

PHARMACEUTICS, PREFORMULATION AND DRUG DELIVERY

Effect of Propylene Glycol on Ibuprofen Absorption into Human Skin *In Vivo*

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ABSTRACT: The objective was to assess the impact of propylene glycol (PG), a common cosolvent in topical formulations, on the penetration of ibuprofen into human skin *in vivo*. Drug uptake into the stratum corneum (SC), following application of saturated formulations containing from 0 to 100% v/v PG, was assessed by tape-stripping. Dermatopharmacokinetic parameters, characterizing drug amount in and diffusivity through the SC, were derived. The solubility behavior of ibuprofen in PG–water mixtures was carefully evaluated, as were a number of other physical properties. Ibuprofen delivery depended on the level of PG in the vehicle, despite all formulations containing the drug at equal thermodynamic activity. PG appeared to alter the solubility of ibuprofen in the SC (presumably via its own uptake into the membrane), the effect becoming more important as the volume fraction of cosolvent in the formulation increased. In summary, tape-stripping experiments, with careful interpretation, can reveal details of a drug's bioavailability in the skin following topical application and may be used to probe the mechanism(s) by which certain excipients influence local drug delivery. © 2007 Wiley-Liss, Inc. and the American Pharmacists Association *J Pharm Sci* 97:185–197, 2008

Keywords: topical drug bioavailability; dermatopharmacokinetics; solubility; partition coefficient; diffusivity; skin; percutaneous absorption

INTRODUCTION

Many drugs developed for the topical treatment of skin disease are poorly water-soluble and difficult to formulate. Furthermore, the elegant vehicles produced commercially often undergo rapid and extensive modification of their composition after application to the skin. For example, volatile components may evaporate and change

the thermodynamic activity of the drug in the formulation;¹ in some instances, the drug may even precipitate on the skin surface. However, as the drug must dissolve into the stratum corneum (SC) to be absorbed, alterations in the properties of the vehicle may significantly impact upon the overall kinetics of drug uptake.

Ideally, to maximize delivery, the largest possible amount of drug should be dissolved in the formulation and become immediately bioavailable to the lipophilic SC.² In the case of water-insoluble drugs, the incorporation of a cosolvent into the formulation is a typical and, in general,

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KINDERFARMS Ex. 1050
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profitable strategy.^{3–6} Propylene glycol (PG) is a particularly useful example of an inexpensive, nontoxic, and well-tolerated cosolvent.⁷ While considerable research into its efficacy and action has been reported in the literature,^{8–13} a detailed examination of its direct mechanism at the formulation-SC interface *in vivo* has not been undertaken. In this study, the tape-stripping procedure in human volunteers is used to probe the manner in which PG as a cosolvent, administered at different levels in simple binary mixtures with water, determines the SC uptake and transport of the model drug, ibuprofen.

MATERIALS AND METHODS

Chemicals

S-(+)-Ibuprofen (Fluka, Buchs, Switzerland) was dissolved at saturation in various PG–water mixtures (Sigma-Aldrich, Steinheim, Germany). Solvents used for ibuprofen extraction and liquid chromatographic (HPLC) analysis were of analytical grade (Sigma-Aldrich). Citric acid monohydrate, sodium hydroxide (Sigma-Aldrich), and hydrochloric acid (Fluka) were used to prepare buffers.

Experimental Procedures

Ten volunteers (7 female, 3 male, 24–46 years) with no history of dermatological disease participated in this study, which was approved by the University of Geneva ethical committee. Informed consent was obtained from all subjects. The treated sites (4 × 5 cm) were non-hairy regions of the ventral forearm surface. Each treatment consisted of a 1.9 mL application of ibuprofen solution on a cellulose gauze (Tela, Basel, Switzerland) which was covered by an occlusive polyester layer (Scotchpak, 3M, St. Louis, MN) and affixed to the skin with an adhesive polyurethane film (Opsite, SmithNephew, Hull, UK). These applications are considered as infinite doses from which negligible drug depletion was anticipated during the experiment. After the chosen application time of 30 min, the patch was removed and excess formulation was gently removed using three dry cellulose swabs without any solvent.

Formulations

The vehicles studied were saturated solutions of ibuprofen in the following v/v mixtures of PG and

water: 0:100, 25:75, 50:50, 75:25, and 100:0. The volume fraction (f) of the cosolvent was defined as $V_{PG}/(V_{PG} + V_{water})$. The saturated solutions were prepared by dissolving the amounts of ibuprofen necessary to fully saturate each PG/water mixture. These amounts were determined from the solubility experiments described below. The solutions were prepared and used immediately.

SC Sampling Protocol

The ibuprofen concentration profile across the SC following application in the different vehicles was determined by sequential removal of the outer skin layer by tape-stripping (Scotch Book Tape, 3M, St. Louis, MN). The SC sampling site was delimited by a template which exposed an area smaller than that treated with the formulation. The template was centered over the drug application site immediately before tape-stripping began. The size of the opening in the template (2 × 2.5 cm) was smaller than the individual tape-strips used. Differential weighing (Mettler AT 261 balance, Greifensee, Switzerland) of tape-strips allowed the amount of SC removed to be estimated. From this mass, and knowing the area of the tape, it was possible to calculate the SC thickness removed (using a SC density of 1 g/cm³)¹⁴ as a function of stripping and hence the corresponding position (or depth, x) within the barrier. The apparent SC thickness (L) was determined as described elsewhere¹⁵ from measurements of transepidermal water loss (TEWL) as a function of SC removed. This permits the drug concentration profile to be expressed as a normalized function of relative position within the SC (x/L) and facilitates the comparison of data originating from different volunteers.^{16–18} The TEWL measurements were made at a site adjacent to the treated skin to avoid residual vehicle effects on the TEWL readings, and to ensure that these measurements did not prolong the experiment to the point that the drug concentration profile could change significantly. Ten to twenty strips were taken from each treated site of each volunteer, the actual number depending most probably upon the efficiency of the tape-stripping process as well as the individual's SC thickness; however, the SC was never completely removed. All tapes were subsequently analyzed for ibuprofen; no strips were discarded, and it was assumed that any drug not removed by the surface cleaning process at the end of the

treatment had been absorbed into the SC and would eventually become bioavailable to the skin.

Extraction and Analysis of Ibuprofen in the Tape-Strips

After re-weighing, each tape-strip was rolled and placed in a 1.5 mL HPLC vial. Ibuprofen was quantitatively extracted with a 90:10 mixture of acetonitrile and 1 M hydrochloric acid during 12 h. Validation of this procedure was evaluated by spiking tape-stripped samples of untreated SC with known amounts of drug in solution, chosen to bracket the expected range of concentrations to be found in the *in vivo* samples. It was not necessary to filter the tape-strip extraction solutions which were completely clear and protein-free (Biorad[®] protein assay, Hercules, CA). Ibuprofen was analyzed by HPLC using a Merck Lichrospher 100 RP18 (5 μ m) column (Darmstadt, Germany) and a model 486 absorbance detector from Waters (Milford, MA) at 227 nm. The isocratic mobile phase was a 55:45 (v/v) mixture of acetonitrile and 0.1 M citrate buffer at pH 2.4. At a flow rate of 1.2 mL/min, and at room temperature, the retention time of ibuprofen was about 5 min. Peak recording and data processing were performed with the built-in system manager. Ibuprofen was determined using the AUC method and calibration plots were generated with the neat compound. The quantification limit was 0.5 μ g/mL.

Experimental Strategy and Data Analysis

The SC concentration (C_x) versus depth (x) profile of ibuprofen was determined following a 30 min treatment of the skin. Data were fitted to the following solution of Fick's 2nd law of diffusion:

$$C_x = K \cdot C_v \left\{ \left(1 - \frac{x}{L} \right) - \frac{2}{\pi} \sum_{n=1}^{\infty} \frac{1}{n} \sin\left(\frac{n\pi \cdot x}{L}\right) \exp\left(\frac{-D \cdot n^2 \cdot \pi^2 \cdot t}{L^2}\right) \right\} \quad (1)$$

The applicable boundary conditions are (i) the applied drug concentration (C_v) remains constant during the treatment period (t); (ii) the viable epidermis acts as a perfect sink for the drug; and (iii) the SC contains no drug at $t=0$. The fitting generates values of K and D/L^2 . The former is the SC/vehicle partition coefficient, a thermodynamic parameter reflecting the affinity of the drug for the

SC relative to the vehicle. The second parameter, the ratio of the drug's diffusivity in the SC (D) to the apparent thickness squared of the barrier, has units of (time)⁻¹ and can be considered a first-order kinetic constant describing drug transport across the SC. Integration of Eq. (1) across the SC thickness (i.e., from $x=0$ to $x=L$) yields the concentration-SC depth profile, that is, the total concentration of drug in the SC ($AUC_{x,x}$), after an application time t :

$$AUC_{x,x} = \int_0^L C_x dx = K \cdot C_v \left\{ \frac{1}{2} - \frac{4}{\pi^2} \sum_{n=0}^{\infty} \frac{1}{(2n+1)^2} \exp\left(-\frac{(2n+1)^2 \cdot \pi^2 \cdot D \cdot t}{L^2}\right) \right\} \quad (2)$$

For each formulation tested, therefore, the C_x versus x profile was individually fitted to yield values of K and D/L^2 . The mean parameters were then compared across vehicles and the resulting dependencies considered in the light of a number of parallel physicochemical measurements now described.

Solubility Determinations

Ibuprofen solubility in each PG–water vehicle was determined (in triplicate) by placing an excess amount of drug in a 20 mL capped tube with the binary mixture. Solubilities were determined in triplicate at each condition. Experiments were performed at 20°C ($\pm 1^\circ$ C) in a temperature-controlled cabinet (Forma Scientific, Marietta, GA). After 96 h, a sample was taken and filtered through 0.45 μ m solvent-resistant filters, and aliquots were diluted for HPLC assay. The results for pure water and for the PG–water mixtures tested in the dermatopharmacokinetic experiments are in Table 1. Densities of the saturated solutions and of the solvent mixtures were determined in triplicate at 20°C ($\pm 1^\circ$ C) in 2 mL pycnometers.

Molar Heat of Fusion (ΔH_m^f) and Analysis of Solid Phase

The molar heat of fusion was determined by differential scanning calorimetry (DSC) (Seiko 220C, Seiko Instruments, Inc., Tokyo, Japan). Samples of solid ibuprofen, obtained after their

Table 1. Experimentally Determined Ibuprofen Solubilities in PG–Water Mixtures at 20°C (Mean ± SD, *n* = 3)

Vehicle (% v/v PG–Water)	Solubility (mg/mL)
0:100	0.14 ± 0.02
25:75	0.30 ± 0.03
50:50	2.56 ± 0.03
75:25	35.7 ± 0.7
100:0	430 ± 13

equilibration with PG–water mixtures containing ≥50% PG, were blotted dry using filter paper to remove excess solvent. The collected solid was dried at room temperature under vacuum for 24 h. The drug (1–4 mg) was then subjected to DSC to determine whether its solid-state properties had been altered during the solute–solvent equilibration process. Thermograms revealed no significant changes (i.e., no polymorphism). The experimentally determined melting point and heat of fusion of ibuprofen, together with related physicochemical parameters from the literature^{19,20} are in Table 2.

Solubility Parameters and Molar Volumes

The solubility parameters and molar volume of ibuprofen (δ_2 ; V_2) and solvents (δ_1 ; V_1) were obtained from the literature and are given in Tables 2 and 3, respectively. Molar volumes of the solvents were readily obtained from their molecular weights and densities at 20°C.

Partition Coefficient of Drug between Isopropyl Myristate (IPM) and PG–Water

IPM has been employed for many years as a model organic medium for SC lipids.²¹ Partition coefficients of ibuprofen between IPM and PG–water mixtures were therefore determined.

Table 2. Melting Point (T_m), Heat of Fusion (ΔH_m^f), Ideal Solubility (X_2^i), Molar Volume (V_2), and Solubility Parameter of Ibuprofen (δ_2)

T_m (K) ^a	ΔH_m^f (cal/mole) ^a	$\ln X_2^i$ ^b (25°)	V_2^c (cm ³ /mole)	δ_2^c (cal/cm ³) ^{1/2}
325.65	6586.9	1.12844	195.5	9.65

^aExperimentally determined by DSC.

^bCalculated from the equation, $-\ln X_2^i = (\Delta H_m^f)/RT[(T_m - T)/T_m]$.⁵

^cFrom Reference 19.

Table 3. Observed and Calculated Mole Fraction Solubilities of Ibuprofen in PG–Water Binary Mixtures at 20°C

Solvent Composition	δ_1^a (cal/cm ³) ^{1/2}	Solution Density g/cm ³	V_1 cm ³ /mole	A^b	W_{obs}	W_{calc}	X_2^{obs}	X_2^{calc}	l_{12}	Residual X_2^c
Water	23.4	1.000	18.02	0.14576	305.1	305.2	1.199E-05	1.264E-05	-0.35	-5.42
10:90	22.53	1.014	23.73	0.14575	286.0	285.8	1.985E-05	1.747E-05	-0.32	11.99
20:80	21.67	1.018	29.40	0.14574	267.6	267.6	3.070E-05	3.189E-05	-0.28	-3.87
30:70	20.81	1.026	35.02	0.14569	250.4	250.5	6.829E-05	7.188E-05	-0.25	-5.26
40:60	19.94	1.032	40.60	0.14554	234.3	234.3	1.791E-04	1.884E-04	-0.22	-5.18
50:50	19.08	1.039	46.13	0.14508	219.1	219.1	5.686E-04	5.471E-04	-0.19	3.77
60:40	18.22	1.039	51.63	0.14384	204.6	204.6	1.751E-03	1.692E-03	-0.16	3.35
70:30	17.35	1.037	57.08	0.14016	190.9	190.8	5.678E-03	5.506E-03	-0.14	3.03
80:20	16.49	1.027	62.48	0.12939	177.8	177.8	1.905E-02	1.911E-02	-0.12	-0.36
90:10	15.63	1.020	67.85	0.09831	165.3	165.4	6.966E-02	7.311E-02	-0.10	-4.95
PG	14.76	1.017	73.17	0.05036	153.6	153.6	2.079E-01	2.054E-01	-0.08	1.23

^aExperimental values from Reference 23.

^b $A = V_2 \cdot \phi_2^2/RT$.

^c% difference between X_2^{obs} and X_2^{calc} .

Experimentally, IPM and PG–water binary mixtures containing drug were stirred at 700 rpm during 96 h, at 20°C ($\pm 1^\circ\text{C}$). The two phases were presaturated with one another for 24 h prior to the partitioning experiment. It should be noted that IPM and PG are immiscible.

Viscosity of PG–Water Formulations

The dynamic viscosity (η) of PG–water binary mixtures and of PG–water mixtures saturated with ibuprofen was evaluated using a Hoeppler falling ball viscometer (Haake, Dreieich, Germany) at 20°C.

RESULTS AND DISCUSSION

Interpretation of Solubility Data

Solubilization can be described by various models from which potential solute–solvent, solute–solute, and solvent–solvent interactions may be highlighted. A simple approach to estimate a nonpolar drug's solubility in a mixture of water and a single cosolvent uses an algebraic mixing rule:²²

$$\ln X_2 = f \ln X_c + (1 - f) \ln X_w \quad (3)$$

where X_2 is the solute's mole fraction solubility in the cosolvent–water mixture, X_c the solubility in neat cosolvent, X_w the solubility in water, and f is the volume fraction of cosolvent. However, the behavior of lipophilic solutes in cosolvent–water mixtures frequently deviates from Eq. (3), and the solubility curve of ibuprofen in PG–water vehicles is an illustration (Fig. 1A). Re-arrangement of Eq. (3) predicts a linear relationship between $\ln(X_2/X_w)$ and f , but the results in Figure 1A show obvious deviation from this dependence, with measured solubilities being less than those predicted. Negative deviations from Eq. (3) imply that cosolvent–water interactions are such that the simple mixing rule is inadequate to explain the solubility behavior (Fig. 1B), an observation that has been made before.^{23,24}

The nonideality must originate either with the solvent or with the solute. In the former case, the nonideality would be independent of the solute and would cause the mixed solvent to be something other than a linear combination of its components. PG has both polar and nonpolar groups and it

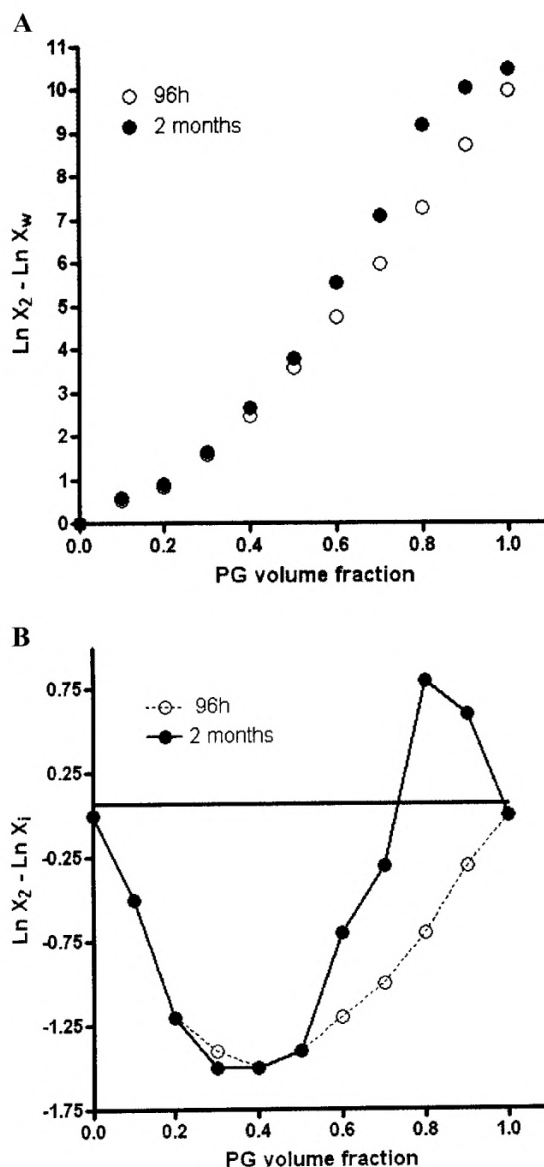


Figure 1. A: Solubility of ibuprofen in propylene glycol–water mixtures (mean values, $n=3$; standard deviations are too small to be visible) 4 days and 2 months post-preparation. B: Deviations of observed ibuprofen solubility (X_2) from ideality (X_1) in PG–water binary mixtures (mean values, $n=3$; standard deviations are too small to be visible) 4 days and 2 months post-preparation.

is expected that the hydrophobic effect operates around the nonpolar portions of the molecule, while hydrogen bonds are formed between polar groups and water. At low PG volume fraction,

the principal cosolvent interactions with water involve hydrogen-bonding via its polar OH-groups, resulting in water-structuring and reduced solvent-solute interactions, as demonstrated by published heat of solution data,²⁵ and a solute solubility that is lower than expected. As the PG volume fraction increases, hydrophobic interactions between the cosolvent become more important,²² relative to H-bonding with water^{26–28} and the solute is able to take greater advantage of the presence of the PG such that its solubility now increases proportionally. In the latter case, nonideality originates from specific interactions between the solute and either itself, or one or both of the solvent components. Deviations from ideality are most likely when the solute is present at its maximum solubility and/or when one of the solvent components is predominant.

As the ibuprofen formulations used in this work were saturated, the possibility of self-association, which is typical of carboxylic acids in organic media,²⁹ was considered. Partitioning of drug between IPM and PG–water mixtures was evaluated as a function of ibuprofen concentration in PG–water (Fig. 2), and was found to increase systematically.

The dashed vertical line in Figure 2 indicates, for each PG–water composition, by how much the partition coefficient increases when the drug

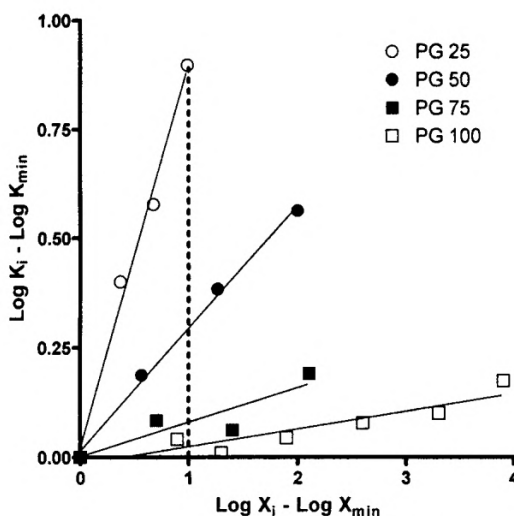


Figure 2. IPM/PG–water partition coefficients of ibuprofen as a function of the initial drug concentration in the PG–water formulation. $X_{\min}$ and $K_{\min}$ are the solubility and partition coefficients, respectively, of the lowest concentration vehicle.

concentration in the cosolvent mixture is increased by a factor of 10. The impact is clearly greatest for the 25:75 v/v PG–water composition and falls off with the increasing presence of PG; it is also noted that $K_{\text{IPM/water}}$ over the same concentration range is constant (data not shown). While the behavior found may be indicative of drug self-association in IPM, no direct evidence was found to support this conclusion,³⁰ and a complete explanation of the results in Figure 2 requires further investigation, for example, using infrared spectroscopy.

Ibuprofen solubility in PG–water systems can also be analyzed with the Hildebrand–Scatchard theory:³¹

$$-\ln X_2 = \frac{\Delta H_f}{R} \left(\frac{T_m - T}{T \cdot T_m} \right) + (\delta_1 - \delta_2)^2 \cdot \frac{V_2 \cdot \phi_1^2}{R \cdot T} \quad (4)$$

where X_2 is the mole fraction solubility of drug, ΔH_f is the heat of fusion, T is temperature, T_m the melting point, V_2 is the molar volume of the drug, R is the gas constant, δ_1 and δ_2 are the solubility parameters of the medium, into which drug is dissolved, and of the drug, respectively, and ϕ_1 is the volume fraction of the solvent. The closer the δ values, the greater is the mutual solubility of the pair. When $\delta_1 \approx \delta_2$, the cohesion forces in the solute and the solvent are the same (provided hydrogen bonding and other complicating interactions are not involved). The principal weakness of Eq. (4) is that the true cohesive energy density between solute and solvent ($\delta_1 \cdot \delta_2$) is not necessarily equal to the geometric mean of the individual solvent and solute values. Regular solution theory has therefore been extended to semi-polar drugs in pure solvents and in polar binary solvents mixtures,^{32–34} and an empirical coefficient l_{12} has been introduced:

$$\begin{aligned} -\ln X_2 = & \frac{\Delta H_f}{R} \left(\frac{T_m - T}{T \cdot T_m} \right) + [\delta_1^2 + \delta_2^2 \\ & + 2\delta_1 \cdot \delta_2(1 - l_{12})] \frac{V_2 \cdot \phi_1^2}{R \cdot T} \\ = & -\ln X_2^i \\ & + [(\delta_1 - \delta_2)^2 - 2 \cdot W_{\text{calc}}] \frac{V_2 \cdot \phi_1^2}{R \cdot T} \end{aligned} \quad (5)$$

where X_2^i is the ideal solubility of the solute expressed in mole fraction and W_{calc} is the solute–solvent interaction energy. From experimental measurements of X_2 , the interaction energies can be determined and regressed against δ_1 in a power series to back-calculate W_{calc} . For ibuprofen in

PG–water:

$$W_{\text{calc}} = 59.72 - 4.12(\delta_1) + 1.11(\delta_1)^2 - 0.038(\delta_1)^3 + 0.0007(\delta_1)^4 \quad (6)$$

Substitution of W_{calc} into Eq. (5) enables a theoretical mole fraction solubility ($X_{2\text{calc}}$) to be calculated and compared to the experimental values (Tab. 3).

Figure 3 shows the measured mole fraction solubilities of ibuprofen as a function of volume fraction of PG in PG–water binary mixtures. The deviation from regular solution theory is clear. In contrast, the extended Hildebrand approach reproduces the solubility of ibuprofen in the solvent mixtures very well. As seen in Table 3, $l_{12} < 0$; that is, solute–solvent association occurs, and a high cohesive energy exists. Thus, for ibuprofen in PG–water mixtures, solubility is always less than ideal but greater than that predicted by regular solution theory. Further, it appears that solutes may adapt their solubility parameters to the solvent environment in which they are found.^{29,35,36}

Finally, measurements of ibuprofen solubility were repeated but, in this case, excess solid drug was equilibrated with solvent for 2 months rather

than 4 days. While no differences were observed for PG–water mixtures with 50% or less PG, those with higher cosolvent levels showed significantly enhanced solubilities (Fig. 1). At 60:40 v/v PG–water, solubility was doubled; for 70:30 and 90:10, the increase was threefold; and, at 80:20, solubility was augmented by a factor of 6. Attempts to physically provoke crystallization in these systems were unsuccessful. It is not clear whether this means that the solutions at 4 days were not fully saturated or that, after 2 months, supersaturation had been achieved. Despite a faint discoloration of the solutions stored for 2 months, neither HPLC nor DSC was able to show any evidence of solute degradation; the DSC thermogram obtained for the undissolved solid after 2 months was identical to that of the original material. It is apparent that the determinants of ibuprofen solubilization in different PG–water mixtures are not straightforward, and that the drug's interactions with itself, and with the cosolvents, are complex.

Dermatopharmacokinetics (DPK)

Figure 4 compares the concentration profiles of ibuprofen across the SC of human volunteers following delivery of the drug from four saturated formulations comprising different PG–water ratios. The ibuprofen concentrations are absolute having been determined by HPLC analysis of the extracted tape-strips removed after a 30 min application of the drug. It is first noteworthy that inter-subject variability (coefficient of variation = 10–20%) is quite low, attesting to a robust methodology. Second, despite the fact that the drug was present at the same thermodynamic activity (i.e., was saturated) in each formulation, the vehicle clearly influenced delivery into the SC: the greater the volume fraction of PG, the higher the amount of drug taken up into the skin. For the 25:75 v/v PG–water formulation, drug levels in the SC were too low to obtain an accurate concentration profile. Third, when the concentration profiles were fitted to Eq. (1), values of the SC-vehicle partition coefficient (K) and the kinetic parameter (D/L^2) were obtained, and were used with Eq. (2) to determine AUC_{xx} (see Tab. 4).

One-way ANOVA of the diffusion parameters (D/L^2) in Table 4 reveals no statistical difference ($p > 0.05$) between formulations. Given that the measured apparent SC thicknesses of the volunteers used in this work were quite similar (the mean values for cohorts studied were either

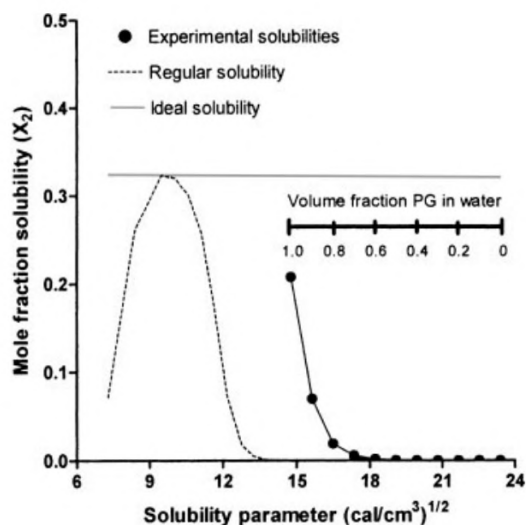


Figure 3. Experimental mole fraction solubilities of ibuprofen (filled circles) in pure PG and binary PG–water solvents (mean values, $n = 3$). The regular solution (—) and extended Hildebrand predictions (---) at 20°C are shown for comparison.

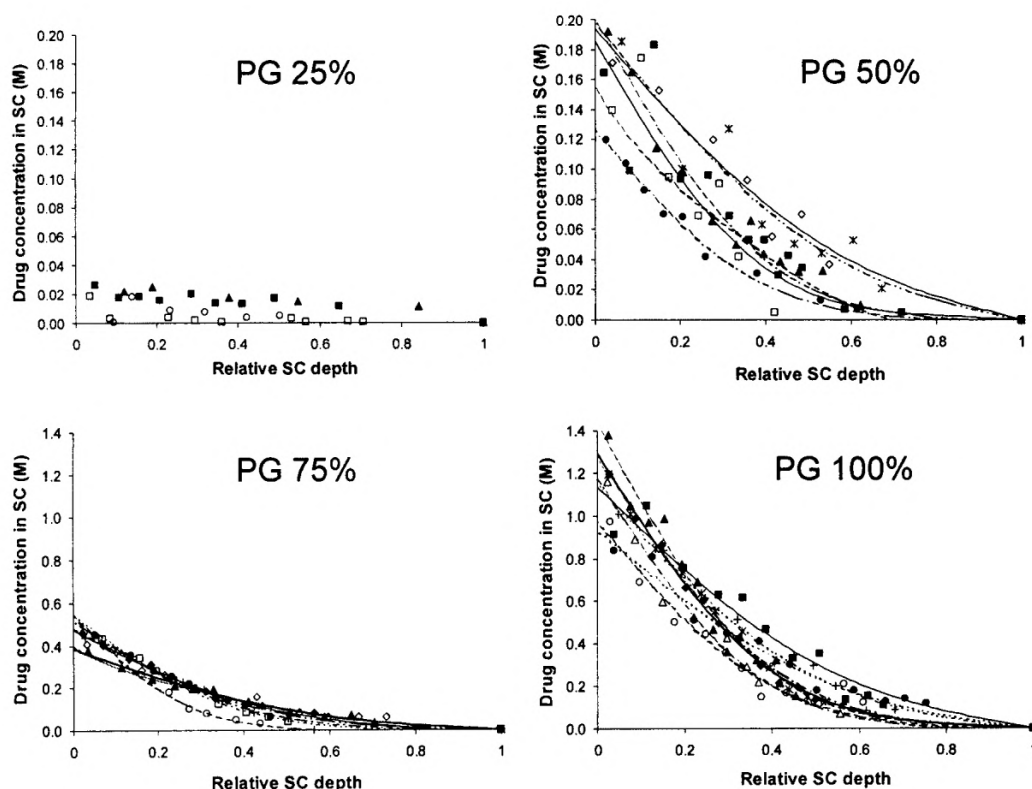


Figure 4. *In vivo* human SC concentration versus relative depth profiles of ibuprofen following 30 min treatment with different PG–water formulations. The lines drawn through the data represent the best fits of Eq. (1) to each set of results.

12 or 13 μm with standard deviations of not more than $\pm 4 \mu\text{m}$), it can be concluded that PG does not influence the diffusivity of ibuprofen across the SC. The observed differences in drug delivery must

be due, therefore, to differences in partitioning. The SC-vehicle partition coefficient may be defined as the ratio of the drug's solubility in the SC to that in the formulation: $K = C_{\text{SC}}^{\text{sat}}/C_{\text{v}}^{\text{sat}}$. This definition

Table 4. Partitioning and Diffusivity Parameters, and Calculated AUC_{xx} (Eqs. (1) and (2)) and $C_{\text{SC}}^{\text{sat}}$ Values, Describing Ibuprofen Uptake into SC Following a 30 min Application of Various PG–Water Vehicles *In Vivo* in Human Volunteers (Mean $\pm$ SD, $n = 7$ –9)

Formulation PG: Water (v/v)	$K^{a,b}$	$D/L^{2a,c}$ (h^{-1})	$\text{AUC}_{\text{xx}}^{b,d}$ (M)	$K * C_{\text{v}}^{\text{sat}} = C_{\text{SC}}^{\text{sat}}$ (mg/mL)
0:100	— ^e	— ^e	— ^e	— ^e
25:75	98 ± 5^f	0.12 ± 0.05^f	0.04 ± 0.01	$29.4 (25.1\text{--}34.0)^g$
50:50	14 ± 2	0.16 ± 0.07	0.05 ± 0.01	$35.8 (30.4\text{--}41.4)^g$
75:25	2.8 ± 0.3	0.12 ± 0.05	0.13 ± 0.02	$100 (87.5\text{--}113)^g$
100:0	0.56 ± 0.07	0.13 ± 0.06	0.33 ± 0.07	$241 (204\text{--}279)^g$

^aValues from the best-fits of Eq. (1) to the results in Figure 4.

^bValues statistically different ($p < 0.05$, unpaired *t*-test).

^cANOVA reveals no significant differences between any of the D/L^2 values.

^dDetermined from Eq. (2) using the corresponding fitted D/L^2 and K for each subject.

^eNot determined.

^fResults from an experiment using a modified drug application procedure (see text and Fig. 6).

^gCalculated from the mean values of K in this table and those of $C_{\text{v}}^{\text{sat}}$ in Table 1; the ranges in parentheses were determined using the $\pm$ SD and $-\text{SD}$ values for these two parameters.

clearly predicts, under ideal circumstances, that $K * C_v^{\text{sat}}$ should be a constant ($= C_{\text{SC}}^{\text{sat}}$), irrespective of the vehicle. However, as shown in Table 4, this is not the case, and the values of $K * C_v^{\text{sat}}$ follow a trend similar to that of AUC_{xx} . Following Hildebrand, K depends mainly on the drug's solubility parameter and on those of the SC and vehicle. However, *a priori* estimation of K is difficult because the molar volume of the SC, and the drug–SC interaction energy, are unknown. Further, although the solubility parameter of the SC has been estimated,² the manner in which it may be modified by the penetration of solvents into the barrier cannot be quantitatively predicted (i.e., K can only be estimated if the SC is unmodified by the formulation and if the SC–drug interaction is small).

The conclusion, therefore, is that PG itself penetrates into the SC and increases its solubility parameter. While no direct measurements have been made to confirm this suggestion, PG flux across human skin *in vitro* is substantial ($50\text{--}150 \mu\text{g}\cdot\text{cm}^{-2}\cdot\text{h}^{-1}$)¹³ and that—at least, at steady-state—a significant presence of the cosolvent in the SC can be anticipated. In addition, as the experiments described here involved occlusive application of the formulations tested, an elevated hydration of the SC was achieved (and confirmed by TEWL measurements—data not shown), again contributing to an increase in δ_{SC} .

The trend in the values of $K * C_v^{\text{sat}}$ as a function of the PG–water composition of the vehicle may also be predicated by the experimental design. The volume of the formulation applied to the treated skin area (1.9 mL over 5 cm^2) is approximately 400-fold greater than the volume of the SC ($\sim 0.005 \text{ mL}$ assuming an SC thickness of $10 \mu\text{m}$). It is possible, therefore, that the SC can become saturated with drug, especially for those vehicles in which the drug concentration is high. In other words, the SC-vehicle partition coefficient of ibuprofen will no longer be independent of the drug's concentration when this value becomes very high, as is the case, for example, in 100% PG. To test this hypothesis, ibuprofen solutions in pure PG were prepared at concentrations of 50, 100, and $200 \mu\text{g/mL}$ and were applied to the forearms of volunteers for 3 h (i.e., for a period sufficient to establish an almost steady-state concentration profile across the SC). The partition coefficients derived from the profiles were compared to those measured following application of the saturated drug formulation (Fig. 5). The results clearly demonstrate that

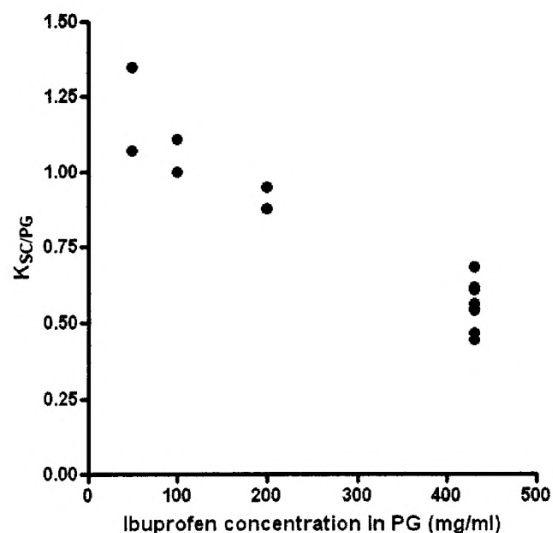


Figure 5. Individual SC/PG partition coefficients measured *in vivo* as a function of ibuprofen concentration in the cosolvent.

$K_{\text{SC/PG}}$ is not independent of drug concentration in the vehicle.

The extent of ibuprofen absorption (AUC_{xx}) was calculated for the formulations containing 50% v/v PG or more using the fitted K and D/L^2 parameters and Eq. (2) (see Tab. 4). For vehicles in which the volume fraction of PG was less than 50%, the fitting procedure was unreliable because the amounts of drug extractable from each strip were below the limit of quantification. As a consequence the strips were combined to facilitate the analysis and to allow an experimental AUC_{xx} to be determined. Figure 6 compares the measured AUC_{xx} values with a linear extrapolation of those determined for the vehicles containing $\geq 50\%$ PG, and a clear difference is observed. It may be hypothesized that the low AUC_{xx} value at 25% v/v PG is due to drug depletion at the SC-vehicle interface. However, this seemed unlikely as the predicted curve in Figure 6 implies that less than 1% of the applied drug would be taken up in the 30 min application period. Nevertheless, because the administration procedure involved saturating a gauze pad with the vehicle, the possibility existed that this matrix might impede the rapid replenishment of drug at the SC surface. A further experiment was therefore performed in which the 25:75 v/v PG–water formulation was applied for 30 min via a small plastic chamber. The liquid vehicle was brought

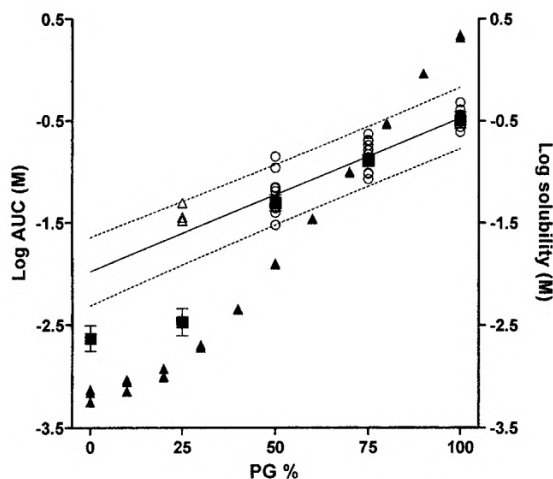


Figure 6. Mean (filled squares) and individual (open circles) AUC_{xx} values determined for various PG–water formulations saturated with ibuprofen. The linear regression shown (solid line) and 95% prediction interval (dashed lines) is based on the individual AUC_{xx} values obtained for the 50:50, 75:25, and 100:0 PG–water formulations. Drug solubility as a function of PG–water composition is shown for comparison (filled triangles; values determined at 4 days); ($n=3$). The AUC_{xx} values depicted as open triangles at 25:75 v/v PG–water were measured when the formulation was administered in the absence of a gauze supporting matrix (see Fig. 7 and text for details).

into contact with the skin, as a result, without the need for a supporting “platform” with which the drug could potentially associate. The resulting SC concentration-depth profiles are in Figure 7;

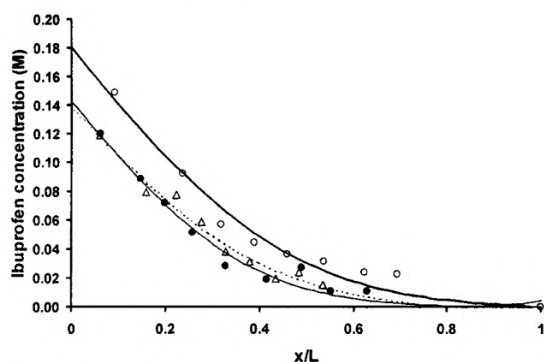


Figure 7. Ibuprofen concentration profiles across the SC *in vivo* following a 30 min application, in the absence of a supporting gauze matrix, of a 25:75 v/v PG–water formulation saturated with drug ($n=3$).

the mean, fitted D/L^2 value ($0.12 \pm 0.05 \text{ h}^{-1}$) is indistinguishable from those in Table 4 for the other PG–water formulations, while K (98 ± 5) was significantly higher than that for the 50:50 v/v PG–water vehicle as expected. The AUC_{xx} , furthermore, was higher than that determined experimentally using the conventional application protocol, with the individual values now falling within the 95% prediction interval predicted by the linear extrapolation of the data from the vehicles with $\geq 50\%$ PG (Fig. 6). The latter suggests that the impact of the gauze pad is saturable and that this matrix has not significantly affected the results for PG–water formulations containing 50% v/v or more PG.

The precise mechanism of action by which PG facilitates drug permeation across the skin has been debated in the literature and studied by a variety of techniques.^{8–13} Under ideal circumstances, the AUC_{xx} at steady-state is given by:

$$AUC_{xx} = \frac{1}{2} \cdot K \cdot C_v = \frac{1}{2} \cdot \frac{C_{SC}^{sat}}{C_v^{sat}} \cdot C_v \quad (7)$$

It follows that, if the formulation is saturated with drug, $C_v = C_v^{sat}$ and $AUC_{xx} = 1/2 \cdot C_{SC}^{sat}$. Hence, if AUC_{xx} increases with changes in the amount of PG in the vehicle (as is observed here), then either the formulation must be altering the drug's solubility in the SC (C_{SC}^{sat}) or the vehicles were not, in fact, equally saturated; that is, supersaturation occurred in the formulations containing higher amounts of PG. Relevant to this point, it has been reported³⁷ that the cosolvent may retard the crystallization process because it increases the viscosity of the formulation, and this was indeed the case for the PG–water vehicles considered in this work (Fig. 8). However, when a deliberately twofold supersaturated solution of drug in 75:25 v/v PG–water was prepared³⁸ and evaluated *in vivo*, its performance was entirely consistent with the results from the conventionally saturated vehicles: K and D/L^2 were essentially identical while AUC_{xx} was approximately doubled (Tab. 5). The data support, therefore, the first explanation, that is, that PG increases C_{SC}^{sat} , an idea explored and argued for in a recent publication,¹² which showed that the cosolvent displaced water from its binding sites within the SC. Taken together, it is evident that the role of PG is complex and that direct measurement of its distribution across the SC following application of different vehicles is required to fully unravel the behavior observed.

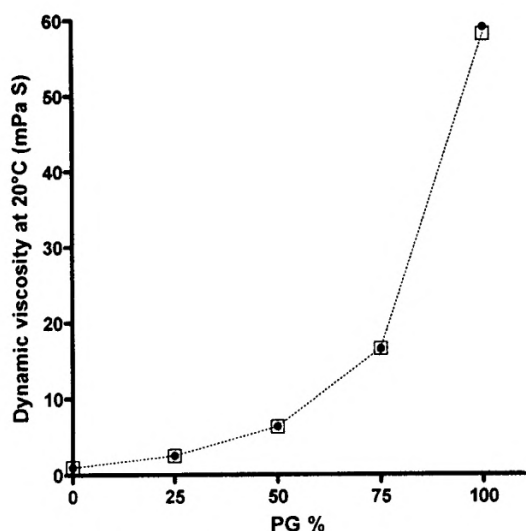


Figure 8. Dynamic viscosity of various PG–water binary mixtures (filled circles) and of the corresponding formulations saturated with ibuprofen (open squares) (mean values, $n = 3$).

As a final point, it is evident that the partitioning and diffusion parameters in Table 4, together with the corresponding values of SC thickness, can be used to calculate the permeability coefficient ($K_p = K \cdot (D/L^2) \cdot L$) and steady-state flux ($J_{ss} = K_p \cdot C_v$) of ibuprofen from each formulation. Given the discussion above, and the results in Table 4, it is not surprising that K_p and J_{ss} track, respectively, the values of K and $K \cdot C_v^{sat}$ (and hence AUC_{xx}); that is, J_{ss} increases, with increasing levels of PG in the formulation (Fig. 9). The predicted steady-state fluxes range from 7 to 44 $\mu\text{g}/\text{cm}^2/\text{h}$, corresponding to 0.14–0.88 mg/h for a 20 cm^2 “patch.” This estimated delivery is at least

of the same order of magnitude as that reported in an *in vivo* study reported in the literature³⁹ where 4.5 mg of ibuprofen and its metabolites were recovered in urine following a 24 h application of 16 g of a 5% w/v gel to the thigh (area of application = 323 cm^2). As another investigation demonstrated similar clinical efficacy between administration of 4 g of a similar gel to the forearm (area not specified; continuous application) and oral delivery of 800 mg of drug,⁴⁰ it can be concluded that the formulations tested in this work would provoke a measurable pharmacological effect *in vivo*. Of course, the attainment and maintenance of steady-state transport in practice is difficult to achieve for many reasons and the actual performance of the formulations in the “real world” would need more rigorous evaluation.

CONCLUSIONS

This study demonstrates that the bioavailability of a model compound—ibuprofen—in the SC can be easily evaluated *in vivo* in humans by tape-stripping. This simple technique, combined with the appropriate analysis of the experimental data, provides kinetic and thermodynamic parameters needed to design rationally a topical formulation. Further, the role of PG, a common cosolvent in topical formulations of moderately lipophilic drugs, has been examined carefully. PG enters the SC in a manner proportional to its level in the applied formulation, and appears to alter ibuprofen’s solubility in the barrier. On the other hand, PG does not affect the diffusivity of the drug across the SC. Quantitative characterization of the uptake of PG itself into the SC from different

Table 5. Partitioning and Diffusivity Parameters, and Calculated AUC_{xx} (Eqs. (1) and (2)), Describing ibuprofen Uptake into SC Following Application of a Saturated and Twofold Supersaturated 75:25 v/v PG–Water Vehicle *In Vivo* in Human Volunteers (Mean $\pm$ SD, $n = 7$ –9)

Degree of Saturation	Application Time (h)	K^a	D/L^2 ^{a,b} (h^{-1})	AUC_{xx}^c (M)
1	0.5	2.8 ± 0.3^d	0.12 ± 0.05	0.13 ± 0.02
	3	3.0 ± 0.3^e	—	0.25 ± 0.04
2	0.5	2.4 ± 0.4^d	0.19 ± 0.09	0.26 ± 0.05
	3	3.1 ± 0.4^e	—	0.49 ± 0.07

^aValues from the best-fits of Eq. (1) to the results in Figure 4.

^bANOVA reveals no significant differences between the D/L^2 values.

^cDetermined from Eq. (2) using the corresponding fitted D/L^2 and K for each subject.

^dValues not statistically different ($p > 0.05$, unpaired t -test).

^eValues not statistically different ($p > 0.05$, unpaired t -test).

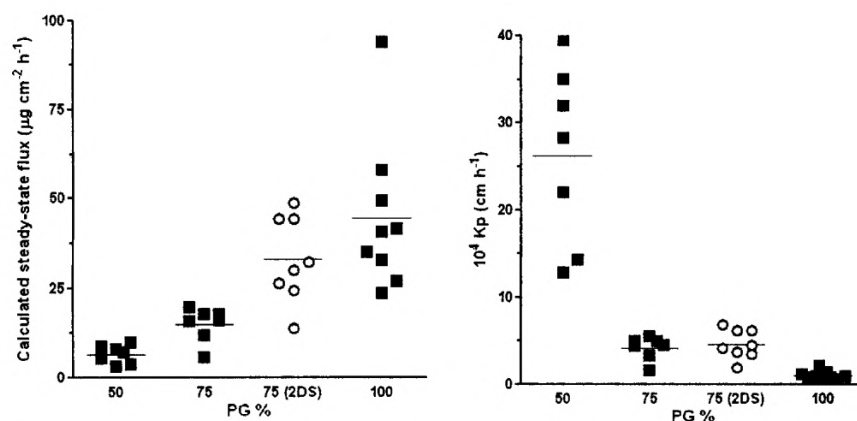


Figure 9. Steady-state fluxes (J_{ss} , left panel) and permeability coefficients (K_p , right panel) of ibuprofen deduced from DPK transport parameters obtained following a 30 min application *in vivo* of various PG–water formulations containing the drug at saturation (averages are indicated by the horizontal lines; $n = 7-9$). ANOVA indicates that, while J_{ss} from the 50% and 75% PG formulations are statistically indistinguishable ($p > 0.05$), they are both significantly different to that from 100% PG ($p < 0.01$). For K_p , the value from 50% PG differs significantly from those at 75% and 100% ($p < 0.001$); the two latter results, on the other hand, are not different ($p > 0.05$).

vehicles represents a logical research effort for future investigation.

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