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Research paper

Evidence that drug flux across synthetic membranes is described by normally distributed permeability coefficients

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Abstract

Over recent decades, the use of *in vitro* diffusion cell studies to assess skin permeability has evolved into a major research tool, providing key insights into the relationships between skin, drug and formulation. Sometimes, such studies involve synthetic membranes as this approach can yield useful inferences with respect to drug–skin partitioning and diffusion phenomena. Yet despite the popularity of such studies, it is still not at all known whether typical solute transport across synthetic barriers results in a normal distribution of permeability coefficients or alternatively some type of skewed distribution. The present study aims to shed light on this issue. To this end, five compounds (testosterone, oestradiol, corticosterone, aldosterone and adenosine) exhibiting a broad range of octanol–water partition coefficient values were selected as test penetrants. The protocol involved taking multiple replicate measurements of each drug's passive steady state flux through poly(dimethylsiloxane) membrane. Each penetrant's resultant permeability coefficient database was subjected to a Kolmogorov–Smirnov (KS) test for normality. It was found that the permeability coefficients of all five drugs were distributed in a Gaussian-normal fashion. The theoretical significance and practical impact of these findings are discussed.

Keywords: Testosterone; Oestradiol; Corticosterone; Aldosterone; Adenosine; Permeability coefficient; Variability; Gaussian distribution; Normal distribution

1. Introduction

There is considerable evidence that the permeability coefficients (k_p), which describe any given *in vitro* transdermal drug penetration process, are not distributed in a Gaussian-normal fashion but rather may follow other patterns. Key research in this area was conducted by Barry's group [1,2], who measured the penetration of both 5-fluorouracil (hydrophilic) and oestradiol (lipophilic) through human epidermal samples. They reported that, in general, the k_p values for both compounds could be more closely fitted to log-normal frequency distributions than to normal frequency distributions. Another team [3] tracked water penetration rates through 539 samples of split-thickness human cadaver skin, derived from seven different donors. It was found that the k_p distribution exhibited a strong positive skew, suggesting a non-normal pattern. Also, analysis of k_p databases describing tritiated water flux through human epidermis again provided evidence for non-normal distributions [4–6].

The main consequence of non-normal k_p behaviour is that it directly impacts the statistical methodologies used to interpret drug penetration data. For instance, use of the *t*-test to evaluate statistical significance assumes that the original population exhibits Gaussian-normality. If this is not the case for k_p values then non-parametric tests such as the Mann–Whitney *U*-test or Wilcoxon's signed rank test should be applied. Another option is to ascertain that the data exhibit Gaussian-normality following a suitable

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mathematical transformation. For example, if it is known that the database fits a log-normal distribution, the mean and standard deviation of the logarithmically transformed data may be used for a valid *t*-test.

Although several groups have analysed the k_p distributions describing drug penetration through skin, negligible work has been published with respect to permeation through synthetic membranes. These are frequently employed to simulate the drug partitioning properties of the skin barrier [7]. The aim of the present study is to shed light on this neglected issue. To this end, membranes fabricated from poly(dimethylsiloxane) (PDMS) were investigated as these have been used quite widely in recent years in transdermal modeling studies [8–13]. In order to investigate the possible influence of partition coefficient ($K_{o/w}$), we selected five different solutes – testosterone, oestradiol, corticosterone, aldosterone and adenosine – exhibiting comparable molecular weights but different $\log K_{o/w}$ values (see Table 1). The drugs were screened by taking multiple replicate measurements of their steady state transmembrane flux rates. Each penetrant's resultant permeability coefficient (k_p) distribution was evaluated for Gaussian-normality by applying a Kolmogorov–Smirnov (KS) test.

2. Materials and methods

2.1. Chemicals

All the radiolabeled penetrants i.e. [2-^{3}H]adenosine (15 Ci/mmol), [1,2-^{3}H]aldosterone (1 mCi/ml), [1,2,6,7-^{3}H] corticosterone (1 mCi/ml), [2,4,6,7-^{3}H]oestradiol (1 mCi/ml) and [1,2,6,7-^{3}H]testosterone (1 mCi/ml) were purchased from Amersham Biosciences (Amersham, UK). The 'cold' penetrants i.e. adenosine, aldosterone, corticosterone, oestradiol, testosterone were purchased from Sigma–Aldrich (Poole, UK) as were phosphate buffered saline (PBS) tablets (pH 7.4) and absolute ethanol. Optiphase HiSafe 3 scintillation fluid and scintillation vials were purchased from Fisher Scientific (Loughborough, UK) and Packard Instrument Co. (Meriden, CT), respectively. Translucent poly(dimethylsiloxane) membranes of stated nominal thickness 300 μm were obtained from Samco Silicone Products Ltd (Nuneaton, UK). All the tested PDMS membranes originated from the same delivered batch. Distilled water was used to make up all solutions.

2.2. Transport studies

Immediately prior to the transport studies, the PDMS material was immersed for 14–16 h in a large volume of receiver solution in order to hydrate the membrane and remove any excipients such as glycerol. Subsequently, sections of PDMS membrane were mounted in Franz diffusion cells (PermeGear, Bethlehem, PA), having a diffusion-available surface area of 0.64 cm^2 and a receptor compartment volume of 5.3 ml. The receiver phase consisted of 10% (v/v) ethanol in PBS (pH 7.4) that had been degassed by sonication for 15 min (Camlab Transsonic T310, Cambridge, UK). These media were stirred at 600 rpm and maintained at 37 ± 0.5°C by the use of a thermostatic water pump (Haake DC10, Karlsruhe, Germany) that circulated water through each chamber jacket. The membranes were initially left in the Franz cells for 1 h. Subsequently, 0.5 ml of donor solution was deposited onto each membrane surface. This donor solution was composed of the test solute dissolved in a 20:80% (v/v) mixture of ethanol:PBS (pH 7.4). The solute concentrations were: 0.01% w/v for aldosterone, corticosterone and testosterone, 0.0008% w/v for oestradiol and 0.1% w/v for adenosine. Each donor compartment was covered with a taut layer of Parafilm® in order to prevent evaporation.

At selected time intervals (0, 1, 2, 3, 4, 5, and 6 h) a 100 μl aliquot was withdrawn from each receiver solution and replaced with the same volume of blank receiver solution. Permeant amounts in the withdrawn solutions were determined by liquid scintillation counting. To this end, each 100 aliquot was vortexed with 3 ml of scintillation cocktail and then placed in a liquid scintillation counter (Packard, TriCarb™ 1600TR). Imposing a minimal threshold of 50 dpm above background (~12 dpm), the emitted activity of each aliquot was converted to a drug concentration value. In order to determine whether donor depletion was occurring, aliquots were taken from the donor compartment at 6 hours and similarly assayed by liquid scintillation counting. Each study consisted of 63 replicate runs.

In a small parallel experiment, twelve hydrated PDMS membrane sections were not mounted in Franz cells but were wiped dry. The thickness of these sections was measured using a Mitutoya model 500-181U digital caliper (Andover, UK).

Table 1
Physicochemical properties and permeability coefficient data of the selected candidate drugs

Penetrant	$\log K_{o/w}$[a]	Molecular weight (Da)	Mean $k_p \pm$ SD (cm/h × 10^{-4})	% CV[b]	KS distance	P value; $* = 0.05$ (passed normality test?)
Adenosine	−1.05	267	0.38 ± 0.17	45.5	0.074	$P > 0.10$ (yes)
Aldosterone	1.08	360	0.84 ± 0.26	30.7	0.090	$P > 0.10$ (yes)
Corticosterone	1.94	346	7.60 ± 1.10	15.1	0.071	$P > 0.10$ (yes)
Oestradiol	2.29[c]	272	85.0 ± 11.1	13.1	0.070	$P > 0.10$ (yes)
Testosterone	3.32	288	270.2 ± 107.5	39.8	0.080	$P > 0.10$ (yes)

[a] $\log K_{o/w}$ values were derived from published experimental data [14,15].
[b] Coefficient of variation expressed as a % = 100(SD/mean).
[c] $\log K_{o/w}$ values for oestradiol vary somewhat in the literature. We have chosen to use a value of 2.29 as described in [1,14,16,17].

2.3. Data analysis

For all experiments, derived concentration values were corrected for the progressive dilution caused by sampling. Subsequently, linear regression was used to determine the gradient of the linear segment of each permeation experiment, thus yielding a k_p value for each individual replicate. All the replicate k_p values for each study were pooled together without the omission of outliers. Each resulting distribution was tested for normality by applying a form of the KS test [18] known as "Dallal and Wilkinson's approximation to Lilliefors' method" [19]. This testing was performed by IBM-compatible software, specifically Prism version 2 (GraphPad Software, San Diego, CA). The null hypothesis was that each k_p database is derived from a normal distribution. A level of 0.05 was deemed acceptable for its rejection.

3. Results

3.1. Drug penetration-time plots

Fig. 1 presents the pooled penetration-time plots for all five investigated permeants. Notably, the profiles for testosterone, oestradiol, corticosterone and aldosterone were characterised by a negligible lag time followed by a well-defined steady state phase. In contrast, the penetration-time plot for adenosine was untypical. The rate of drug flux was higher over the first hour than over subsequent hours, yielding in effect a 'negative lag time'. This can be explained by a three way interaction between the adenosine, membrane and solvent. Importantly, sink conditions were maintained throughout all five studies. At 6 h, mean solute concentrations in the receiver compartment were only; 2.4% (testosterone), 0.61% (oestradiol), 0.06% (corticosterone), 0.03% (aldosterone), or <0.01% (adenosine) of that in the donor compartment at time zero. There were no significant donor depletion effects and sink conditions were maintained. In other words, we can validate that the experimental systems permitted free diffusion of all the candidate drugs.

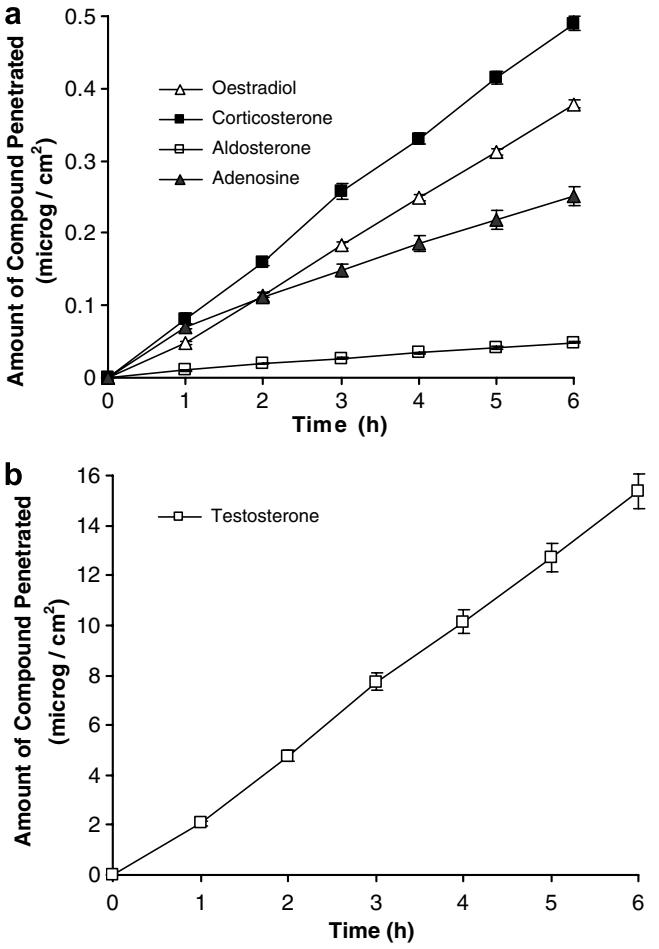

Fig. 1. Penetration of adenosine, aldosterone, corticosterone and oestradiol (a) as well as testosterone (b) through PDMS membranes as a function of time. Error bars represent standard error of the mean values, with $n = 63$.

3.2. Mean values of permeability coefficients

Table 1 presents the derived mean k_p values for each of the five permeation studies. It can be seen that mean drug permeability increased with increasing octanol–water partition coefficient. This would be expected since greater solute lipophilicity will tend to accelerate both drug partitioning from the donor solution into the PDMS membrane as well as the transmembrane diffusion rate.

3.3. Distribution of permeability coefficients

Figs. 2–6 present the k_p frequency distributions for testosterone, oestradiol, corticosterone, aldosterone and adenosine, respectively. Table 1 presents the derived statistical parameters describing each k_p data set. It can be seen that the magnitude of data dispersion, as evaluated by the coefficient of variation (CV), varied between 45.5% (for

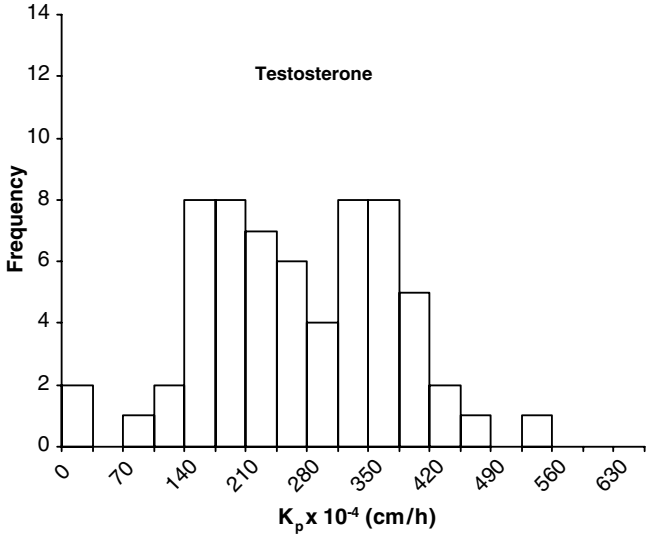

Fig. 2. Frequency distribution of testosterone permeability coefficients.

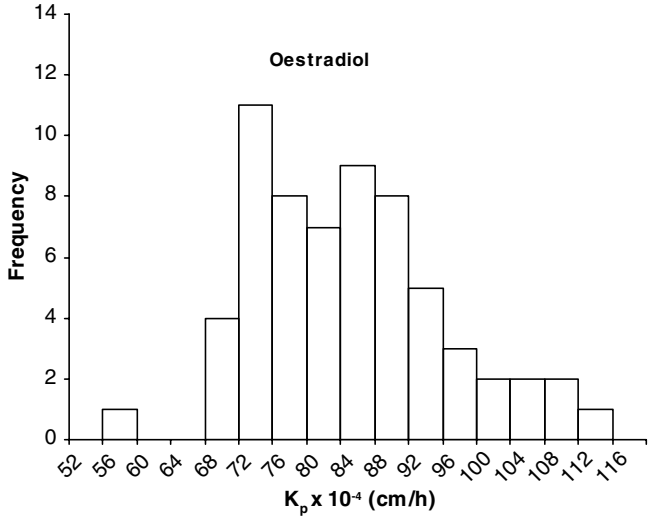

Fig. 3. Frequency distribution of oestradiol permeability coefficients.

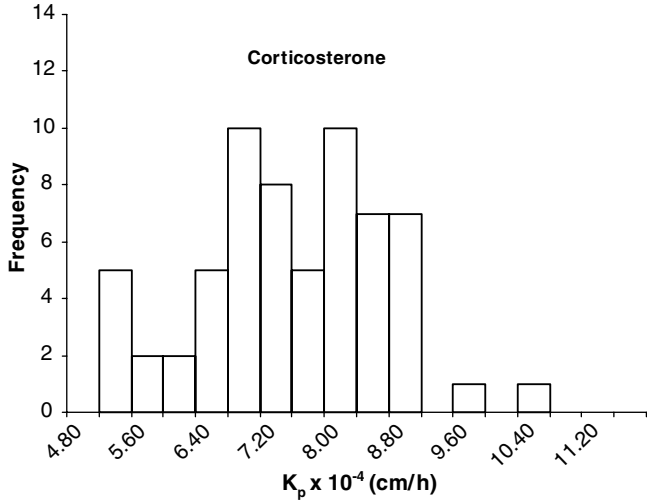

Fig. 4. Frequency distribution of corticosterone permeability coefficients.

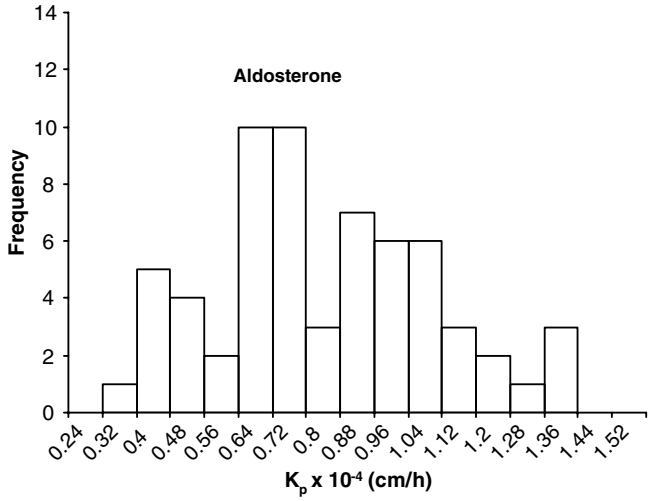

Fig. 5. Frequency distribution of aldosterone permeability coefficients.

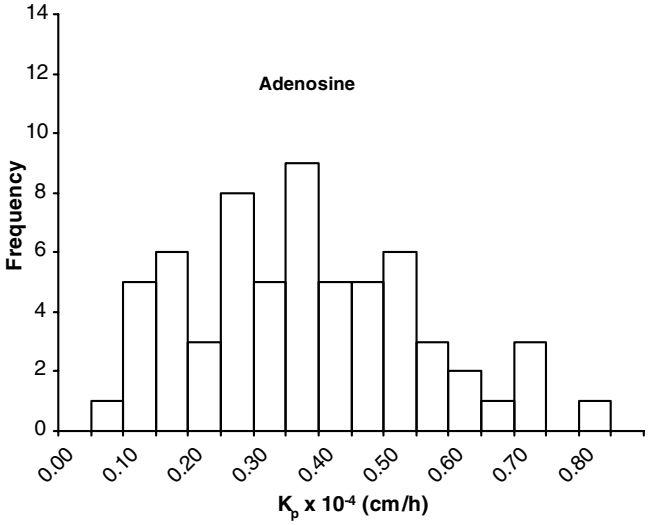

Fig. 6. Frequency distribution of adenosine permeability coefficients.

adenosine) and 13.1% (for oestradiol). However, there did not seem to be any clear trend relating this parameter to any of the solute's physicochemical properties. Importantly, caliper measurements indicated that mean PDMS membrane thickness was 259 μm and that CV was 2.6%. This indicates that k_p variability was being caused by variability in membrane composition rather than thickness.

The most interesting parameter is probably the KS distance. If each permeant's k_p values followed a perfect normal distribution, the KS distance would equal zero. Larger values of the KS distance correspond to larger deviations from an ideal normal distribution. Crucially, the KS distance values did not vary greatly between each of the five drugs as values ranged between 0.070 and 0.090. Additionally, there was no discernable correlation between partition coefficient and KS distance. The *P* value answers the question: if the parent population was really Gaussian, what is the chance that a sample of 63 values would have a KS distance as large or larger than observed? A level of 0.05 was deemed acceptable for rejection. As Table 1 shows, the permeability coefficient databases of all five drugs could be easily fitted to a normal distribution. It should be noted that when the *P* value was calculated to be greater than 0.1, the software did not give a precise value but just stated that this was the case.

4. Discussion

Our results showed that for all five test drugs, k_p variability could be easily fitted into a Gaussian-normal distribution. Furthermore, the extent of each k_p database's fit to the normal distribution was relatively similar in magnitude. These findings were obtained despite the greater than 10,000-fold difference in octanol–water partition coefficient between the most hydrophilic and most lipophilic penetrant. This difference in lipophilicity was also associated with huge concomitant differences in the

mean permeability coefficient values. Given these results, it seems likely that passive solute flux through most synthetic membrane systems will be described by normally distributed permeability coefficients. Yet in order to be certain of this, similar studies would have to be conducted using other differently sourced membranes. It is also just possible that other forms of variability may occur with penetrants exhibiting extreme partition coefficient behaviour and/or molecular weights. Further work would be required in order to eliminate these caveats.

Intriguingly, the results of this study contrast quite sharply with those reported for biological skin systems. The k_p distributions for animal and human skins or split-skins were found to be mostly non-Gaussian [1–6,20–22] with log-normal patterns sometimes predominating. It is difficult to explain this differential behaviour as very little is known about the underlying principles of membrane flux variability right across the sciences [23]. However, variability in carboxyfluorescein permeation through corneal epithelium [24] could be fitted to a Gaussian distribution as could the transport of both glucose and a small zwitterion through synthetic lipid bilayers [25]. In contrast, coefficients describing water and organic salt mobility through lipophilic plant cuticles could not be fitted to a Gaussian distribution [26]. Therefore, it seems probable that drug permeation through biological membranes likely to include pores, follicles, thickness variations and other defects may show skewed variability. In contrast, barriers relatively free of such imperfections such as artificial membranes or corneal epithelia would tend to show normally distributed permeability coefficients.

If synthetic membrane flux is invariably described by normally distributed k_p values, this means that it is statistically permissible to use *t*-tests and *F*-tests to assess the data generated in such studies. In practice, many transdermal delivery workers tend to remove obvious outliers and then perform *t*-tests or *F*-tests on the residual permeability data. The current study suggests that such an approach may make some practical sense in the case of flux through biological skin, where population k_p variability is likely to be non-Gaussian. Yet this approach may be inappropriate for most synthetic membrane systems where population k_p variability is probably Gaussian.

References

[1] A.C. Williams, P.A. Cornwell, B.W. Barry, On the non-Gaussian distribution of human skin permeabilities, Int. J. Pharm. 86 (1992) 69–77.
[2] P.A. Cornwell, B.W. Barry, Effects of penetration enhancer treatment on the statistical distribution of human skin permeabilities, Int. J. Pharm. 117 (1995) 101–112.
[3] G.B. Kasting, W.R. Francis, T.G. Filloon, M.P. Meredith, 1992. Improving the sensitivity of in vitro skin penetration studies. AAPS Annual Meeting, San Antonio TX.
[4] A.C. Watkinson, K.R. Brain, K.A. Walters, R.S. Grabarz, R.K. Sharma, Is it logical to reject skin samples based on water permeability data? in: K.R. Brain, K.A. Walters (Eds.), Perspectives in Percutaneous Penetration, vol. 6A, STS Publishing, Cardiff, UK, 1998, p. 62.
[5] C.S. Roper, A.G. Simpson, S. Madden, B.D. Cameron, Tritiated water permeability coefficient assessment and rejection criteria for barrier function of human skin, in: K.R. Brain, K.A. Walters (Eds.), Perspectives in Percutaneous Penetration, vol. 7A, STS Publishing, Cardiff, UK, 2000.
[6] W.J. Fasano, L.A. Manning, J.W. Green, Rapid integrity assessment of rat and human epidermal membranes for in vitro dermal regulatory testing: correlation of electrical resistance with tritiated water permeability, Toxicol. In Vitro 16 (2002) 731–740.
[7] J.C. Smith, W.J. Irwin, Ionisation and the effect of absorption enhancers on transport of salicylic acid through silastic rubber and human skin, Int. J. Pharm. 210 (2000) 69–82.
[8] M.T. Cronin, J.C. Dearden, R. Gupta, G.P. Moss, An investigation into the mechanism of flux across polydimethylsiloxane membranes by use of quantitative structure–permeability relationships, J. Pharm. Pharmacol. 50 (1998) 143–152.
[9] J. Du Plessis, W.J. Pugh, A. Judefeind, J. Hadgraft, The effect of the nature of H-bonding groups on diffusion through PDMS membranes saturated with octanol and toluene, Int. J. Pharm. 15 (2002) 63–69.
[10] M.M. Dias, S.L. Raghavan, M.A. Pellet, J. Hadgraft, The effect of beta cyclodextrins on the permeation of diclofenac from supersaturated solutions, Int. J. Pharm. 263 (2003) 173–181.
[11] X. Gu, S. Kasichayanula, D.J. Fediuk, F.J. Burczynski, In-vitro permeation of the insect repellent *N*,*N*-diethyl-*m*-toluamide (DEET) and the sunscreen oxybenzone, J. Pharm. Pharmacol. 56 (2004) 621–628.
[12] R. Chilcott, N. Barai, A. Beezer, S. Brain, M. Brown, A. Bunge, S. Burgess, S. Cross, C. Dalton, M. Dias, A. Farinha, B. Finnin, S. Gallagher, D. Green, H. Gunt, R. Gwyther, C. Heard, C. Jarvis, F. Kamiyama, G. Kasting, E. Ley, S. Lim, G. McNaughton, A. Morris, M. Nazemi, M. Pellett, J. DuPlessis, Y. Quan, S. Raghavan, M. Roberts, W. Romonchuk, C. Roper, D. Schenk, L. Simonsen, A. Simpson, B. Traversa, L. Trottet, A. Watkinson, S. Wilkinson, F. Williams, A. Yamamoto, J. Hadgraft, Inter- and intra-laboratory variation of in vitro diffusion cell measurements: an international multi-centre study using quasi-standardised methods and materials, J. Pharm. Sci. 94 (2005) 632–638.
[13] V. Sarveiva, J.F. Templeton, H.A. Benson, Effect of lipophilic counter-ions on membrane diffusion of benzydamine, Eur. J. Pharm. Sci. 26 (2005) 39–46.
[14] N.A. Megrab, A.C. Williams, B.W. Barry, Oestradiol permeation through human skin and silastic membrane: effects of propylene glycol and supersaturation, J. Control. Rel. 36 (1995) 277–294.
[15] C. Hansch, D. Hoekman, A. Leo, L.T. Zhang, P. Li, The expanding role of quantitative structure–activity relationships (QSAR) in toxicology, Toxicol. Letts. 79 (1995) 45–53.
[16] E.A. Essa, M.C. Bonner, B.W. Barry, Human skin sandwich for assessing shunt route penetration during passive and iontophoretic drug and liposome delivery, J. Pharm. Pharmacol. 54 (2002) 1481–1490.
[17] G.M.M. El Maghraby, A.C. Williams, B.W. Barry, Drug interaction and location in liposomes: correlation with polar surface areas, Int. J. Pharm. 292 (2005) 179–185.
[18] J.C. Miller, J.N. Miller, Statistics for Analytical Chemistry, John Wiley, Chichester, UK, 1986.
[19] G.E. Dallal, L. Wilkinson, An analytic approximation to the distribution of Lilliefors' test for normality, Am. Stat. 40 (1986) 294–296.
[20] G.M. Khan, Y. Frum, O. Sarheed, G.M. Eccleston, V.M. Meidan, Assessment of drug permeability distributions in two different model skins, Int. J. Pharm. 303 (2005) 81–87.
[21] B.P. Wenkers, B.C. Lippold, The statistical distribution of in vivo skin permeability, Pharm. Pharmacol. Commun. 5 (1999) 425–427.

[22] P. Liu, J. Nightingale, T. Kurihara-Bergstrom, Variation of in vitro skin permeation data for ionic compounds, Pharm. Res. 8 (Suppl.) (1991) S137.
[23] E. Limpert, W.A. Stahel, M. Abbt, Log-normal distributions across the sciences: keys and clues, Bioscience 51 (2001) 341–352.
[24] B.E. McCarey, T. Reaves, Noninvasive measurement of corneal epithelial permeability, Curr. Eye Res. 14 (1995) 505–510.
[25] S.G. Clerc, T.E. Thompson, Permeability of dimyristoyl phosphatidylcholine/dipalmitoyl phosphatidylcholine bilayer membranes with coexisting gel and liquid–crystalline phases, Biophys. J. 68 (1995) 2333–2341.
[26] P. Baur, Log-normal distribution of water permeability and organic solute mobility in plant cuticles, Plant Cell Environ. 20 (1997) 167–177.