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Research paper

Dermatopharmacokinetics of betamethasone 17-valerate: Influence of formulation viscosity and skin surface cleaning procedure

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ABSTRACT

The objective was to compare the *in vivo* distribution profiles of betamethasone 17-valerate (BMV) across the stratum corneum (SC) following (a) delivery from gelled and un-gelled formulations, and (b) two different skin cleaning procedures at the end of the application period. BMV was dissolved in gelled and un-gelled vehicles comprising either medium chain triglycerides (MCT) or a brand microemulsion (ME). The BMV concentration was adjusted to 80% of saturation and applied to the forearms of healthy volunteers. After 2 h, the treated skin site was cleaned either with a dry paper towel or with an isopropyl alcohol swab, and the SC was then progressively removed by repeated adhesive tape-stripping. BMV distribution profiles across the SC showed reasonable reproducibility, and that delivery from the ME was significantly superior to that from MCT. Gelled vehicles were less efficiently removed from the skin surface by dry wiping than un-gelled formulations. Removing excess formulation more aggressively with isopropyl alcohol resulted in a lower apparent uptake of drug into the SC. Excess gelled formulation may be trapped in the skin 'furrows', and requires an efficient skin cleaning procedure to ensure its complete removal.

1. Introduction

The tape-stripping, or dermatopharmacokinetic (DPK), method is attracting increasing attention as a method with which to assess the rate and extent of topical drug bioavailability in the stratum corneum (SC) [1–3]. This approach, first described by the U.S. Food and Drug Administration (FDA), has been proposed for the bioequivalence testing of topically applied drugs [4,5]. In 2002, however, the Draft Guidance was withdrawn because of doubts regarding the reproducibility between laboratories [6] and, currently, the method is being re-evaluated and improved.

The DPK concept involves determination of the amount of drug present in the SC as a function of time post-application and post-removal of the formulation under examination. A key experimental requirement before this evaluation is made is to ensure that the residual formulation on the skin surface is completely removed before tape-stripping is commenced. The cleaning procedure must be efficient, but not so aggressive as to 'drive' material into the barrier nor to extract it therefrom. Clearly, different types of formulation pose different levels of challenge: for example, it might be anticipated that a simple lotion will be easier to clean off than an oleaginous ointment. The SC surface is not flat; it possesses macroscopic furrows, which run parallel to the surface of the skin [7]. It has been argued that the quantified amount of drug in the SC tape-strips will include the portion deposited in 'furrows' as well as that which has penetrated into the SC. The putative excess formulation in the 'furrows' may complicate the interpretation of data obtained after tape-stripping.

The aim of this study, therefore, was to investigate whether the cleaning procedure of the treated skin site before stripping altered the DPK profile of a typical topical drug (betamethasone 17-valerate) applied in two different formulations. Specifically, cleaning the surface with a dry paper towel (as described by the FDA) was compared to wiping with an isopropyl alcohol swab. In addition, the SC concentration profiles of the same drug, resulting from the administration of the same formulations, which were either applied as liquids, or as semi-solids following gelling with an appropriate polymeric excipient, were evaluated.

2. Materials and methods

2.1. Materials

Betamethasone 17-valerate (BMV) (Crystal Pharma, Boecillo, Spain) was dissolved either in the reference vehicle consisting of medium chain triglycerides (MCT) (Mygliol 812N, Synopharm, Barsbüttel, Germany) or in the microemulsion Mikro 100® (ME) (Sebapharma, Boppard, Germany). When appropriate, MCT and ME were gelled with 15% (w/w) polypropylene, and 10% (w/w)

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Aerosil 200® (Sigma–Aldrich, Steinheim, Germany), respectively. The BMV concentration was adjusted to 80% of the saturation level $C_{s,V}$ (i.e., to a constant thermodynamic activity), equivalent to 1.7 mg/ml in MCT and 9.3 mg/ml in ME. The saturation level was determined by stirring a suspension of BMV in the liquid vehicles until equilibrium, followed by centrifugation and analysis of the drug in the resulting supernatant [8].

2.2. Tape-stripping procedure

Six healthy Caucasian volunteers, 4 females, 2 males (23–41 years of age), with no history of dermatological disease, participated in the study, which was approved by the Local Research Ethics Committee (Salisbury Research Ethics Committee, Wiltshire, UK). Informed consent was obtained from each subject.

A single infinite dose of 600 μl of the gelled formulations was applied using a 1.8 cm diameter Hill Top Chamber® (Hill Top Research, Cincinnati, OH, USA) on the forearm, at least 4 cm from either the wrist or the bend of the elbow. In a first series of experiments, after an application time of 2 or 6 h, the SC at the treated skin site was cleaned with a dry paper towel. Then, the SC was progressively removed by repeated adhesive tape-stripping (Scotch Book Tape, 3M, St. Paul, MN, USA). In a second set of studies, the same formulations were applied and the same procedure was adopted, except that the skin surface cleaning involved first wiping with a dry paper towel followed by twice wiping a 70% (v/v) isopropyl alcohol swab (Sterets swab, Seton Healthcare, Oldham, UK) across the treated skin site. In a third protocol, the MCT and ME formulations were applied as un-gelled liquids. A foam tape (3M, St. Paul, MN, USA), into which a 2.0 cm diameter hole had been cut, acted as a template to constrain the vehicles, and the system was covered by an occlusive tape (Blenderm™, 3M, Neuss, Germany) to prevent any loss. After a 2-h application, the template was removed and excess formulation was cleaned away with a dry paper towel.

Briefly, the tape-stripping procedure involved the following steps. A piece of polypropylene foil, into which a predefined hole had been cut, was placed onto the cleaned, treated skin site and affixed by a piece of self-adhesive tape. This template ensured that all tape-strips were removed from the same site. The tape (2.5 × 2.5 cm) was applied to this template, pressed down with a constant pressure (140 g/cm^2) using a weighted roller (6 cm width) and then removed. Up to 20 strips were taken from each treated site, such that the SC was never completely removed. To check skin barrier function, transepidermal water loss (TEWL) measurements were performed (AquaFlux V4.7, Biox Systems Ltd., London, UK) during the stripping procedure, which was stopped if TEWL reached 60 g/m^2 h. Each tape was carefully weighed before and after stripping on a 10-μg precision balance (Mettler AT 261, Greifensee, Switzerland) to determine the mass and thickness of the SC layer removed [9]. BMV in the tape-strips was subsequently extracted quantitatively and analyzed by high-performance liquid chromatography (HPLC) (see method below). The amount of BMV on each strip could then be converted to a concentration within that removed layer of the SC.

To calculate the total thickness of the SC, the same tape-stripping procedure was performed at an adjacent, untreated skin area with periodic measurements of TEWL after each tape-strip [9]. Pre-weighed tapes were used, which were re-weighed after a SC layer had been detached to assess the amount of SC removed. From this mass, and knowing the stripping area and the density of the SC [10], it was possible to calculate the total thickness of the SC from the x-axis intercept of a graph of 1/TEWL versus the cumulative thickness of the SC removed. In this way, the drug concentration profile across the SC could be displayed in a consistent fashion for all subjects, as a function of relative position (or depth) into the barrier [11,12].

2.3. Extraction and HPLC analysis of BMV

Each tape was placed into a 2 ml vial, and was extracted with 1.0 ml of 60:40 (v/v) acetonitrile/water by shaking overnight. Validation of the extraction process involved spiking tape-stripped samples of untreated SC with 20 μl of a solution of known drug concentration. Drug recovery was 96.9% (±3.4) (n = 5). BMV in the various samples was quantified by HPLC (Dionex, Sunnyvale, CA, USA) with UV detection at 240 nm using a Lichrospher® 100 RP-18 column (4 × 125 mm) (Hichrom, Reading, UK). The mobile phase was degassed acetonitrile/water (60:40 v/v) delivered at a flow rate of 1 ml/min in a 50-μl sample loop. The retention time of BMV at 25 °C was ~3.8 min. A calibration curve was generated using solutions of the neat compound at five different concentrations. The detection limit was 0.03 μg/ml. The amount of BMV recovered from the tape-strips was expressed in terms of amount of drug per unit SC volume (mg/cm^3).

2.4. Analysis of the SC distribution profile

The SC concentration (C_x) versus normalized depth (x/L) profiles of BMV were fitted to the appropriate solution of Fick's second law of diffusion [13]:

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

The boundary conditions necessary are: (i) the SC contains no drug at t = 0, (ii) the drug concentration at the skin surface is constant (infinite dose conditions), and (iii) the viable epidermis at the lower surface of the SC provides perfect sink conditions for the drug. C_V is the BMV concentration in the vehicle. From the best fit of the experimental values to Eq. (1), the SC-vehicle partition coefficient (K) and the diffusivity parameter (D/L^2) across the SC of path-length L were obtained. Subsequently, using the derived parameters, K and D/L^2, Eq. (1) was integrated across the SC thickness (i.e., from x/L = 0 to x/L = 1) to yield the amount of drug per area per unit of SC thickness (Q):

$$Q = \int_0^1 C_x d\left(\frac{x}{L}\right) = KC_V\left[\frac{1}{2} - \frac{4}{\pi^2}\sum_{n=0}^{\infty}\frac{1}{(2n+1)^2}\exp\left(-\frac{D}{L^2}(2n+1)^2\pi^2 t\right)\right] \quad (2)$$

The derived values of Q were used to compare the relative bioavailability of BMV from the two vehicles, and from their quotient (Q_{ME}/Q_{MCT}) a penetration enhancement factor (EF) was calculated.

2.5. Statistics

Statistical differences were assessed by two-tailed, Student's t-test and by ANOVA, followed by a Bonferroni's multiple comparison test, using GraphPad Prism 4.01 software (San Diego, CA, USA).

3. Results and discussion

Several factors, such as skin hydration, vehicle composition, cohesion between corneocytes, and inter-individual differences in total SC thickness [11,14–16], can influence the amount of SC that is removed by a single tape-strip. It follows that this quantity is not linearly proportional to the number of tape-strips removed and that normalization of the SC thickness removed is a prerequisite to facilitate comparison between subjects and between formulations.

The individual SC distribution profiles of BMV after a 2-h application time of the gelled MCT and ME formulations are in Fig. 1.

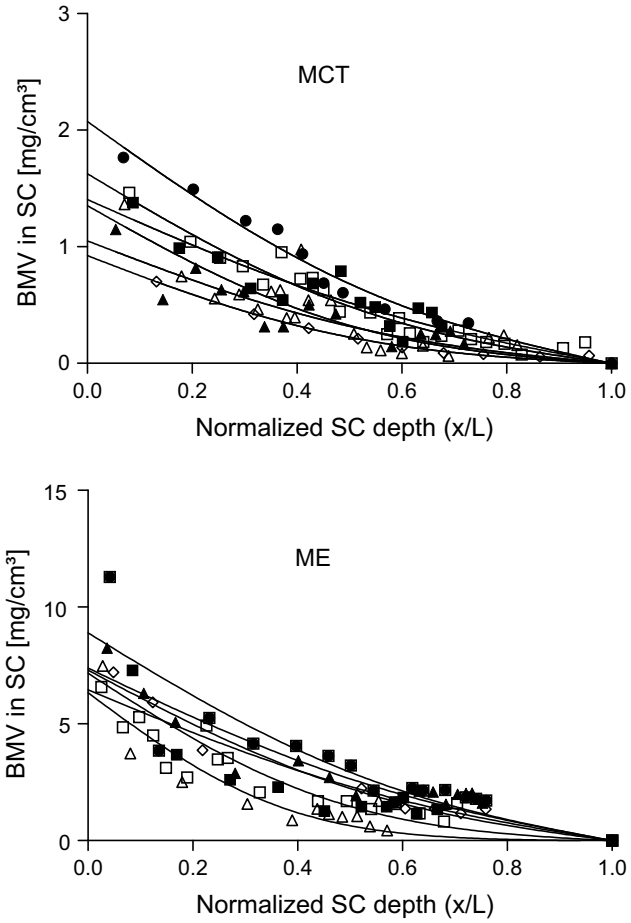

Fig. 1. SC distribution profiles of BMV delivered from gelled MCT and ME formulations following a 2-h application. The skin surface was cleaned by wiping with a dry paper towel. The best fits of Eq. (1) to the individual experimental data points are shown ($n = 6$).

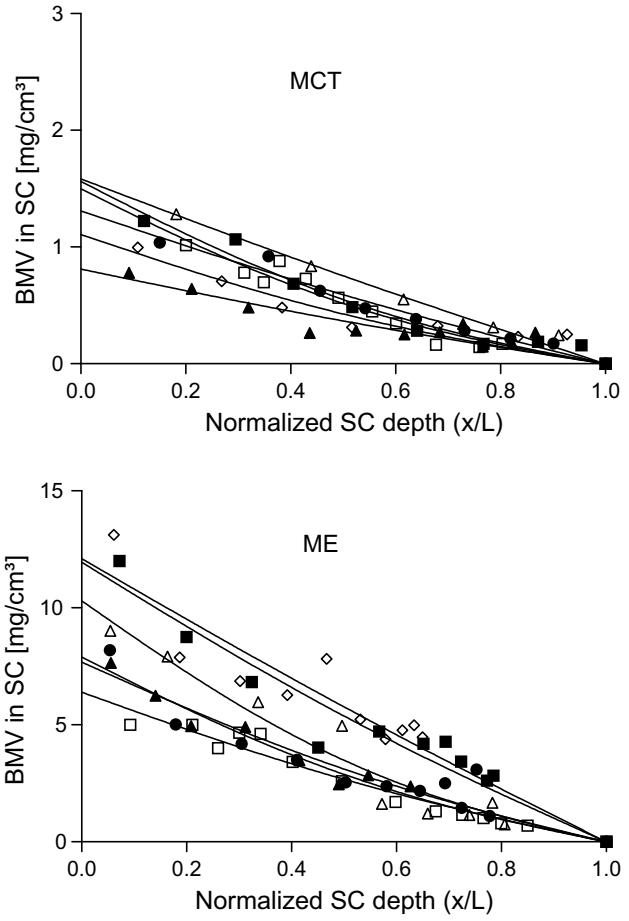

Fig. 2. SC distribution profiles of BMV delivered from gelled MCT and ME formulations following a 6-h application. The skin surface was cleaned by wiping with a dry paper towel. The best fits of Eq. (1) to the individual experimental data points are shown ($n = 6$).

The ME clearly and significantly (two-tailed t-test, $P < 0.05$) increased the extent of drug delivery into the SC, relative to that achieved from the MCT vehicle, as reflected in the Q values observed: 2.61 (±0.67) and 0.51 (±0.18) mg/cm^3 for ME and MCT, respectively, corresponding to an EF of 5.6 ± 2.2.

When the same formulations were administered for a longer period of 6 h, BMV from both MCT and ME penetrated further into the SC and the profiles became more linear (indicating the approach to steady-state transport conditions) (Fig. 2).

Fitting the data to Eq. (1) allowed values of the partitioning (K) and diffusivity (D/L^2) parameters to be derived from the experiments performed after drug application times of 2 and 6 h. These results and the Q values, which were determined as described above, are collected in Table 1. It was also possible, using the K and D/L^2 parameters from the 2-h experiments to predict, using Eq. (2), the values of Q anticipated following a longer application period of 6 h. These calculations are also in Table 1.

There were no significant differences (ANOVA, $P > 0.05$) between the deduced values of either K or D/L^2 for MCT and ME following either 2 or 6 h of BMV delivery. However, there was a significant enhancement of drug uptake into the SC from ME relative to MCT: EF values were 5.6 (±2.2) and 7.6 (±2.9) at 2 and 6 h, respectively. This was the result (see Table 1) of a significantly increased apparent saturation level of BMV in the SC caused, presumably, by the concomitant penetration of ME constituents into the barrier. This phenomenon has been reported previously for ibuprofen delivered from vehicles containing different proportions of propylene glycol and water [17,18]. The predicted values of Q at 6 h based on the derived values of K and D/L^2 from the 2-h experiments were in good agreement with the experimental findings for BMV uptake from both formulations. This confirms the potential of the technique, as has been proposed [19,20], to reduce the number of measurements necessary, for example, to establish bioequivalence between different drug products.

Table 1
Partitioning (K) and diffusivity (D/L^2) parameters, as well as experimental and predicted amounts of drug per area per unit of SC thickness (Q_{expt} and Q_{pred}, respectively), and calculated saturation levels of BMV in the SC ($C_{s,SC}$), from the drug concentration profiles following application of gelled MCT and ME formulations to human volunteers (mean ± SD, $n = 6$).

Formulation	MCT	MCT	ME	ME
Application time [h]	2	6	2	6
K[a]	0.83 ± 0.24	0.77 ± 0.18	0.78 ± 0.10	1.01 ± 0.26
D/L^2 [h^{-1}][a]	0.058 ± 0.013	0.037 ± 0.013	0.056 ± 0.021	0.036 ± 0.012
$C_{s,SC}$ [mg/ml][b]	1.7 ± 0.5	1.6 ± 0.4	9.1 ± 1.2	11.8 ± 3.0
Q_{expt} [mg/cm^3][c]	0.51 ± 0.18	0.58 ± 0.14	2.61 ± 0.67	4.18 ± 1.27
Q_{pred} [mg/cm^3][d]	–	0.68 ± 0.21	–	3.38 ± 0.65

[a] Deduced from the fit of Eq. (1) to the experimental distribution profiles.
[b] $C_{s,SC} = K \cdot C_{s,V}$.
[c] Determined from Eq. (2) with the deduced values of K and D/L^2.
[d] Predicted from Eq. (2) using the values of K and D/L^2 deduced from the 2-h data.

The results from the second set of experiments, in which the skin was more aggressively cleaned with an isopropyl alcohol swab, following a 2-h application of the gelled MCT and ME formulations, are in Fig. 3. Similarly, Fig. 4 presents the SC uptake profiles of BMV, when applied as un-gelled (liquid) formulations in the third series of studies; in this case, skin surface cleaning at the end of the administration period was accomplished with a dry paper towel. Analysis of these two sets of results using Eqs. (1) and (2) generated the additional information (partitioning and diffusivity parameters, Q values, saturation levels in the SC and EFs) collected in Table 2.

It is immediately apparent that an isopropyl alcohol wipe of the skin surface alters the extent of apparent BMV delivery into the SC. Values of Q were significantly reduced with respect to the values reported in Table 1. This was manifested in an approximately 50% reduction in the SC-vehicle partition coefficients of the drug and a ~2-fold lowering in the deduced saturation levels of BMV in the SC. The values of D/L^2, on the other hand, were not significantly affected by the surface cleaning procedure, suggesting that the brief exposure of the skin served only to remove sequestered formulation more efficiently from skin 'furrows' and did not artefactually 'drive' drug into the SC. Nevertheless, the basic differences between BMV delivery from MCT and ME formulations remained unchanged, with the latter still provoking a significant enhancement in uptake.

These conclusions were reinforced by the final experiments which considered BMV uptake into the SC from un-gelled, liquid formulations and skin cleaning with a dry paper towel. Q values were again lower, as reflected in the lower values of K; in turn, these translated into reduced saturation levels in the SC. The order of magnitude of these changes was very similar to what had been seen when the gelled vehicles were removed with isopropyl alcohol. Clearly, the simpler, dry-wiping procedure is quite effective

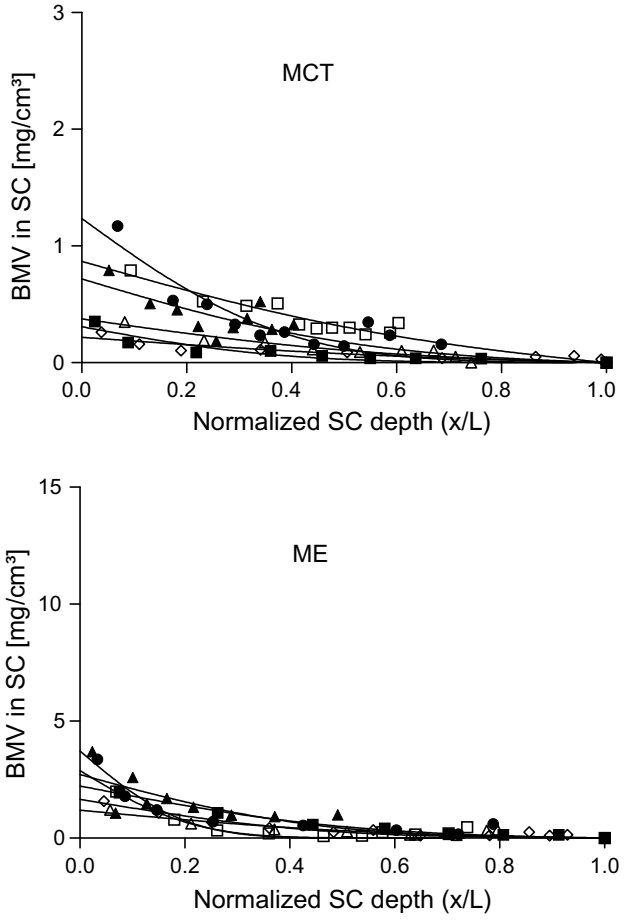

Fig. 3. SC distribution profiles of BMV delivered from gelled MCT and ME formulations following a 2-h application. The skin surface was cleaned using an isopropyl alcohol swab. The best fits of Eq. (1) to the individual experimental data points are shown ($n = 6$).

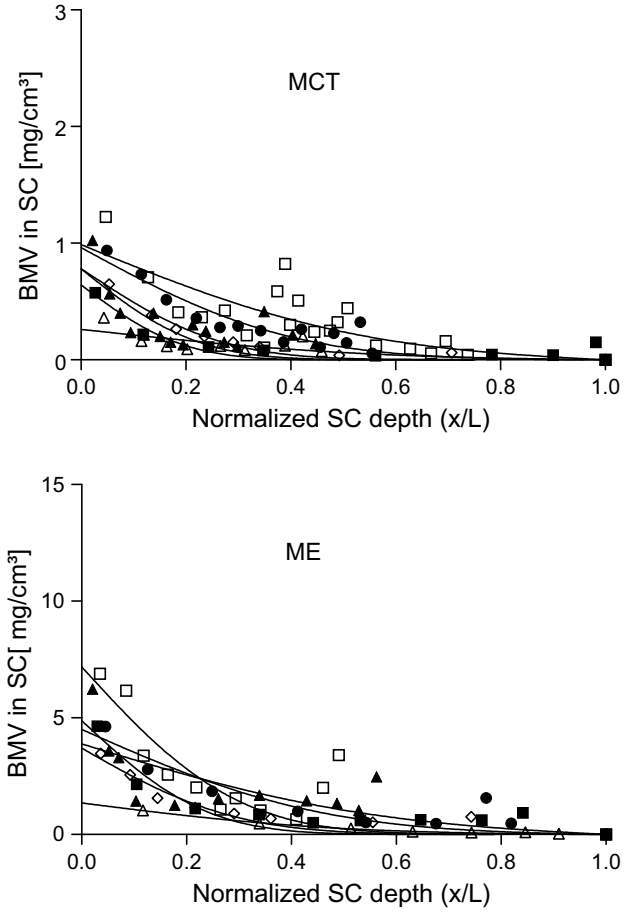

Fig. 4. SC distribution profiles of BMV delivered from un-gelled MCT and ME formulations following a 2-h application. The skin surface was cleaned with a dry paper towel. The best fits of Eq. (1) to the individual experimental data points are shown ($n = 6$).

Table 2
Partitioning (K) and diffusivity (D/L^2) parameters, as well as experimental amounts of drug per area per unit of SC thickness (Q_{expt}), and calculated saturation levels of BMV in the SC ($C_{s,SC}$) and enhancement factors (EF), from the drug concentration profiles subsequent to a 2-h application to human volunteers (mean ± SD, $n = 6$) of (i) gelled MCT and ME formulations followed by skin surface cleaning with isopropyl alcohol, and (ii) un-gelled vehicles followed by skin wiping with a dry paper towel.

Formulation Experimental conditions	MCT (i) IPA[a]	ME (i) IPA[a]	MCT (ii) Un-gelled[b]	ME (ii) Un-gelled[b]
K[c]	0.37 ± 0.23	0.26 ± 0.10	0.43 ± 0.16	0.46 ± 0.20
D/L^2 [h^{-1}][c]	0.049 ± 0.023	0.028 ± 0.018	0.023 ± 0.018	0.026 ± 0.017
$C_{s,SC}$ [mg/ml][d]	0.8 ± 0.5	3.0 ± 1.2	0.9 ± 0.3	5.3 ± 2.4
Q_{expt} [mg/cm^3][e]	0.20 ± 0.12	0.54 ± 0.16	0.16 ± 0.10	0.97 ± 0.42
EF	–	4.0 ± 3.2[f]	–	6.9 ± 3.4[f]

[a] Skin surface cleaned using an isopropyl alcohol (IPA) swab post-application of gelled vehicles.
[b] Skin surface cleaned using a dry paper towel post-application of un-gelled (liquid) vehicles.
[c] Deduced from the fit of Eq. (1) to the experimental distribution profiles.
[d] $C_{s,SC} = K \cdot C_{s,V}$.
[e] Determined from Eq. (2) with the deduced values of K and D/L^2.
[f] $EF = Q_{ME}/Q_{MCT}$.

in removing material from deeper regions of the skin's surface topology for low-viscosity, liquid formulations; in contrast, a more aggressive approach is warranted for semi-solid products which are typically used in practice.

In summary, therefore, this research has provided further information essential for the evolution of the DPK, tape-stripping method as a tool for the measurement of bioavailability and bioequivalence. It is clear that quantitative comparisons between formulations must be performed 'on a level playing field' where it is certain that the SC uptake deduced from the analysis of the tape-strips is not contaminated by drug formulation trapped in skin 'furrows'. The careful evaluation and validation of an efficient skin cleaning procedure at the end of the application period is a prerequisite.

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References

[1] A.L. Stinchcomb, F. Pirot, G.D. Touraille, A.L. Bunge, R.H. Guy, Chemical uptake into human stratum corneum in vivo from volatile and non-volatile solvents, Pharm. Res. 16 (1999) 1288–1293.
[2] J.-C. Tsai, S.-A. Chuang, M.-Y. Hsu, H.-M. Sheu, Distribution of salicylic acid in human stratum corneum following topical application in vivo: a comparison of six different formulations, Int. J. Pharm. 188 (1999) 145–153.
[3] L.K. Pershing, B.S. Silver, G.G. Krueger, V.P. Shah, J.P. Skelley, Feasibility of measuring the bioavailability of topical betamethasone dipropionate in commercial formulations using drug content in skin and a skin blanching assay, Pharm. Res. 9 (1992) 45–51.
[4] V.P. Shah, G.L. Flynn, A. Yacobi, H.I. Maibach, C. Bon, N.M. Fleischer, et al., Bioequivalence of topical dermatological dosage forms-methods of evaluation of bioequivalence, Pharm. Res. 15 (1998) 167–171.
[5] FDA, Guidance for Industry: Topical dermatologic drug product NDAs and ANDAs-in vivo bioavailability, bioequivalence, in vitro release, and associated studies, Draft Guidance, U.S. Department of Health and Human Services, Food and Drug Administration, Center of Drug Evaluation and Research, 1998.
[6] FDA, Guidance for Industry on special protocol assessment; Availability, Fed. Reg. 67: 35122, U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research, 2002.
[7] M. Fiedler, W.D. Meier, U. Hoppe, Texture analysis of the surface of the human skin, Skin Pharmacol. 8 (1995) 252–265.
[8] S. Wiedersberg, C.S. Leopold, R.H. Guy, Pharmacodynamics and dermatopharmacokinetics of betamethasone 17-valerate: assessment of topical bioavailability, Br. J. Dermatol. (2008), doi:10.1111/j.1365-2133.2008.08757.x.
[9] Y.N. Kalia, F. Pirot, R.H. Guy, Homogeneous transport in a heterogeneous membrane: water diffusion across human stratum corneum in vivo, Biophys. J. 71 (1996) 2692–2700.
[10] R.L. Anderson, J.M. Cassidy, Variations in physical dimensions and chemical composition of human stratum corneum, J. Invest. Dermatol. 61 (1973) 30–32.
[11] Y.N. Kalia, I. Alberti, N. Sekkat, C. Curdy, A. Naik, R.H. Guy, Normalization of stratum corneum barrier function and transepidermal water loss in vivo, Pharm. Res. 17 (2000) 1148–1150.
[12] Y.N. Kalia, I. Alberti, A. Naik, R.H. Guy, Assessment of topical bioavailability in vivo: the importance of stratum corneum thickness, Skin Pharmacol. Appl. Skin Physiol. 14 (2001) 82–86.
[13] J. Crank, Diffusion in a plane sheet, in: J. Crank (Ed.), The Mathematics of Diffusion, Oxford University Press, London, 1975, pp. 44–68.
[14] R.G. van der Molen, F. Spies, J.M. van't Noordende, E. Boelsma, A.M. Mommaas, H.K. Koerten, Tape stripping of human stratum corneum yields cell layers that originate from various depths because of furrows in the skin, Arch. Dermatol. Res. 289 (1997) 514–518.
[15] S.J. Bashir, A.-L. Chew, A. Anigbogu, F. Dreher, H.I. Maibach, Physical and physiological effects of stratum corneum tape stripping, Skin Res. Technol. 7 (2001) 40–48.
[16] U. Jacobi, N. Meykadeh, W. Sterry, J. Lademann, Effect of the vehicle on the amount of stratum corneum removed by tape stripping, JDDG 1 (2003) 884–889.
[17] C. Herkenne, A. Naik, Y.N. Kalia, R.H. Guy, Effect of propylene glycol on ibuprofen absorption into human skin in vivo, J. Pharm. Sci. 97 (2008) 185–197.
[18] C. Herkenne, A. Naik, Y.N. Kalia, J. Hadgraft, R.H. Guy, Pig ear skin ex vivo as a model for in vivo dermatopharmacokinetic studies in man, Pharm. Res. 23 (2006) 1850–1856.
[19] I. Alberti, Y.N. Kalia, A. Naik, R.H. Guy, Assessment and prediction of the cutaneous bioavailability of topical terbinafine, in vivo, in man, Pharm. Res. 18 (2001) 1472–1475.
[20] C. Herkenne, A. Naik, Y.N. Kalia, J. Hadgraft, R.H. Guy, Dermatopharmacokinetic prediction of topical drug bioavailability in vivo, J. Invest. Dermatol. 127 (2007) 887–894.