ELSEVIER

International Journal of Pharmaceutics 181 (1999) 255–263

international journal of pharmaceutics

Effect of ethanol/propylene glycol on the in vitro percutaneous absorption of aspirin, biophysical changes and macroscopic barrier properties of the skin

Angela K. Levang, Kaidi Zhao, Jagdish Singh *

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

Received 10 February 1998; received in revised form 23 November 1998; accepted 29 January 1999

Abstract

The effect of the solvent systems ethanol (EtOH), propylene glycol (PG) and combinations thereof was examined on the in vitro percutaneous absorption of the antithrombotic, aspirin, through porcine epidermis. Biophysical changes in the stratum corneum lipids were studied through the use of Fourier transform infrared (FTIR) spectroscopy. Macroscopic barrier properties of the epidermis were examined through the use of in vitro transepidermal water loss (TEWL). The flux of aspirin increased with increasing concentrations of EtOH in the solvent systems. The maximum flux of aspirin was achieved by 80% EtOH in combination with 20% PG beyond which (i.e. 100% EtOH) there was no increase in the flux. FTIR spectroscopic study was enacted in order to determine the biophysical properties of the stratum corneum when the solvents were applied. The FTIR spectra of the stratum corneum treated with 80% EtOH/20% PG showed a maximum decrease in absorbance for the asymmetric and symmetric C–H peaks, which suggests a greater loss of the lipids in the stratum corneum layers. In vitro TEWL studies allowed an investigation into the macroscopic barrier integrity properties of the stratum corneum. The TEWL results indicated that each of the solvent systems significantly enhanced ($P < 0.05$) in vitro TEWL in comparison to the control. In conclusion, 80% EtOH/20% PG enhanced the percutaneous absorption of aspirin by perturbing the macroscopic barrier integrity of the stratum corneum and through a loss of stratum corneum lipids.

Keywords: Aspirin; Fourier transform infrared spectroscopy; Penetration enhancer; Percutaneous absorption; Transepidermal water loss

* Corresponding author: Tel.: +1-701-231-7943; fax: +1-701-231-7606.
E-mail address: jsingh@plains.nodak.edu (J. Singh)

0378-5173/99/$ - see front matter
PII: S0378-5173(99)00055-1

1. Introduction

Aspirin is the most extensively prescribed analgesic, antipyretic and anti-inflammatory agent in the treatment of pain, fever and infection. In addition, aspirin is being utilized in the prevention of cardiovascular diseases and cancer. Because of its antiplatelet actions, aspirin has been shown to be effective in the management of myocardial infarction, atrial fibrillation, angina, ischemic stroke and coronary artery bypass grafts (Antiplatelet Trialists' Collaboration, 1994; Hennekens, 1997). However, there has been a reluctance to recommend aspirin as a primary antithrombotic measure, in part, because of its gastrointestinal (GI) toxicity (McAdam et al., 1996). Transdermal diffusion would provide a means to avoid the GI toxicity associated with administration by the oral route. The skin provides a medium through which low doses of aspirin can be given slowly and continuously. Such a transdermal route would be particularly useful in patients with tendencies for GI irritation and/or bleeding.

The primary barrier to transdermal diffusion is the stratum corneum. A well-known approach, which reduces the barrier function of the stratum corneum, involves the use of enhancers that can partition into and interact with skin constituents to induce a temporary, reversible increase in skin permeability (Williams and Barry, 1992). Ethanol (EtOH), with its history as a cosolvent and its well-established systemic toxicology and local tolerability, is currently contained in commercial transdermal delivery systems as an enhancer for estradiol (Good et al., 1985) and fentanyl (Gale et al., 1986). Propylene glycol (PG) is widely used as a cosolvent for lipophilic drugs and potential enhancers, but the literature provides conflicting results as to whether this molecule is able to increase skin permeability. An increase in solution capacity within the stratum corneum is supposed to be the action of PG (Barry, 1988; Williams and Barry, 1992).

Fourier transform infrared (FTIR) spectroscopy provides information on the vibrational modes of its components and probes the structure on a molecular level (Casal and Mantsch, 1984). Of particular interest in lipid studies are the IR absorbances near 2850 and 2920 cm^{-1} due to symmetric and asymmetric methylene group (H–C–H) stretching, respectively. Solvent extraction of the stratum corneum lipids results in reduction of the methylene group stretching absorbances. FTIR spectroscopy results (Goates and Knutson, 1994) suggest a correlation between enhanced permeation of mannitol and extraction of lipids from human skin in the presence of 75% (v/v) aqueous alcohol solutions.

Transepidermal water loss (TEWL) provides a method for assessing macroscopic changes in the barrier properties of the stratum corneum (Abrams et al., 1993). TEWL can be considered a determinant indicative of the functional state of the cutaneous barrier (Wilson and Maibach, 1982; Maibach et al., 1984; Rougier et al., 1989). Biophysical evidence suggests that stratum corneum lipid domains are the primary barrier to both water loss and the penetration of compounds into the skin (Van Duzee, 1971). Measurement of TEWL is a relevant parameter for the prediction of the percutaneous penetration of substances (Lotte et al., 1987).

Smith and Anderson (1995) developed an in vitro method that allows an investigation into the mechanism(s) of solute transport enhancement across the skin by a model enhancer under equilibrium conditions. The classical in vitro method, in which the enhancer is added to the donor side of the skin and enhancer-free aqueous buffers are placed in the receiver side, takes time to equilibrate the skin with enhancer. The Smith and Anderson (1995) method places the enhancer in both the donor and receiver compartments. This method is important for studying the percutaneous penetration enhancement mechanism(s) because it avoids enhancer concentration gradients, organic vehicle gradients and water gradients, which may interfere with reaching a steady state. Moreover, in contrast to the classical method, the Smith and Anderson (1995) method allows the skin, donor phase and receiver phase to be at equilibrium with respect to the enhancer.

In this study, we investigated the effects of solvent/solvent systems under equilibrium conditions on the in vitro percutaneous absorption of

aspirin, biophysical changes in the stratum corneum lipids and in vitro TEWL. We also correlated the biophysical and macroscopic barrier changes with the in vitro percutaneous absorption of aspirin in order to understand the mechanism(s) of percutaneous penetration.

2. Materials and methods

2.1. Materials

Acetylsalicylic acid was obtained from Sigma Chemical Company, St. Louis, MO, USA. The solvents, ethanol (Sigma Diagnostics, St. Louis, MO, USA) and propylene glycol (Gallipot Inc., St. Paul, MN, USA) were used. Also used were HPLC grade acetonitrile (Fisher Chemical, Fair Lawn, NJ, USA), glacial acetic acid (Mallinckrodt Chemical, Inc., Paris, KT, USA), 1-heptane sulfonic acid and formic acid (Sigma Chemical Company, St. Louis, MO, USA). All other chemicals used were of analytical grade. All commercially obtained chemicals were used as received without further purification.

2.2. Preparation of the epidermis

Porcine ears were obtained from a local slaughter house and, after cleaning under cold running water, the outer region of the ear was cut. The whole skin was removed carefully from the underlying cartilage with the help of a scalpel. The method of Kligman and Christophers (1963), with slight modification, was adopted to remove the epidermis. The epidermis was prepared by soaking the whole skin in water at 60°C for 45 s. The skin was removed from water, blotted dry and pinned with the dermal side down. The intact epidermis was then teased off from the dermis with forceps, washed with water and used in the in vitro permeability studies (Bhatia and Singh, 1996).

2.3. Preparation of the stratum corneum

The epidermis was incubated for 4 h in a 0.5% trypsin solution in phosphate-buffered saline (PBS) (pH 7.4) at 37°C. The tissue was then smoothed out on a flat surface and the mushy epidermis removed by rubbing with a moistened cotton tipped applicator. The transparent stratum corneum obtained was briefly floated on water, blotted dry and used in the FTIR spectroscopic studies.

2.4. In vitro studies

Franz diffusion cells were used in all permeability studies. The epidermis was sandwiched between the cells with the stratum corneum facing the donor compartment. The maximum capacity for the donor and receiver compartments was 1 ml and 5 ml, respectively. The surface area of the epidermis exposed to the solution was 0.785 cm^2. The Smith and Anderson (1995) method was used to study the in vitro percutaneous absorption of aspirin under equilibrium conditions. The donor compartment contained 1 ml of 100 mg/ml aspirin in solvent/solvent system solution and the receiver contained 5 ml of the solvent/solvent system. The cells were maintained at 37 ± 0.5°C by a PMC Dataplate® stirring digital dry block heater (Crown Bioscientific Inc., NJ, USA). The content of the receiver compartment was stirred with the help of a magnetic bar at 100 rpm. At specified intervals, 0.5-ml samples were withdrawn from the receiver compartment and an equivalent amount of solvent/solvent system was added in order to maintain a constant volume. All experiments were run for 10 h.

The samples were assayed using high-performance liquid chromatography (HPLC). A Hewlett-Packard series 1050 liquid chromatograph (Hewlett-Packard GmbH, Waldbronnz, Germany) was used with a Zorbax ODS (Wilmington, DE) C_{18} column (5 μm, 150×4.6 mm). The mobile phase consisted of a 15:85 (v/v) acetonitrile–water mixture adjusted to pH 3.4 by glacial acetic acid. The flow rate was 3 ml/min. A 10-μl sample size was injected. Aspirin was detected using a variable wavelength UV detector at 280 nm and results were quantified through use of the equation derived from the slope of the standard curve prepared for aspirin ($r = 0.994$) at 280 nm.

2.5. Fourier transform infrared spectroscopy

The stratum corneum was soaked in solvent/solvent system solution for 10 h, washed with water and blotted dry. The FTIR experiments were then performed on the stratum corneum. Each of the FTIR experiments was performed in triplicate. FTIR (Nicolet Instrument Corporation, Madison, WI, USA) was used for the study. Emphasis was placed on peaks which appeared at approximately 2850 and 2920 cm^{-1} and are due to symmetric and asymmetric C–H stretching, respectively. OMNIC® FTIR software (Nicolet Instrument Corporation, Madison, WI, USA) was used to calculate the peak heights and areas. For each stratum corneum sample, peak height and area were measured before and after the solvent/solvent system treatment. This experimental strategy allowed each sample to serve as its own control.

2.6. Transepidermal water loss

Franz diffusion cells were used in all TEWL studies. Porcine epidermis was soaked in the solvent/solvent system and shaken for 10 h. The epidermis was then washed with water, blotted dry and sandwiched between the diffusion cells with the stratum corneum facing the donor compartment. The epidermal side was exposed to air and the dermal side was exposed to isotonic saline (0.9% sodium chloride solution) which was placed in the receiver compartment. The surface area of the epidermis exposed for TEWL was 0.785 cm^2. The temperature of the diffusion cells was maintained at 37 ± 0.5°C. The epidermis was allowed to equilibrate for 4 h before measurements were taken with Tewameter™ (Courage & Khazaka, Colgne and Academ, Menlo Park, CA, USA.). The measurements were performed by holding the Tewameter probe over the donor compartment until a stable TEWL value was achieved. The experiments were performed in a setting with a temperature range between 29 and 32.5°C and relative humidity between 47.1% and 62.7%. All experiments were performed in triplicate, with results expressed as the mean ± S.D. Experiments were performed in the same manner without solvent/solvent system treatment to serve as a control.

2.7. Data analysis

The aspirin concentration of the receiver was corrected for sample removal by use of the equation derived by Hayton and Chen (1982):

$$C_n^1 = C_n \frac{V_t}{V_t - V_s} \cdot \frac{C_{n-1}^1}{C_{n-1}}$$

where C_n^1 and C_n are the corrected and measured aspirin concentrations, respectively. V_t is the total volume of the receiver compartment and V_s is the volume of the sample. C_{n-1}^1 and C_{n-1} are the corrected and measured drug concentrations before sampling.

The cumulative amount of aspirin permeated per unit skin surface area was plotted against the time and slope of the linear portion of the graph was estimated as steady-state flux (J_{ss}). We also determined the solubility of aspirin in donor solution and corrected the flux from fractional solubility adjustment (Baker, 1987; Gao and Singh, 1998):

$$\text{Corrected flux} = \frac{J_{ss}}{C_v/C_s}$$

where C_s is the saturated solubility of aspirin in control/solvent system and C_v is the donor concentration of aspirin.

Statistical comparisons were made using the Student's t-test, Mann–Whitney U-test and analysis of variance (ANOVA). The level of significance was taken as $P < 0.05$.

3. Results and discussion

The effect of solvent systems on the in vitro percutaneous absorption of aspirin through porcine epidermis is shown in Fig. 1. The greatest amount of aspirin transport was achieved with 80% EtOH/20% PG. The flux of aspirin with various solvent/solvent systems is shown in Table 1. The flux of aspirin was significantly different ($P < 0.05$, ANOVA) among treatment groups. The flux of aspirin increased with increasing con-

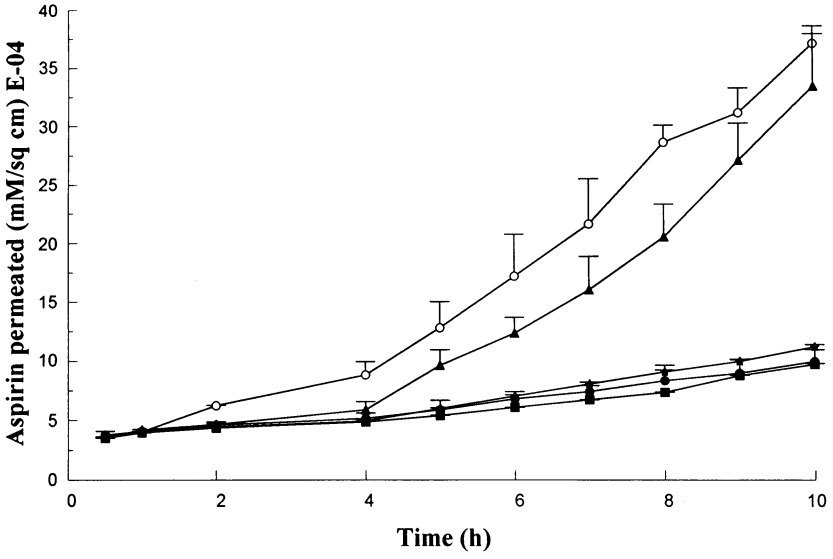

Fig. 1. The effect of solvent/solvent systems on the in vitro transport of aspirin through porcine epidermis. Each data point is the mean ± S.D. of three determinations. ○, PBS; □, 100% PG; △, 20% EtOH/80% PG; ◇, 40% EtOH/60% PG; ●, 80% EtOH/20% PG; ▲, 100% EtOH.

centrations of EtOH in the solvent system. The maximum flux of aspirin was obtained with 80% EtOH/20% PG beyond which there was no enhancement of flux found with 100% EtOH. The flux of aspirin with 80% EtOH/20% PG was not significantly different ($P > 0.05$) than 100% EtOH as determined by the Mann–Whitney *U*-test.

Water is the only known plasticizer for skin or keratin (Moellgaard and Hoelgaard, 1983). At low water concentrations, the keratins shrink and the epidermis becomes more impermeable (Moellgaard and Hoelgaard, 1983). At a 100% concentration of neat ethanol, the stratum corneum dehydrates. Consequently, the solute (aspirin) transport does not increase ($P > 0.05$) beyond 80% concentrations of ethanol.

The flux is actually proportional to a gradient of thermodynamic activity rather than concentration. At a constant drug concentration, drug activity will be reduced as solubility in a

Table 1
Flux and solubility of aspirin

Treatment[a]	Flux[b] (mmol/cm^2/h) × 10^5	Solubility (mg/ml)	Corrected flux (mmol/cm^2/h) × 10^5
PBS	7.30 ± 0.05	11.03 ± 0.37	7.30 ± 0.05
PG	7.10 ± 0.17 ($P > 0.05$)	42.10 ± 1.10	7.10 ± 0.17
20% EtOH/80% PG	7.90 ± 0.09 ($P < 0.05$)	102.69 ± 3.73	8.10 ± 0.10
40% EtOH/60% PG	10.10 ± 0.05 ($P < 0.05$)	123.56 ± 4.72	12.60 ± 0.06
80% EtOH/20% PG	46.80 ± 2.20 ($P < 0.05$)	184.20 ± 3.45	86.20 ± 4.10
EtOH	44.40 ± 7.00 ($P < 0.05$)	195.64 ± 2.14	87.00 ± 13.80

[a] PBS, phosphate-buffered saline (pH 7.4); EtOH, 100% ethanol; PG, propylene glycol.

[b] ANOVA was used to determine the difference in the flux of aspirin among five treatment groups. There was a significant difference ($P < 0.05$, ANOVA) in the aspirin flux among the treatment groups. Student's *t*-test was used to determine the difference in the flux of aspirin between control (PBS) and treatment groups. The difference in the flux of aspirin with 100% EtOH and 80% EtOH/20% PG was not significant ($P > 0.05$) as determined by the Mann–Whitney *U*-test.

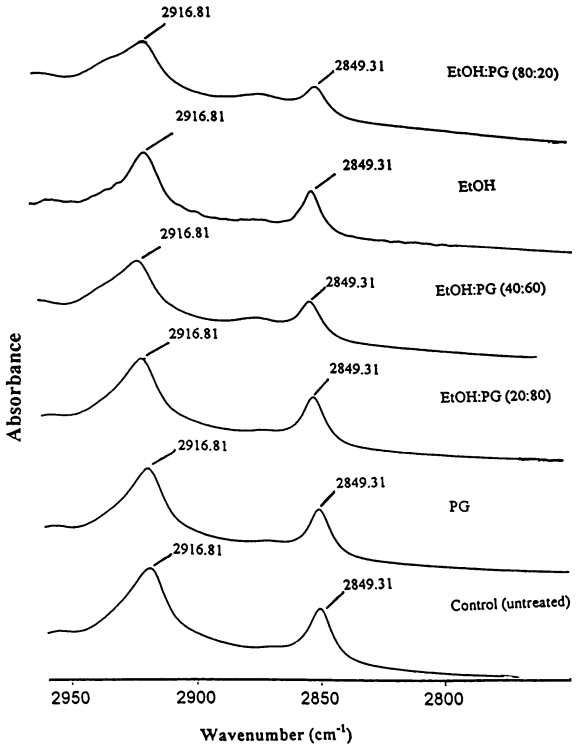

Fig. 2. FTIR spectrum of porcine stratum corneum treated with solvent/solvent systems.

solvent/solvent system increased. In all vehicles that contain saturated solutions of the drug, the thermodynamic activity of the drug is also maximal. Therefore, we determined the solubility of aspirin in solvent/solvent system and corrected the flux from fractional solubility adjustment (Baker, 1987; Gao and Singh, 1998). The results are presented in Table 1.

In an effort to limit GI toxicity and inhibition of endothelial prostaglandin PGI_2 formation, aspirin has been administered in very low doses, and it is possible to achieve cumulative inhibition of platelet thromboxane TXA_2 formation with oral doses as low as 30 mg/day (Patrignani et al., 1982). The oral bioavailability of aspirin as unhydrolyzed aspirin was found to be about 70% (American Society of Health-system Pharmacists, 1998). Therefore, 21 mg/day of aspirin is required to be systemically available for inhibition of platelet formation. The corrected flux of aspirin through the epidermis with 80% EtOH/20% PG was 86.2 mmol/cm^2/h (0.16 mg/cm^2/h or 3.84 mg/cm^2/day). If we use a patch size of 10 cm^2, the amount of aspirin delivered will be 38.4 mg/day. Thus, it would easily reach the target delivery rate for inhibition of platelet formation.

With respect to the stratum corneum, the most informative FTIR lipid absorbances are those originating from the hydrophobic alkyl chain (Naik and Guy, 1997). Of these, the most extensively studied are the carbon–hydrogen stretching vibrations. Fig. 2 shows the FTIR spectrum of porcine stratum corneum. The prominent peaks near 2850 cm^{-1} and 2920 cm^{-1} are the result of asymmetric and symmetric stretching modes, respectively. The FTIR spectra of porcine stratum corneum did not show any shift in either the asymmetric or symmetric absorbance peaks due to treatment with EtOH/PG solvent systems. Goates and Knutson (1994) also demonstrated that the stratum corneum lipid conformation and mobility were not significantly altered in the presence of short chain alcohols at 75% (v/v) alcohol concentrations.

Tables 2 and 3 show the effect of solvent/solvent systems on the peak height and area of asymmetric and symmetric absorbance, respectively. We observed that increasing concentrations of ethanol in the solvent systems decreased peak height and peak area under both asymmetric and symmetric absorbances until 80% EtOH/20% PG. Further increases in the concentration of EtOH (i.e. 100% EtOH) did not correspondingly decrease the peak heights and areas under asymmetric and symmetric absorbances. However, we did not observe a difference ($P > 0.05$) in the flux of aspirin between 80% EtOH/20% PG and 100% EtOH.

The stratum corneum membranes were examined by FTIR in the presence of aqueous alcohols to identify molecular level structural changes in the membrane (Goates and Knutson, 1994). The above demonstrated that the lipids do not become more fluid in the presence of high concentrations of short chain alcohols. Rather, the decrease in CH_2 stretching bandwidths, accompanied by a decrease in CH_2 stretching band intensity, suggested an overall extraction of stratum corneum lipids.

Table 2
Percent decrease in the peak height and peak area of asymmetric C–H stretching absorbance of the porcine SC

Penetration enhancer[a]	Asymmetric C–H stretching					
	Peak height			Peak area		
	Control	Treatment	% Decrease[b]	Control	Treatment	% Decrease[b]
PG	0.29 ± 0.00	0.27 ± 0.00	6.9	6.65 ± 0.08	6.06 ± 0.07	8.9
EtOH:PG (20:80)	0.38 ± 0.00	0.35 ± 0.00	7.9	7.82 ± 0.00	7.21 ± 0.01	7.8
EtOH:PG (40:60)	0.45 ± 0.04	0.29 ± 0.01	35.6	9.93 ± 0.99	6.17 ± 0.17	37.9
EtOH	0.39 ± 0.00	0.19 ± 0.00	51.3	8.03 ± 0.00	3.56 ± 0.01	55.7
EtOH:PG (80:20)	0.33 ± 0.01	0.11 ± 0.01	66.7	6.98 ± 0.34	2.49 ± 0.39	64.3

[a] PG, 100% propylene glycol; EtOH, 100% ethanol.
[b] % Decrease in peak height or area is calculated from the following equation:

$$\%\ \text{Decrease in peak height or peak area} = \frac{(\text{Peak height or area from enhancer} - \text{treated SC}) - (\text{Peak height or area from control})}{\text{Peak height or area from control}} \times 100.$$

TEWL results are shown in Table 4. Treatments of the epidermis with the solvent/solvent systems significantly enhanced the in vitro TEWL ($P < 0.05$) in comparison to their respective controls. TEWL was most enhanced by 80% EtOH/20% PG and 100% EtOH and there was no significant difference ($P > 0.05$) in TEWL through 80% EtOH/20% PG and 100% EtOH treated epidermis. TEWL measurements are regarded as an indicator of barrier function; a high TEWL generally indicates barrier perturbation. Several investigators (Wertz and Downing, 1982; Elias, 1983) have suggested that the high resistance of the stratum corneum to water flux is due to the extended multilamellar lipid domains present intercellularly in the stratum corneum. Accordingly, water molecules must traverse the hydrocarbon regions of these lamellae in order to diffuse across this barrier. It is generally accepted that a solvent, such as ethanol, removes intercellular material, which results in cutaneous barrier disruption (Scheuplein and Blank, 1971). Rougier et al. (1989) observed a linear relationship between transepidermal water loss and percutaneous absorption of molecules. Thus, an increase in the percutaneous absorption of aspirin by the solvent

Table 3
Percent decrease in the peak height and peak area of symmetric C–H stretching absorbance of the porcine SC

Penetration enhancer[a]	Symmetric C–H stretching					
	Peak height			Peak area		
	Control	Treatment	% Decrease[b]	Control	Treatment	% Decrease[b]
PG	0.19 ± 0.00	0.18 ± 0.00	5.3	2.13 ± 0.14	1.83 ± 0.12	14.1
EtOH:PG (20:80)	0.26 ± 0.00	0.23 ± 0.00	11.5	2.83 ± 0.04	2.54 ± 0.01	10.2
EtOH:PG (40:60)	0.30 ± 0.03	0.15 ± 0.00	50.0	3.53 ± 0.41	1.62 ± 0.03	54.1
EtOH	0.26 ± 0.00	0.12 ± 0.00	53.8	2.94 ± 0.01	1.22 ± 0.00	58.5
EtOH:PG (80:20)	0.20 ± 0.00	0.05 ± 0.00	75.0	2.41 ± 0.11	0.48 ± 0.07	80.1

[a] PG, 100% propylene glycol; EtOH, 100% ethanol.
[b] See Table 2.

Table 4
In vitro TEWL through porcine epidermis

Treatment	TEWL ($g/m^2/h$, mean ± S.D.)		
	Treated	Control	Treated −control[a]
100% EtOH	23.33 ± 0.83 ($P<0.01$)	5.73 ± 0.45	17.60 ± 0.38
80% EtOH/ 20% PG	23.67 ± 1.67 ($P<0.01$)	5.27 ± 0.90	18.40 ± 0.77
40% EtOH/ 60% PG	16.0 ± 0.22 ($P<0.01$)	4.57 ± 1.05	11.43 ± 0.84
20% EtOH/ 80% PG	10.70 ± 0.62 ($P<0.01$)	5.87 ± 0.56	4.83 ± 0.07
100% PG	10.73 ± 1.58 ($P<0.05$)	5.50 ± 1.08	5.23 ± 0.50

[a] TEWL through solvent-treated epidermis minus TEWL through the control epidermis. Student's *t*-test was used to find the difference in TEWL of treated group from the control.

system (80% EtOH/20% PG) can be attributed to the barrier perturbation as is evident by an increased in vitro TEWL. In conclusion, FTIR and in vitro TEWL findings with the solvent systems used in the study suggest that 80% EtOH/20% PG increases the percutaneous absorption of aspirin by increasing lipid extraction and perturbing the macroscopic barrier properties of the skin.

Acknowledgements

We acknowledge the support of the Glaxo Wellcome–AFPE 'Gateway' Research Scholarship to Angela K. Levang for this work.

References

Abrams, K., Harvell, J.D., Shriner, D., Wertz, P., Maibach, H., Maibach, H.I., Rehfeld, S.J., 1993. Effect of organic solvents on in vitro human skin water barrier function. J. Invest. Dermatol. 101, 609–613.

American Society of Health-system Pharmacists, 1998. AHFS Drug Information. American Society of Health-system Pharmacists, pp. 1584–1592.

Antiplatelet Trialists' Collaboration, 1994. Collaborative overview of randomised trials of antiplatelet therapy. 1. Prevention of death, myocardial infarction and stroke by prolonged anti-platelet therapy in various categories of patients. Br. Med. J. 308, 81–106.

Baker, R.W., 1987. Controlled Release of Biologically Active Agents. Wiley, New York Chapter 2.

Barry, B.W., 1988. Action of skin penetration enhancers—lipid protein partitioning theory. Int. J. Cosmet. Sci. 10, 284–293.

Bhatia, K.S., Singh, J., 1996. Pig ear skin as a model for predicting percutaneous absorption in man. Pharm. Sci. 2, 1–2.

Casal, H.L., Mantsch, H.H., 1984. Polymorphic phase behavior of phospholipid membranes studied by infrared spectroscopy. Biochim. Biophys. Acta 779, 381–401.

Elias, P.M., 1983. Epidermal lipids, barrier function, and desquamation. J. Invest. Dermatol. 80, 44s–49.

Gale, R.M., Goetz, V., Lee, E.S., Taskovich, L.T., Yum, S.I., 1986. Device for delivering fentanyl across the skin at a constant rate to maintain analgesia over a long period. US Patent 4588580.

Gao, S., Singh, J., 1998. In vitro percutaneous absorption enhancement of a lipophilic drug tamoxifen by terpenes. J. Control. Release 51, 193–199.

Goates, C.Y., Knutson, K., 1994. Enhanced permeation of polar compounds through human epidermis. 1. Permeability and membrane structural changes in the presence of short chain alcohols. Biochim. Biophys. Acta 1195, 169–179.

Good, W.R., Powers, M.S., Campbell, P., Schenicel, L., 1985. A new transdermal delivery system for estradiol. J. Control. Release 2, 89–97.

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

Hennekens, C.H., 1997. Aspirin in the treatment and prevention of cardiovascular disease. Sore Rev. Public Health 18, 37–49.

Kligman, A.M., Christophers, E., 1963. Preparation of isolated sheets of human stratum corneum. Arch. Dermatol. 88, 702–705.

Lotte, C., Rougier, A., Wilson, D.R., Maibach, H.I., 1987. In vivo relationships between transepidermal water loss and percutaneous penetration of some organic compounds in man: effect of anatomic site. Arch. Dermatol. Res. 279, 351–356.

Maibach, H.I., Bronaugh, R., Guy, R., Turr, E., Wilson, D., Jacques, S., Chaing, D., 1984. Noninvasive techniques for determining skin function. In: Drill, V.A., Lazar, P. (Eds.), Cutaneous Toxicity. Raven Press, New York, pp. 63–97.

McAdam, B., Keimowitz, R.M., Maher, M., Fitzgerald, D.J., 1996. Transdermal modification of platelet function: an aspirin patch system results in marked suppression of platelet cyclooxygenase. J. Pharmacol. Exp. Ther. 277, 559–564.

Moellgaard, B., Hoelgaard, A., 1983. Vehicle effect on topical drug delivery. I. Influence of glycol and drug concentration on skin transport. Acta Pharm. Suec. 20, 433–442.

Naik, A., Guy, R.H., 1997. Infrared spectroscopic and differential scanning calorimetric investigations of the stratum corneum barrier function. In: Potts, R.O., Guy, R.H. (Eds.), Mechanisms of Transdermal Drug Delivery. Marcel Dekker, New York, pp. 87–162.

Patrignani, P., Filabozzi, P., Patrono, C., 1982. Selective cumulative inhibition of platelet thromboxane production by low-dose aspirin in healthy subjects. J. Clin. Invest. 69, 1366–1372.

Rougier, A., Lotte, C., Maibach, H.I., 1989. In vivo relationship between percutaneous absorption and transepidermal water loss. In: Bronaugh, R.L., Maibach, H.I. (Eds.), Percutaneous Absorption. Marcel Dekker, New York, pp. 175–190.

Scheuplein, R.J., Blank, I.H., 1971. Permeability of the skin. Physiol. Rev. 57, 702–747.

Smith, S.W., Anderson, B.D., 1995. Human skin permeability enhancement by lauric acid under equilibrium aqueous conditions. J. Pharm. Sci. 84, 551–556.

Van Duzee, B.F., 1971. Thermal analysis of human stratum corneum. J. Invest. Dermatol. 65, 702–747.

Wertz, P.M., Downing, D.T., 1982. Glycolipids in mammalian epidermis: structure and function in the water barrier. Science 217, 1261–1262.

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

Wilson, D.R., Maibach, H.I., 1982. A review of transepidermal water loss: physical aspects and measurements as related to infants and adults. In: Maibach, H.I., Bouititis, E.K. (Eds.), Neonatal Skin. Marcel Dekker, New York, pp. 83–100.