliquett and Weaver, 1996) enhancers. The goal of the present study was to investigate the effect of lipid extraction of SC by chloroform:methanol (2:1) (C:M (2:1)) for various time intervals on the in vitro percutaneous absorption of hydrophilic solutes. The C:M (2:1) is a well established solvent combination used for lipid extraction. Radiolabeled water, urea and inulin of molecular weight 18, 60, and 5200, respectively, were chosen as model hydrophilic solutes.

2. Materials and methods

2.1. Materials

[^{3}H]Water (specific activity 200 Ci mmol^{-1}), [^{14}C]urea (specific activity 0.055 Ci mmol^{-1}), [^{3}H]inulin (specific activity 2.6 Ci mmol^{-1}) were obtained from Amersham Life Sci. (Cleveland, OH). Radio chemical purity of [^{14}C] urea and [^{3}H] inulin was 95–99% and 96.7–98%, respectively. Chloroform and methanol were obtained from Sigma Chemical Co. (St. Louis, MO) and Fisher Scientific (Pittsburgh, PA), respectively. All other chemicals used were of analytical grade.

2.2. Preparation of epidermis and stratum corneum

Porcine ears were obtained from a local slaughterhouse. The epidermis was prepared by a heat separation technique (Bhatia et al., 1997b). The whole skin was soaked in water at 60 °C for 45 s, followed by careful removal of the epidermis. The epidermis was washed with water and used in the in vitro percutaneous absorption studies. SC samples from epidermis were prepared using the trypsin digestion method (Bhatia et al., 1997b).

2.3. Fourier transform infrared spectroscopy

After C:M (2:1) treatment of the SC for different time intervals, the samples were vacuum-dried (650 mmHg) at 21 ± 1 °C for 3 days and stored in a desiccator to evaporate the solvent (Okamoto et al., 1988). The samples were then subjected to Fourier transform infrared (FT-IR) spectroscopic study. The SC samples were squeezed between the KBr plates, clamped and mounted in the enclosed sample chamber, away from moisture to get their spectra. FT-IR (Nicolet 210, Nicolet Instrument Corporation, Madison, WI) was used to accom-

plish this study. For each SC sample, the peak areas of C–H asymmetric and symmetric stretching absorbances were measured before and after the solvent treatment. This experimental strategy allowed each sample to serve as its own control. Spectra were obtained in the frequency range 3050–2750 cm^{-1} in absorbance mode. Attention was focused on characterizing the occurrence of peaks near 2848 and 2915 cm^{-1}, which were due to the symmetric and asymmetric C–H stretching absorbances, respectively. All spectra analyzed represent an average of 64 scans with resolution of 4 cm^{-1}, Happ–Genzel apodization and zero filling factor of none. FT-IR experiments with each condition were performed in triplicate with samples coming from different subjects. OMNIC® FT-IR software (Nicolet Instrument Corporation) was used to calculate the peak areas of C–H stretching absorbances.

2.4. In vitro percutaneous absorption

Franz diffusion cells were used in the in vitro percutaneous absorption studies. The C:M (2:1)-treated or untreated (control) epidermis was sandwiched between the cells with the SC facing the donor compartment. The maximum capacity of the donor and receiver compartments was 1 and 5 ml, respectively. The effective diffusional area was 0.785 cm^2. The donor compartment contained 1 ml solution of model solute (0.2 μCi of solute in 1 ml of normal saline) and the receiver compartment was filled with 5 ml of normal saline. Thus, 0.018, 218.18, and 400 ng/ml, respectively, were used as donar solution for water, urea and inulin. All donor concentrations of solutes were below their solubility limits.

The cells were maintained at 37 ± 0.5 °C by a PMC Dataplate® stirring digital dry block heater (Crown Bioscientific Inc., Somerville, NJ). The contents of the receiver compartment were stirred with the help of a magnetic bar at 100 revolution min^{-1}. At appropriate times, 0.5 ml samples were withdrawn from the receiver compartment and transferred to scintillation vials. An equivalent amount of normal saline (0.5 ml) was added to the receiver compartment to maintain a constant volume. Each sample was mixed with 10 ml of scintillation cocktail (Econosafe®, Research Products International Corp., Mount Prospect, IL) and was counted in a liquid scintillation counter (Packard, Tri Carb® 2100 TR, Downers Grove, IL) for quantification of ^3H or ^{14}C in disintegrations per minute (dpm). The instrument was programmed to give counts for 10 min. Net dpm for the samples was obtained by subtracting background dpm. The results were expressed as the mean $\pm$ SD of three experiments.

The permeability coefficient of leuprolide acetate through 40 min C:M (2:1) treated epidermis was determined with a donor concentration of 5 mg/ml. The other conditions of in vitro percutaneous absorption were similar to test solutes as described above. Samples were withdrawn from the receiver compartment and analyzed for leuprolide acetate by high performance liquid chromatography (Singh et al., 2000).

2.5. Epidermal resistance

The electric resistance of the epidermis treated with C:M (2:1) for different time intervals and the control (no treatment) was investigated. Franz diffusion cells modified for iontophoresis were used to determine the epidermal resistance. The donor and receiver compartment contained 1 and 5 ml of normal saline, respectively. Scepter™ iontophoretic power source (Keltronics Corp., Oklahoma City, OK) and Ag/AgCl electrodes were used. Scepter™ provides multichannel prescription set constant voltage or constant current source with an accuracy of 0.1%. DC electric current 0.2 mA cm^{-2} was applied by placing anode in the donor and cathode in the receiver solution. The potential difference across the epidermal sample was noted. The resistance was calculated according to Ohm's law.

2.6. Data treatment

The receiver compartment concentration of solute was corrected for sample removal by using the equation given by Hayton and Chen (1982). The cumulative amount of solute permeated per unit skin surface area was plotted against time and the slope of the linear portion of the plot was

estimated as the steady state flux (J_{ss}). The permeability coefficient, P was calculated as (Scheuplein, 1978):

$$P = \frac{J_{ss}}{C_v}, \quad (1)$$

where C_v is donor concentration of solutes.

Enhancement ratio of the permeability coefficients (ER) can be expressed as:

$$ER = \frac{P_{treatment}}{P_{control}}, \quad (2)$$

where, $P_{treatment}$ is the permeability coefficient of solute through solvent treated epidermis and $P_{control}$ is the permeability coefficient of solute through control epidermis. Statistical comparisons were made using the students t-test. The level of significance was taken as $p < 0.05$.

3. Results and discussion

Fig. 1 gives the FT-IR spectra and Table 1 presents the data derived from it. There was a

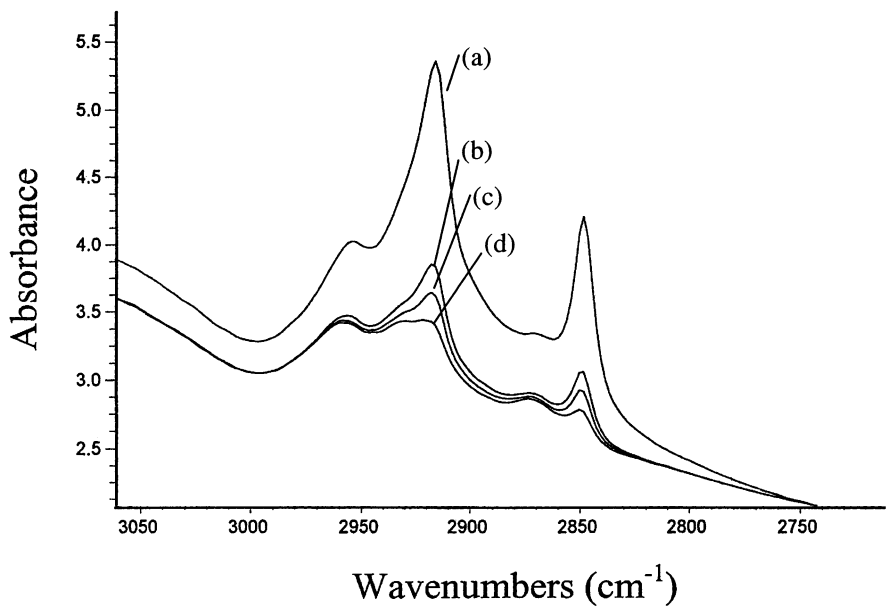

Fig. 1. FT-IR spectrum of porcine SC treated with C:M (2:1) for various time intervals; (a) control, (b) 10 min, (c) 20 min, and (d) 40 min.

Table 1
Changes in symmetric and asymmetric C–H stretching absorbance peak areas of the SC after treatment with C:M (2:1)

Treatment time (min)	Peak area (mean ± SD, $n = 3$)					
	Asymmetric			Symmetric		
	Control	Treatment	Decrease (%)	Control	Treatment	Decrease (%)
10	6.48 ± 0.41	2.08 ± 0.60	68.11	2.20 ± 0.54	0.71 ± 0.30	65.95
20	6.46 ± 0.50	1.69 ± 1.22	74.65	2.50 ± 0.24	0.57 ± 0.22	77.31
40	6.09 ± 0.21	1.12 ± 0.94	75.94	2.36 ± 0.62	0.23 ± 0.10	89.94

Decrease (%) = 100 − [(absorbance peak area due to treatment/absorbance peak area due to control) × 100].

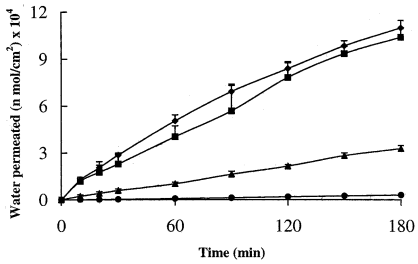

Fig. 2. Effect of C:M (2:1) treatment for different time intervals on the in vitro transport of [^{3}H]water through porcine epidermis. Each data point is the mean ± SD of three determinations. Key: control (●); 10 min (▲); 20 min (■); and 40 min (◆).

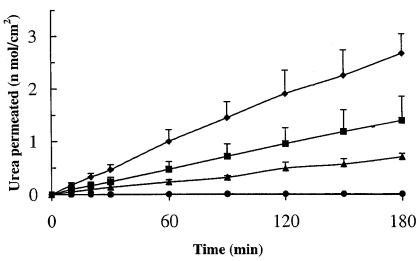

Fig. 3. Effect of C:M (2:1) treatment for different time intervals on the in vitro transport of [^{14}C]urea through porcine epidermis. Each data point is the mean ± SD of three determinations. Key: control (●); 10 min (▲); 20 min (■); and 40 min (◆).

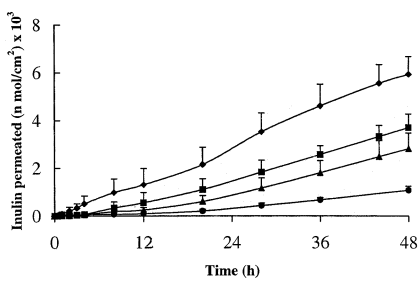

Fig. 4. Effect of C:M (2:1) treatment for different time intervals on the in vitro transport of [^{3}H]inulin through porcine epidermis. Each data point is the mean ± SD of three determinations. Key: control (●); 10 min (▲); 20 min (■); and 40 min (◆).

greater decrease in peak areas of the asymmetric and symmetric C–H stretching absorbances (i.e. increase in lipid extraction) with increasing exposure times of the SC with C:M (2:1). After 40 min treatment, asymmetric and symmetric C–H stretching peak areas showed a decrease of 75.9 and 89.9%, respectively. Increasing the exposure time beyond 40 min did not cause further changes in peak areas.

The above observations strongly agree with the results obtained by other workers. Raykar et al. (1988) have mentioned that >80–90% of the ester containing lipids appeared to get removed by the C:M (2:1) extraction (after 1 and 19 days of extraction, to examine the completeness of removal of extractable lipids). Scheuplein and Blank (1971) have reported 20–30% removal of dry mass of the SC tissue as lipids and lipoproteins and an 85% decrease in long chain lipids (indicated by intensity of C–H stretching bands) on 24 h treatment. Also, Potts et al. (1991) have reported a 'dramatic' decrease in intensity for C–H stretching peaks after chloroform–methanol treatment.

Figs. 2–4 present the transport profiles and Table 2 shows the epidermal resistance, permeability coefficient, and enhancement ratio of permeability coefficients for the test solutes. Permeability of the epidermis increased with increasing lipid extraction for water, urea, and inulin, as detailed in the Table 2. Permeability coefficients through 40 min C:M (2:1) treated epidermis for water, urea, and inulin were 418.02×10^{-3}, 229.73×10^{-3}, and 15.78×10^{-4} cm h^{-1}, respectively. Enhancement in the permeability coefficient, through 40 min C:M (2:1) treated epidermis in comparison to the control for water, urea, and inulin was 48.72, 215.65, and 3.90, respectively. The above results agree with the findings of other workers (Elias, 1981), which suggest that rates of percutaneous transport correlate inversely with lipid content. Ogiso et al. (1995) found an increase in the flux of solutes with removal of ceramides from rat skin by chemical enhancers, indicating that the removal of intercellular lipids would cause dramatic dilations between adherent cornified cells. This can facilitate the passive transport of hydrophilic solutes due to the resultant increase in free volume.

There was a large (one order of magnitude) decrease in resistance of the epidermis when treated with C:M (2:1) for 10 min, which further

Table 2
Epidermal resistance, permeability coefficient (P) of [^{3}H]water, [^{14}C]urea and [^{3}H] inulin, and enhancement ratio (ER) through C:M (2:1) treated epidermis

Treatment time (min)	Resistance (kΩ cm^2)	Water		Urea		Inulin	
		P (cm/h) $\times 10^3$	ER	P (cm/h) $\times 10^3$	ER	P (cm/h) $\times 10^4$	ER
Control[a]	10.27 ± 1.84	8.58 ± 0.78	–	1.07 ± 0.44	–	4.04 ± 0.61	–
10	1.08 ± 0.03	102.60 ± 14.16*	11.96	66.57 ± 13.89*	62.85	10.82 ± 1.91*	2.67
20	0.90 ± 0.05	338.76 ± 92.34*	39.48	129.05 ± 44.60*	121.13	12.23 ± 0.60*	3.02
40	0.80 ± 0.05	418.02 ± 27.36*	48.72	229.70 ± 25.42*	215.65	15.78 ± 3.85*	3.90

[a] No treatment.
* Significantly greater than respective controls ($P<0.05$).

decreased with increasing the treatment time. This agrees with the work of Srinivasan et al. (1990) and Inamori et al. (1994) where ethanol pretreatment had decreased the resistance to a large extent. Decrease in resistance of the epidermis, treated with C:M (2:1) for different time intervals, follows similar trends as decrease in symmetric and asymmetric C–H absorbance peak areas, with accompanying increase in permeability coefficients of test solutes (Fig. 5 and Tables 1 and 2).

A lag time of ~ 14 h for inulin indicates that the pathway is tortuous and discontinuous. It was observed that the lag time for inulin decreased with increase in lipid-extraction, coming down to 2.6 h for 40 min treatment with C:M (2:1). Permeant size is a strong determinant of SC transport. Permeability data obtained for a number of compounds demonstrate that transport through the SC decreases exponentially with the increasing molecular volume of the permeant (Potts and Francoeur, 1993). The permeability coefficient of leuprolide acetate through 40 min C:M (2:1) treated epidermis was 4.31×10^{-2} cm/h. Plot of log (permeability coefficient) through delipidized membrane and log (mol. wt.) for test solutes including leuprolide acetate (Fig. 6) turned out to be inversely related ($R^2 = 0.9974$). An inverse relationship between the flux and molecular weight of a substance, through a non-delipidized membrane, has been suggested (Idson, 1975). Moreover, the present study suggests that the same inverse relationship between flux and molecular weight of hydrophilic solutes exists through delipidized epidermal membrane.

4. Conclusions

Our findings provide evidence that the SC lipid extraction increased the permeability of hydrophilic solutes. Therefore it is reasonable to assume that the SC lipids extraction creates additional free volume to enhance the permeability of hydrophilic solutes through the epidermis. Finally, this study provides the basis of using a large number of non-toxic penetration enhancers in transdermal delivery systems, which can extract the SC lipids and enhance the percutaneous absorption of hydrophilic drugs.

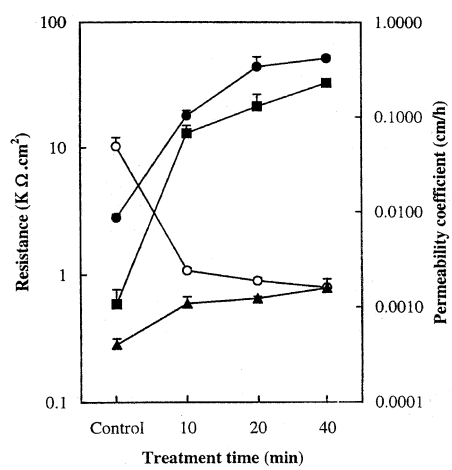

Fig. 5. Relationship between the resistance of epidermis and the permeability coefficients of test solutes through C:M (2:1) treated epidermis. Key: permeability coefficient of inulin (▲), urea (■), and water (●); resistance of epidermis (○).

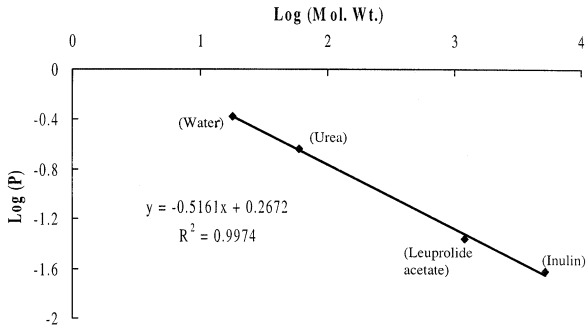

Fig. 6. Plot of log (P) versus log (mol. wt.) for water, urea, leuprolide acetate, and inulin through 40 min treated porcine epidermis. In vitro transport study of leuprolide acetate through 40 min C:M (2:1) treated porcine epidermis was also carried out and the log (P) value of leuprolide acetate was incorporated in the plot.

Acknowledgements

We acknowledge the financial support from the US Air Force, Office of Scientific Research grant no. F49620-99-1-0223.

References

Bhatia, K.S., Gao, S., Freeman, T.P., Singh, J., 1997a. Effect of penetration enhancers and iontophoresis of the ultrastructure and cholecystokinin-8 permeability through porcine skin. J. Pharm. Sci. 86, 1011–1015.

Bhatia, K.S., Gao, S., Singh, J., 1997b. Effect of penetration enhancers and iontophoresis in the FT-IR spectroscopy and LHRH permeability through porcine skin. J. Control. Rel. 47, 81–89.

Bhatia, K.S., Singh, J., 1998. Mechanism of transport enhancement of LHRH through porcine epidermis by terpenes and iontophoesis: permeability and lipid extraction studies. Pharm. Res. 15, 1857–1862.

Bronaugh, R.L., Maibach, H.I. (Eds.), 1999. Percutaneous Absorption. Marcel Dekker, New York.

Cornwell, P.A., Barry, B.W., 1993. The routes of penetration of ions and 5-fluorouracil across human skin and the mechanisms of action of terpene skin penetration enhancers. Int. J. Pharm. 94, 189–194.

Cussler, E.L., Huges, S.E., Ward, W.J. III, Aris, R., 1988. Barrier membrane. J. Membrane Sci. 38, 161–174.

Elias, P.M., 1981. Lipids and the epidermal permeability barrier. Arch. Dermatol. Res. 270, 95–117.

Elias, P.M., Friend, D.S., 1975. The permeability barrier in mammalian epidermis. J. Cell Biol. 65, 180–191.

Friend, D., Catz, P., Heller, J., Reid, J., Baker, R., 1988. Transdermal delivery of levonorgestrel I: alkanols as permeation enhancers in vitro. J. Control Rel. 7, 243–250.

Frithiof, L., Wersall, J., 1965. A highly ordered structured in keratinizing human oral epithelium. J. Ultrastruct. Res. 12, 371–379.

Ganga, S., Ramarao, P., Singh, J., 1996. Effect of Azone on the iontophoretic transdermal delivery of metprolol tartrate through human epidermis in vitro. J. Control. Rel. 42, 57–64.

Ghanem, A.H., Mahmound, H., Higuchi, W.I., Rohr, U.D., Borsadia, S., Liu, P., Fox, J.L., Good, W.R., 1987. The effects of ethanol on the transport of β-estradiol and other permeants in the hairless mouse skin. II. A new quantitative approach. J. Control Rel. 6, 75.

Hatanaka, T., Manabe, E., Sugibayashi, K., Morimoto, Y., 1994. An application of the hydrodynamic pore theory to percutaneous absorption of drugs. Pharm. Res. 11, 654–658.

Hayton, W.L., Chen, T., 1982. Correction of perfusate concentration for sample removal. J. Pharm. Sci. 71, 820–821.

Hayward, A.F., 1978. Ultrastructural changes in contents of membrane-coating granules after extrusion from epithelial cells of hamster cheek pouch. Cell Tissue Res. 187, 323–331.

Idson, B., 1975. Percutaneous absorption. J. Pharm. Sci. 64, 901.

Inamori, T., Ghanem, A-H., Higuchi, W.I., Srinivasan, V., 1994. Macromolecule transport in and effective pore size of ethanol pretreated human epidermal membrane. Int. J. Pharm. 105, 113–123.

Kai, T., Mak, V.H.W., Potts, R.O., Guy, R.H., 1988. Mechanism of skin penetration enhancement: effect of n-alkanols on the permeability barrier. Proc. Int. Symp. Control. Rel. Bioactive Mater. 15, 211.

Lavker, R.M., 1976. Membrane-coating granules: the fate of the discharged lamellae. J. Ultrastruct. Res. 55, 79–96.

Li, S.K., Suh, W., Parikh, H.H., Ghanem, A-H., Mehta, S.C., Peck, K.D., Higuchi, W.I., 1998. Lag time data for characterizing the pore pathway of intact and chemically pretreated human epidermal membrane. Int. J. Pharm. 170, 93–108.

Mershon, M.M., 1975. In: Baier, R.E. (Ed.), Applied Chemistry at Protein Interfaces. American Chemical Society, Washington, DC, pp. 41–73 Adv. chem. ser. no. 145.

Micheals, A.S., Chandrasekaran, S.K., Shaw, J.E., 1975. Drug permeation through human skin: theory and in vitro experimental measurement. AIChE J. 21, 985–996.

Okamoto, H., Hashida, M., Sezaki, H., 1988. Structure-activity relationship of 1-alkyl- or 1-alkenylazacycloalkanone derivatives as percutaneous penetration enhancers. J. Pharm. Sci. 77, 418–424.

Ogiso, T., Iwaki, M., Paku, T., 1995. Effects of various enhancers on transdermal penetration of indomethacin and urea, and relationship between penetration parameters and enhancement factors. J. Pharm. Sci. 84, 482–488.

Peck, K.D., Ghanem, A-H., Higuchi, W.I., 1994. Hindered diffusion of polar molecules through and effective pore radii estimates of intact and ethanol treated human epidermal membrane. Pharm. Res. 11, 1306–1314.

Pliquett, U., Weaver, J.C., 1996. Transport of charged molecule across the human epidermis due to electroporation. J. Control. Rel. 38, 1–10.

Potts, R.O., Francoeur, M.L., 1993. Infrared spectroscopy of stratum corneum lipids: in vitro results and their relevance to permeability. In: Walters, K.A., Hadgraft, J. (Eds.), Pharmaceutical Skin Penetration Enhancement. Marcel Dekker, New York, pp. 269–291.

Potts, R.O., Francoeur, M.L., 1991. The influence of stratum corneum morphology on water permeability. J. Invest. Dermatol. 96, 495–499.

Potts, R.O., Golden, G.M., Francoeur, M.L., Mak, V.H.M., Guy, R.H., 1991. Mechanism and enhancement of solute transport across the stratum corneum. J. Control. Rel. 15, 249–260.

Raykar, P.V., Fung, M.C., Anderson, B.D., 1988. The role of protein and lipid domains in the uptake of solutes by human stratum corneum. Pharm. Res. 5, 140–150.

Scheuplein, R.J., 1978. Skin as barrier. In: Jarret, A. (Ed.), The Physiology and Pathophysiology of Skin. Academic Press, New York, pp. 1693–1730.

Scheuplein, R.J., Blank, I.H., 1971. Permeability of skin. Physiol. Rev. 51, 702–747.

Singh, J., Bhatia, K.S., 1996. Topical iontophoretic drug delivery: pathways, principles, factors and skin irritation. Med. Res. Rev. 16, 285–296.

Singh, J., Rastogi, S.K., Singh, S.N., Bhatia, J.S., 2000. Quantification of leuprolide acetate by high performance liquid chromatography. J. Liq. Chrom. Rel. Technol. 23, 3023–3031.

Smith, E.W., Maibach, H.I. (Eds.), 1995. Percutaneous Penetration Enhancers. CRC Press, Boca Raton, FL.

Srinivasan, V., Su, M.H., Higuchi, W.I., Behl, C.R., 1990. Iontophoresis of polypeptides: effect of ethanol pretreatment on human skin. J. Pharm. Sci. 79, 588–591.

Sweeny, T.M., Downing, D.T., 1970. The role of lipids in the epidermal barrier to water diffusion. J. Invest. Dermatol. 55, 135–140.

Wertz, P.W., Kremer, M., Squier, C.A., 1992. Comparison of lipids from epidermal and palatal stratum corneum. J. Invest. Dermatol. 98, 375–378.

Williams, A.C., Barry, B.W., 1992. Skin absorption enhancers. Crit. Rev. Ther. Drug Carrier Syst. 9, 305–353.

Williams, M.L., Elias, P.M., 1987. The extracellular matrix of stratum corneum: role of lipids in normal and pathological function. CRC Crit. Rev. Ther. Drug Carrier Syst. 3, 95–112.