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The relationship between transepidermal water loss and skin permeability

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ABSTRACT

Transepidermal water loss (TEWL) is a measure of the steady-state water vapour flux crossing the skin to the external environment and it has been used extensively to characterise skin barrier function. We have previously hypothesised that *in vivo* TEWL is directly related to the reciprocal of the diffusional permeation pathlength through the *stratum corneum* (SC). The aim of the present paper is to validate experimentally this hypothesis. Ninety volunteers were recruited and TEWL and corneocyte surface areas were measured for six anatomic sites. The number of cell layers in the SC was calculated for each anatomic site in order to estimate the geometric pathlength for water efflux. Significant anatomic site variability was found for both TEWL and corneocyte surface area which were inversely correlated. A direct reciprocal relationship between TEWL and pathlength was determined, with TEWL values tending to zero when corneocytes are infinitely large. In general, skin sites with smaller corneocytes have fewer cell layers, with shorter permeation pathlengths and higher TEWL values. The results confirm our previous hypothesis and suggest that TEWL may be used to characterise the permeation routes for different anatomic sites.

1. Introduction

The barrier properties of the skin reside, primarily, in the outer 15 μm of the epidermis, the *stratum corneum* (SC) (Landmann, 1988). Despite its barrier function, there is constant evaporation of water, mostly from the underlying tissues, and also from the sweat glands, through the SC to the outside environment. Low temperatures, high relative humidity and lack of air across the skin decrease the rate of evaporation, but even under these conditions, water can still be lost by slow diffusion through the entire SC (Blank, 1952). Transepidermal water loss (TEWL) corresponds to the steady-state water vapour flux density permeating the SC to the exterior. TEWL measurements are routinely used in the pharmaceutical and cosmetic industries to provide information on the skin response to irritation (Cua et al., 1990; Heylings et al., 2003). Recent advances in instrumentation design have also led to the application of TEWL to characterise barrier properties of human skin both *in vitro* and *in vivo* (Imhof et al., 2009).

The predominant pathway for water and drug permeation through the skin is considered to be the intercellular route, i.e. through the rigid bilayers of lipids in which the individual corneocytes of the SC are embedded (Albery and Hadgraft, 1979). Variation in permeability characteristics with anatomic site has been reported by a number of authors (Rougier et al., 1986; Wester and Maibach, 1992). Rougier et al. (1988) have further observed that anatomic sites with relatively higher permeability are associated with smaller corneocyte sizes. TEWL values for the sites studied by these workers also followed an inverse trend with corneocyte size, i.e. higher TEWL values were recorded for the more permeable sites which had smaller corneocytes.

Skin turnover and corneocyte size is a function of the proteases and protease inhibitors responsible for normal skin desquamation which occurs every 14 days (Cork et al., 2009). Higher protease activities should result in a shorter period for cell proliferation and thus fewer mature corneocytes might be expected in sites with higher permeability. Voegeli et al. (2007) reported higher SC serine protease activities in the cheek relative to the forearm in line with the smaller corneocytes and thinner, more permeable skin in the facial area when compared with the forearm.

The actual diffusional pathlength for a molecule through the SC will be a function for both the number of cell layers and the corneocyte size at a particular skin site. If the number of skin layers and corneocyte sizes for various anatomic sites are known it is possible to calculate the permeation pathlength at specific anatomic sites (Hadgraft and Lane, 2009). Assuming that water flux through the skin may be described by Fick's first law we hypothesised that there should be a direct reciprocal relationship between the permeation pathlength and respective TEWL values for anatomic sites. The aim of the present study is to test this hypothesis. TEWL and CS datasets are reported for six anatomic sites from a much larger pool of volunteers than previously studied. The distribution of the data is assessed for normality and the relationship between the two datasets is investigated. The significance of the findings for topical and transdermal delivery is discussed. The implications of

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Table 1
Number, age, gender and ethnicity of study subjects.

Group	Number of volunteers	Age	Gender	Ethnicity
I	19	20–30	Female	Caucasian
II	15	20–30	Female	Asian
III	19	20–30	Male	Caucasian
IV	9	50–60	Male	Caucasian

protease activity for variation in skin permeability are also considered.

2. Materials and methods

2.1. Study group

Studies were performed on 90 volunteers, males and females, aged 20–60 years, Caucasians and Asians, with no history of dermatological disease. The research protocol was approved by the Camden and Islington Community Local Research Ethics Committee (06/Q0511/26). Other than daily washing, the anatomic sites received no self-administered application of any topical product such as lotions, creams or emollients over the 24 h period prior to the study. Volunteers were divided into four groups on the basis of age, gender and ethnicity (Table 1). Six anatomic sites were studied: ventral wrist, ventral mid forearm close to the ventral wrist (FA1), ventral mid forearm close to the ventral elbow (FA2), ventral elbow, forehead and abdomen.

2.2. Measurement of corneocyte surface area

Corneocytes were collected by tape-stripping the skin with Scotch Tape™ (3M) as reported previously (Choi et al., 2003). The tapes were flushed with HPLC-grade hexane followed by sonication of the suspension for 15 min to break up desmosomes and lipid cohesion between individual corneocytes. A droplet of this suspension was observed using light microscopy (Nikon microscope, 20× magnification) and corneocytes were photographed. Corneocyte surface area (A) was measured using ImageJ® software (NIH-Image, USA) and a mean value was calculated from 20 measured corneocytes for each anatomic site.

2.3. TEWL

Volunteers remained physically inactive for 15 min for acclimatisation prior to measurement at ambient conditions (21 °C, 45% relative humidity). TEWL was measured with a closed chamber device (Aqua Flux™, Biox Ltd., UK). Measurements (expressed in g/m^2/h) were performed in triplicate at each site for 3 min and Aqua Flux® V6.0 software was used for data analysis.

2.4. Data analysis

The software package SPSS 17® (SPSS Ltd, Chicago, USA) was used for all statistical analyses. Normality assessment was performed using the Kolmogorov–Smirnov test. A one-way between group analysis of variance (ANOVA) with a post hoc test was used to compare the different anatomic sites. A probability of $p < 0.05$ was considered statistically significant.

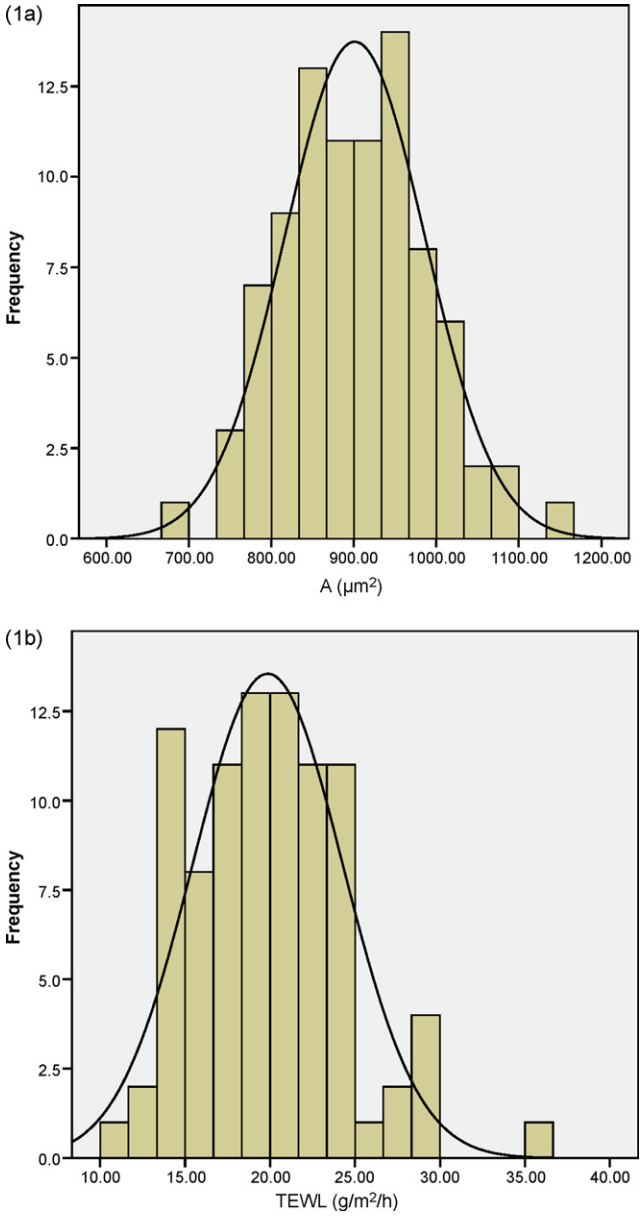

Fig. 1. (a) Corneocyte surface area (A) and (b) TEWL for the wrist with corresponding Gaussian probability density function ($p > 0.05$, $n = 88$).

3. Results and discussion

3.1. Frequency distribution of corneocyte surface area and TEWL data

The data for corneocyte surface area (A) and TEWL followed a normal distribution for all anatomic sites. Fig. 1a and b illustrates representative data for the TEWL and corneocyte size measurements for the wrist.

The TEWL results appear to contradict previous *in vitro* (Williams et al., 1992; Meidan and Roper, 2008) and *in vivo* (Wenkers and Lippold, 1999) studies which reported log-normal distributions for drug permeation and tritiated water permeability. *In vitro* studies generally involve storage, handling and technical preparation of the skin prior to use and therefore the observed non-Gaussian distribution associated with such data may be artefactual as observed by Williams et al. (1992). The *in vivo* study sampled only 24 volunteers in contrast to the 90 volunteers in this study

Table 2
Corneocyte surface area (A, μm^2) for different anatomic sites.

		A (μm^2) (Mean ± SD)
Wrist	(n = 88)	901 ± 85
FA1	(n = 88)	1095 ± 118
FA2	(n = 88)	1130 ± 107
Elbow	(n = 88)	1106 ± 97
Forehead	(n = 85)	825 ± 76
Abdomen	(n = 59)	1236 ± 95

Table 3
TEWL values for each anatomic site.

		TEWL ($g/m^2/h$) (Mean ± SD)
Wrist	(n = 90)	19.8 ± 4.5
FA1	(n = 90)	13.0 ± 2.9
FA2	(n = 90)	12.5 ± 2.5
Elbow	(n = 90)	13.1 ± 2.8
Forehead	(n = 84)	25.9 ± 6.4
Abdomen	(n = 59)	12.0 ± 3.0

and it is possible that this small sample size accounts for the skewed distribution of data reported. Because the data followed a normal distribution parametric tests and arithmetic means were used for subsequent data analyses.

3.2. Corneocyte surface area

For the six anatomic sites studied corneocyte surface area (A) ranged from $825 \pm 76\,\mu m^2$ (forehead) to $1236 \pm 95\,\mu m^2$ (abdomen). Table 2 shows the mean values for each anatomic site, as well as the standard deviations.

A one-way between-groups ANOVA confirmed that there was a statistically significant difference ($p < 0.05$) when testing for the influence of anatomic site on corneocyte size. Post hoc comparisons using the Tukey HSD test indicated that corneocytes in the forehead were significantly smaller than for all other anatomic sites, followed by the wrist, for which corneocytes were also significantly smaller than the remaining sites studied. FA1, FA2 and elbow showed no significant differences. Abdominal corneocytes were significantly larger than those from other anatomic sites. Corneocyte size can therefore be ranked as follows: forehead < wrist < FA1 = FA2 = elbow < abdomen. These differences are clearly illustrated in Fig. 2.

These findings are in line with those of Plewig and Marples (1970) and Rougier et al. (1988) who observed smaller corneocytes in facial areas relative to the forearm and abdomen. When comparing different races, ages and gender, no significant differences ($p > 0.05$) were found between the groups (data not shown).

3.3. TEWL

The TEWL values ranged from $12.0 \pm 3.0\,g/m^2/h$ to $25.9 \pm 6.4\,g/m^2/h$. The mean ± standard deviations for TEWL values for all anatomic sites are given in Table 3. The influence of the anatomic site on TEWL was determined by conducting a one-way between-groups ANOVA. There was a statistically significant difference at the $p < 0.05$ level. Post hoc comparisons using the Tukey HSD test showed that although the lowest TEWL values were obtained for the abdomen these were not significantly different from FA1, FA2 and elbow. These sites had significantly lower TEWL values compared with the wrist and the forehead. The TEWL values for the forehead were significantly higher than values for all other anatomic sites. Thus, TEWL for the sites may be ranked as follows: forehead > wrist > FA1 = FA2 = elbow = abdomen.

Lotte et al. (1987), Schnetz et al. (1999) and Marrakchi and Maibach (2007), have also reported higher TEWL values for the forehead relative to the forearm. TEWL results for the wrist are in agreement with Jang et al. (1996) and Chilcott and Farrar (2000) who reported higher values at this site relative to the mid forearm. In general, sites with higher TEWL values are associated with smaller corneocyte sizes (Tables 2 and 3).

When performing ethnicity, age and gender comparisons no statistically significant differences ($p > 0.05$) were found for TEWL between the groups.

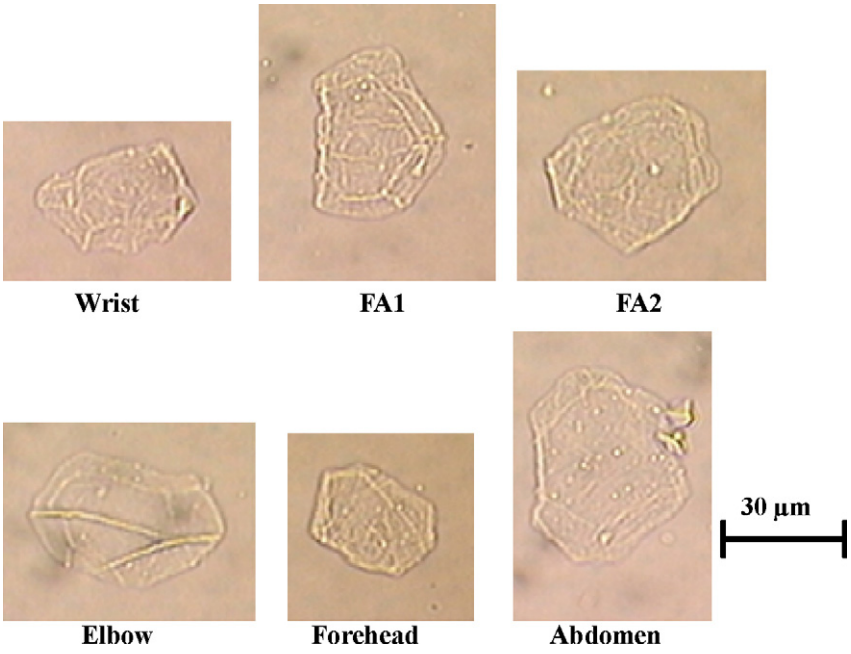

Fig. 2. Representative images of corneocytes from all anatomic sites for a single volunteer.

Table 4
Number of cell layers (n) for each anatomic site.

	n
Wrist	13
Forearm 1	17
Forearm 2	18
Elbow	18
Forehead	12
Abdomen	20

3.4. The relationship between corneocyte size and TEWL

The relationship between TEWL and corneocyte surface area (A) was investigated using the Pearson product-moment correlation coefficient. A correlation of −0.722 was obtained ($p < 0.01$) suggesting that TEWL is highly dependent on corneocyte surface area.

For each anatomic site, the number of cell layers (n) can be calculated from the surface area (A) of the corneocytes (Table 2) using Eq. (1) (Rougier et al., 1988; Ya-Xian et al., 1999; Hadgraft and Lane, 2009):

$$n = \frac{\sqrt{A} - 20}{0.75} \quad (1)$$

The number of cell layers for each anatomic site, calculated using Eq. (1), is reported in Table 4 with the highest number of cell layers in the abdomen and the smallest number in the forehead. The data for the abdomen and forearm are in line with previously reported values for these sites (Holbrook and Odland, 1974; Weigand et al., 1974; Elias et al., 1981).

Multivariate analysis of n and corneocyte size, $\sqrt{A}$, confirmed that the best fit of the data was obtained with a refined value of $n = 18$ for FA1, and $n = 11$ for the forehead. Fig. 3 illustrates these data plotted with the refined values for n. From Fig. 3, a modified equation (Eq. (2)) may be used to calculate the number of cell layers for each anatomic site

$$n = \frac{\sqrt{A} - 21}{0.7} \quad (2)$$

The geometric intercellular pathlength (μm) is calculated from Eq. (3) (Hadgraft and Lane, 2009):

$$\text{Pathlength} = n + \frac{\sqrt{A}(n-1)}{2} \quad (3)$$

A plot of TEWL versus the reciprocal of the pathlength for water is shown in Fig. 4. The robustness of the approach is further confirmed by the observation that TEWL tends to zero when the pathlength for diffusion becomes infinitely long.

Our data suggest that corneocyte size and TEWL measurements may be used to characterise variation in permeability to water transport at different anatomic sites. The application of a simple biophysical measurement such as TEWL to characterise skin structure should have implications for both the cosmetic and pharmaceutical industries. It is also of interest to note that many cosmetic formulations are currently tested on the forearm rather than the face. From our results it is clear that these sites are very different in terms of permeability and this will be of concern both for the delivery of actives and possible irritancy effects from any of the formulation components.

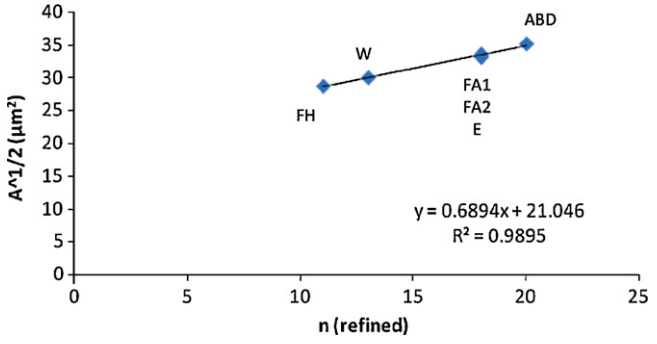

Fig. 3. Plot of corneocyte size ($\sqrt{A}$) vs. number of cell layers (n); refined n values for forearm and forehead (FH: forehead; W: wrist; FA: forearm; E: elbow and ABD: abdomen).

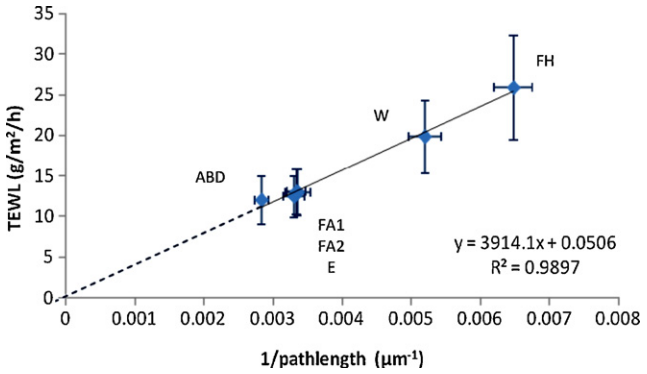

Fig. 4. TEWL (g/m^2/h) vs. 1/pathlength (μm^{-1}) (FH: forehead; W: wrist; FA: forearm; E: elbow and ABD: abdomen).

Since corneocyte size is a consequence of enzymatic activity in the skin these measurements should also help to characterise skin permeability in diseases where this activity is disrupted e.g. atopic eczema. This knowledge should also inform the improved design of formulations for amelioration of such conditions.

4. Conclusions

The data reported in the present investigation confirm that experimentally determined TEWL data is inversely correlated with the diffusional pathlength for water movement in skin. The proposed relationship takes account of previously reported regional ultrastructural variability in anatomic sites. The data are also consistent with variation in enzymatic activity from site to site which is expected to contribute to differences in corneocyte size and maturity. TEWL data and corneocyte size were normally distributed which has not been previously reported in the literature.

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References

Albery, W.J., Hadgraft, J., 1979. Percutaneous absorption: in vivo experiments. J. Pharm. Pharmacol. 31, 140–147.
Blank, I.H., 1952. Factors which influence the water content of the stratum corneum. J. Invest. Dermatol. 18, 433–440.
Chilcott, R.P., Farrar, R., 2000. Biophysical measurements of human forearm skin in vivo: effects of site, gender, chirality and time. Skin Res. Technol. 6, 64–69.
Choi, I.M.J., Zhai, H., Loffler, H., Dreher, F., Maibach, H.I., 2003. Effect of tape stripping on percutaneous penetration and topical vaccination. Exog. Dermatol. 2, 262–269.
Cork, M.J., Danby, S.G., Vasilopoulos, Y., Hadgraft, J., Lane, M.E., Moustafa, M., Guy, R.H., Macgowan, A.L., Tazi-Ahnini, R., Ward, S.J., 2009. Epidermal barrier dysfunction in atopic dermatitis. J. Invest. Dermatol. 129, 1892–1908.
Cua, A.B., Wilhelm, K.P., Maibach, H.I., 1990. Cutaneous sodium lauryl sulphate irritation potential: age and regional variability. Br. J. Dermatol. 123, 607–613.
Elias, P.M., Cooper, E.R., Korc, A., Brown, B.E., 1981. Percutaneous transport in relation to stratum corneum structure and lipid composition. J. Invest. Dermatol. 76, 297–301.
Hadgraft, J., Lane, M.E., 2009. Transepidermal water loss and skin site: a hypothesis. Int. J. Pharm. 373, 1–3.
Heylings, J.R., Diot, S., Esdaile, D.J., Fasano, W.J., Manning, L.A., Owen, H.M., 2003. A prevalidation study on the in vitro skin irritation function test (SIFT) for predic-

tion of acute skin irritation in vivo: results and evaluation of ECVAM Phase III. Toxicol. In Vitro 17, 123–138.
Holbrook, K.A., Odland, G.F., 1974. Regional differences in the thickness (cell layers) of the human stratum corneum: an ultrastructural analysis. J. Invest. Dermatol. 62, 415–422.
Imhof, R.E., De Jesus, M.E.P., Xiao, P., Ciortea, L.I., Berg, E.P., 2009. New developments in skin barrier measurements. In: Rawlings, A.V., Leyden, J.J. (Eds.), Skin Moisturization, Cosmetic Science and Technology Series, 2nd edition. Informa Healthcare, New York, pp. 463–479.
Jang, H.Y., Park, C.W., Lee, C.H., 1996. A study of transepidermal water loss at various anatomical sites of the skin. Korean J. Dermatol. 34, 402–406.
Landmann, L., 1988. The epidermal permeability barrier. Anat. Embryol. (Berl). 178, 1–13.
Lotte, C., Rougier, A., Wilson, D.R., Maibach, H.I., 1987. In vivo relationship between transepidermal water loss and percutaneous penetration of some organic compounds in man: effect of anatomic site. Arch. Dermatol. Res. 279, 351–356.
Marrakchi, S., Maibach, H.I., 2007. Biophysical parameters of skin: map of human face regional, and age-related differences. Contact Dermat. 57, 28–34.
Meidan, V.M., Roper, C.S., 2008. Inter- and intra-individual variability in human skin barrier function: a large scale retrospective study. Toxicol. In Vitro 22, 1062–1069.
Plewig, G., Marples, R.R., 1970. Regional differences of cell sizes in the human stratum corneum. Part I. J. Invest. Dermatol. 54, 13–18.
Rougier, A., Dupuis, D., Lotte, C., Roguet, R., Wester, R.C., Maibach, H.I., 1986. Regional variation in percutaneous absorption in man: measurement by the stripping method. Arch. Derm. Res. 278, 465–469.
Schnetz, E., Kuss, O., Schmitt, J., Diepgen, T.L., Kuhn, M., Fartashi, M., 1999. Intra- and inter-individual variations in transepidermal water loss on the face: facial locations for bioengineering studies. Contact Dermat. 40, 243–247.
Voegeli, R., Rawlings, A.V., Doppler, S., Heiland, J., Schreier, T., 2007. Profiling of serine protease activities in human stratum corneum and detection of a stratum corneum tryptase-like enzyme. Int. J. Cosmet. Sci. 29, 191–200.
Weigand, D.A., Haygood, C., Gaylor, J.R., 1974. Cell layers and density of Negro and Caucasian stratum corneum. J. Invest. Dermatol. 62, 563–568.
Wester, R.C., Maibach, H.I., 1992. Percutaneous absorption of drugs. Clin. Pharmacokinet. 23, 253–266.
Rougier, A., Lotte, C., Corcuff, P., Maibach, H.I., 1988. Relationship between skin permeability and corneocyte size according to anatomic site, age and sex in man. J. Soc. Cosmet. Chem. 39, 15–26.
Wenkers, B.P., Lippold, B.C., 1999. The statistical distribution of in-vivo skin permeability. Pharm. Pharmacol. Commun. 5, 425–427.
Williams, A.C., Cornwell, P.A., Barry, B.W., 1992. On the non-Gaussian distribution of skin permeabilities. Int. J. Pharm. 86, 69–77.
Ya-Xian, Z., Suetake, T., Tagami, H., 1999. Number of cell layers of the stratum corneum in normal skin—relationship to the anatomical location on the body, age, sex and physical parameters. Arch. Dermatol. Res. 291, 555–559.