Finite and Infinite Dosing

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3.1 Introduction

The aim of permeation studies is to determine how much of a preparation should be applied to the skin surface in order to achieve the desired bioavailability. In practice, many topical preparations are applied to the skin as a finite dose, often in the form of a cream, gel or ointment. However, when examining the fundamental permeation behaviour of a substance or when investigating the effects of penetration enhancers on percutaneous absorption, infinite dosing is usually employed to maintain a constant rate of absorption of the test compound through the skin, that is, the so-called steady-state flux.

With infinite dosing, it is generally assumed that there is no change in permeant concentration within the formulation throughout the experiment. When using Franz diffusion cells, this corresponds to a constant permeant concentration (or, more accurately, no change in thermodynamic activity of the permeant) in the donor phase. In practice, this can be achieved by regularly replenishing the donor solution or, more usually, by the addition of a small excess of solid permeant to a saturated donor solution; assuming that dissolution of the solid is not rate-limiting, then as the permeant leaves the donor solution, it is replaced by molecules entering the solution from the solid excess (Megrab et al. 1995). Alternatively, infinite dosing is commonly assumed by administering an amount of the

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formulation large enough to preclude any 'significant' reduction in the test compound or any other component in the donor phase during the course of the experiment (Franz et al. 1993). In these cases, a 'significant reduction' usually occurs when the permeant concentration in the donor phase declines by more than 10 % of the initial value (Kielhorn et al. 2006).

Under finite dose conditions, permeant concentration in the formulation changes during the experiment, due to penetration of the permeant into and permeation through the skin barrier, or due to evaporation. Generally, the experimental conditions should mimic as closely as possible the in vivo situation in order to provide suitable data. The amount and concentration of permeant formulation to be applied to the skin surface, as well as the duration and procedure of the protocols, depend on the study aims. For example, a small amount of permeant formulation may be applied in order to mimic in vivo application of an ointment. According to the Organisation for Economic Co-operation and Development (OECD), the application of ≤10 µl cm^{-2} of a liquid or 1–5 mg cm^{-2} of a solid formulation should be used for finite dose studies (OECD 2004a). For infinite dose, amounts of >100 µl cm^{-2} or >10 mg cm^{-2} are required.

3.2 Skin Absorption Kinetics

Kinetically, the absorption of a substance into or through the skin is regarded as a passive diffusion process. It is also assumed that the skin is a pseudohomogeneous membrane providing a single transport route. Thus, both infinite dose and finite dose experiments can be described by Fick's laws of diffusion.

Typically, the parameters describing the absorption of a substance through the skin barrier (such as the permeability coefficient and lag time) are obtained by evaluating the time-dependency of its cumulative permeated amount. An important parameter is the flux, J, which is proportional to the concentration gradient according to Fick's first law of diffusion (Eq. 3.1). J can be estimated from the cumulative amount of permeant passing through a unit area of membrane at time t (Crank 1975):

$$J = -D\frac{\delta c}{\delta x} \tag{3.1}$$

where J is the flux of the permeant (mass per cm^2), D is the diffusion coefficient and $\delta c/\delta x$ is the concentration gradient. To determine how diffusion affects the permeant concentration with time, Fick's second law of diffusion (Eq. 3.2), which is derived from Fick's first law of diffusion and the differential mass balance, can be employed:

$$\frac{\delta c}{\delta t} = D\frac{\delta^2 c}{\delta x^2} \tag{3.2}$$

This equation assumes that the permeant does not bind, nor is it metabolised. It is also assumed that the diffusion coefficient does not vary with the position or composition of the permeant, and that the barrier properties of the skin remain constant over time (Crank 1975).

3.2.1 Permeation Kinetics

3.2.1.1 Infinite-Dose Permeation

A typical infinite dosing regimen will produce a permeation profile of cumulative amount permeated across a unit area of membrane versus time, as shown in Fig. 3.1. Initially, when a permeant is applied to the skin, molecules penetrate into and diffuse through the stratum corneum. Depending on the permeants' physical and chemical properties (e.g. lipophilicity, hydrogen bond acceptor or donor potential), it may penetrate and diffuse rapidly or may bind to stratum corneum components. A lag phase is seen where the amount of permeant in the receptor compartment increases exponentially due to binding and the increasing concentration of permeant in the stratum corneum. After a sufficient time (again dependent on the nature of the permeant, typically shorter for lipophilic non-bound molecules, longer for hydrophilic molecules), binding sites become fully occupied (or at steady-state equilibrium), and a steady-state concentration gradient of the permeant develops across the membrane. Under these conditions, the flux profile becomes essen-

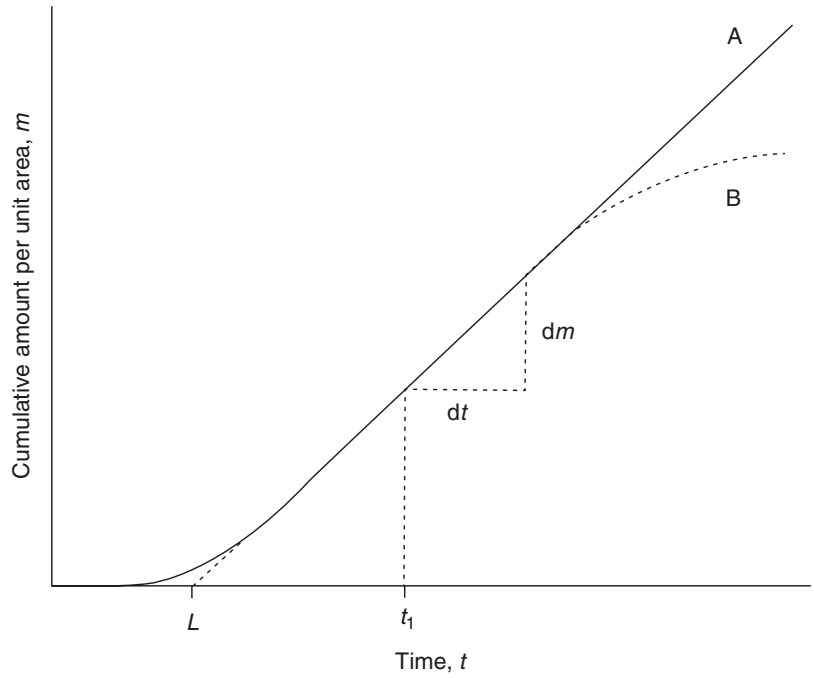

Fig. 3.1 Graph A (solid line) represents a typical permeation profile for an infinite dose regimen. The intercept on the horizontal axis, L, gives the lag time. The steady-state flux (J_{ss}) is obtained from the gradient (dm/dt) of the linear portion of the graph, from $t = t_1$, where $t_1 \geq 2.7\ L$. Graph B shows an alternate situation where a plateau is reached following prolonged incubation, indicating deviation from infinite dose conditions, due possibly to donor depletion or non-sink conditions

tially constant, and the curve approaches a straight line. The linear portion of the graph represents the steady-state flux (J_{ss}) and can be determined by simple linear regression of the linear portion of the graph (Crank 1975; Scheuplein and Blank 1971).

Typically, the steady-state flux is assessed from an in vitro experiment under infinite dose conditions, with 'perfect' sink conditions where the receptor compartment is at zero concentration throughout. J_{ss} can also be estimated by

$$J_{ss} = \frac{DC_0}{h} \tag{3.3}$$

where C_0 is the concentration in the outermost first layer of the skin and h is the membrane thickness. Practically, it is very difficult to measure C_0. However, the concentration of the permeant in the donor vehicle, C_v, is easily obtainable or is usually known. Since C_0 and C_v are related to the partition coefficient between donor and membrane (K_m), where

$$C_0 = K_m C_v \tag{3.4}$$

then substitution of Eq. 3.4 into Eq. 3.3 gives Eq. 3.5, which is the most widely applied mathematical model in examining skin permeation data:

$$J_{ss} = \frac{DK_m C_v}{h} \tag{3.5}$$

The lag time can be obtained by extrapolating the steady-state (linear) portion of the permeation graph to the intercept on the horizontal axis. Crank (1975) showed that the lag time, L, can be determined mathematically by

$$L = \frac{h^2}{6D} \tag{3.6}$$

It has been estimated that the time required for most permeants to achieve steady-state flux is about 2.7 times the lag time (Barry 1983).

Permeant transport across the skin is sometimes described in terms of the permeability coefficient (K_p), essentially a measure for the speed of transport across a membrane (often as cm/h):

$$K_p = \frac{K_m D}{h} = \frac{J_{ss}}{C_v} \tag{3.7}$$

Typically, the steady-state flux J_{ss} and the permeability coefficient K_p are obtained from an in vitro

infinite dose experiment. As described above, J_{ss} is obtained from the gradient of the linear portion of the permeation profile, and therefore, if the concentration of the permeant in the applied vehicle (C_v) is known, then K_p can be determined. K_p is often used to characterise the skin permeation of permeants, as calculations for other parameters such as D and K_m can be problematic as the membrane thickness (h), or the diffusional path length, is often unknown.

Estimates of steady-state flux and permeability coefficients should only be derived from data points beyond 2.7 times the lag time when (pseudo-) steady-state conditions are established (Kielhorn et al. 2006); using data before steady state is established leads to false estimates of permeability coefficients (Shah et al. 1994). It has been recommended that infinite dose permeation experiments should last for at least 24 h (OECD 2004a). However, an increase in exposure time may alter the integrity of the skin barrier (Kleszczyński and Fischer 2012). Typically, experiments are performed for 24–48 h (Boonen et al. 2012; Karadzovska et al. 2012; Fasano et al. 2012; Brain et al. 2005; Walters et al. 1997), although shorter durations have also been reported (Chen et al. 2011). It has been suggested that 72 h or even longer may be needed in some cases for an infinite dose to establish steady-state flux, especially for permanents that have very low fluxes or present difficulties for detection (Franz et al. 1993; Howes et al. 1996).

Although Eqs. 3.5, 3.6 and 3.7 are commonly used to evaluate infinite dose experiments, it is assumed that the skin membrane acts as a homogeneous membrane and that permeation through the stratum corneum is the rate-limiting factor for transdermal transport. This assumption is usually valid for most permeants, but for highly viscous vehicles, highly lipophilic or highly hydrophilic molecules, partitioning behaviour may become the limiting factor (Cross et al. 2001). Also, the equations assume perfect sink conditions throughout the experiment in order to ensure drug permeation is not affected by solubility in the receptor phase. For in vitro studies, flow-through diffusion cells maintain sink conditions by constant replenishment of the receptor fluid, but this may lead to dilution of the penetrant below the detection limit. For static diffusion cells, it is commonly regarded that sink conditions are maintained when the receptor fluid does not contain more than 10 % of the saturated concentration of the penetrant (Ng et al. 2010; Skelly et al. 1987). The most widely used receptor fluid is isotonic buffered saline, pH 7.4. However, for highly lipophilic compounds, the solubility in the receptor fluid may become the rate-determining step in skin absorption and may have a significant effect on the total flux measured. When solubility is a concern, the receptor phase can include lipophilic solvents which do not affect the skin barrier or a solubilising agent (e.g. 50 % ethanol, 6 % polyethylene glycol, 20 % oleyl ether or 5 % bovine serum albumin) (Skelly et al. 1987; OECD 2004a).

In reality, even under infinite dose conditions, depletion of the donor phase, non-sink receptor conditions and deterioration of the skin can occur with time. These factors may result in inaccuracies in steady-state flux and lag time estimations (Moody 2000). Therefore, particular caution has to be exercised in experiments where prolonged incubation times are necessary.

3.2.1.2 Finite-Dose Permeation

In vitro percutaneous absorption studies often utilise an infinite dose regimen to define a permeant's properties, that is, steady-state flux, permeability coefficient and lag time. However, a major limitation of infinite dosing is its failure to mimic the application of topical drug formulations in common clinical situations, where a relatively small amount (i.e. a finite dose) of the formulation is used. Under these circumstances, steady-state permeation seldom occurs and, therefore, the permeability coefficient cannot be determined. Most in vivo experiments are based on finite dosing, although in some cases, infinite dose conditions may result when finite doses are applied repeatedly.

In contrast to the infinite-dose permeation profile, finite-dose application may result in a 'pseudo steady-state' condition, where the amount permeated may be transiently linear but then reaches a plateau, beyond which the amount

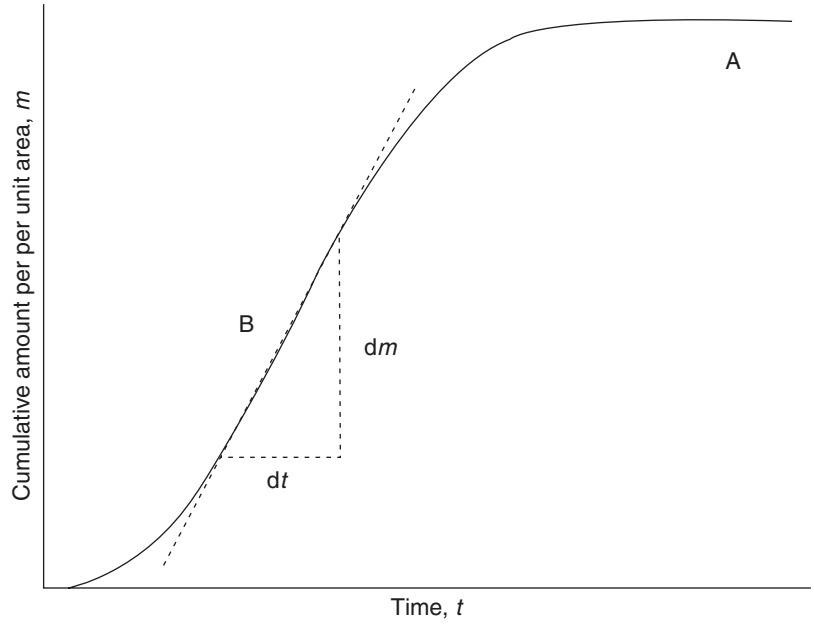

Fig. 3.2 A typical permeation profile for a finite-dose regimen. Depletion of the donor phase gives the characteristic plateau in region A. The maximum flux (J_{max}) is obtained from the gradient (dm/dt) of the near-linear portion of the curve in region B. Given that steady-state flux may not be obtained before donor depletion becomes significant, the maximum flux may not represent steady-state flux

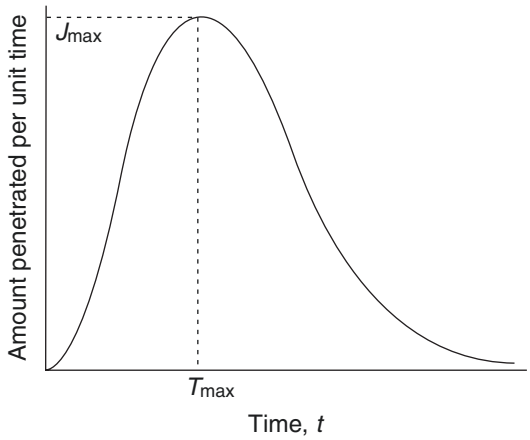

Fig. 3.3 Estimation of maximum flux (J_{max}) from a graph of amount penetrated per unit time (assuming constant permeation area) versus time. In this case, the vertical axis represents instantaneous flux. The time taken to reach maximum flux is referred to as T_{max}

permeated remains constant due to donor depletion (Fig. 3.2).

Alternatively, by plotting the amount penetrated between the time points (i.e. instantaneous flux) against time (Figs. 3.3 and 3.4), a peak is observed which corresponds to the maximum flux before appreciable donor depletion (Franz 1983; Kasting 2001). The maximum flux (J_{max}) and the time to maximum flux (T_{max}) are the most commonly reported parameters in finite dosing. These can be represented by (Scheuplein and Ross 1974; Crank 1975; Kasting 2001)

$$J_{max} = \frac{1.85DC_0\delta}{h^2} \tag{3.8}$$

$$T_{max} = \frac{h^2 - \delta^2}{6D} \tag{3.9}$$

Here, D is the apparent diffusion coefficient, C_0 is the concentration of the permeant in the first layer of the stratum corneum, h is the thickness of the stratum corneum and δ is the thickness of the finite dose layer on the skin surface. For a finite dose, since δ is considerably smaller in comparison with h, δ can be neglected, leading to

$$T_{max} = \frac{h^2}{6D} \tag{3.10}$$

Thus, it is possible to estimate the apparent diffusion coefficient (D), if the values of J_{max} and T_{max} are known. In addition to this mathematical model, J_{max} and T_{max} are sometimes obtained graphically from experimental data (Figs. 3.2 and 3.3) (van de Sandt et al. 2004; Wilkinson et al. 2006).

Due to the nature of finite dosing, experimental problems can ensue, since applying a small

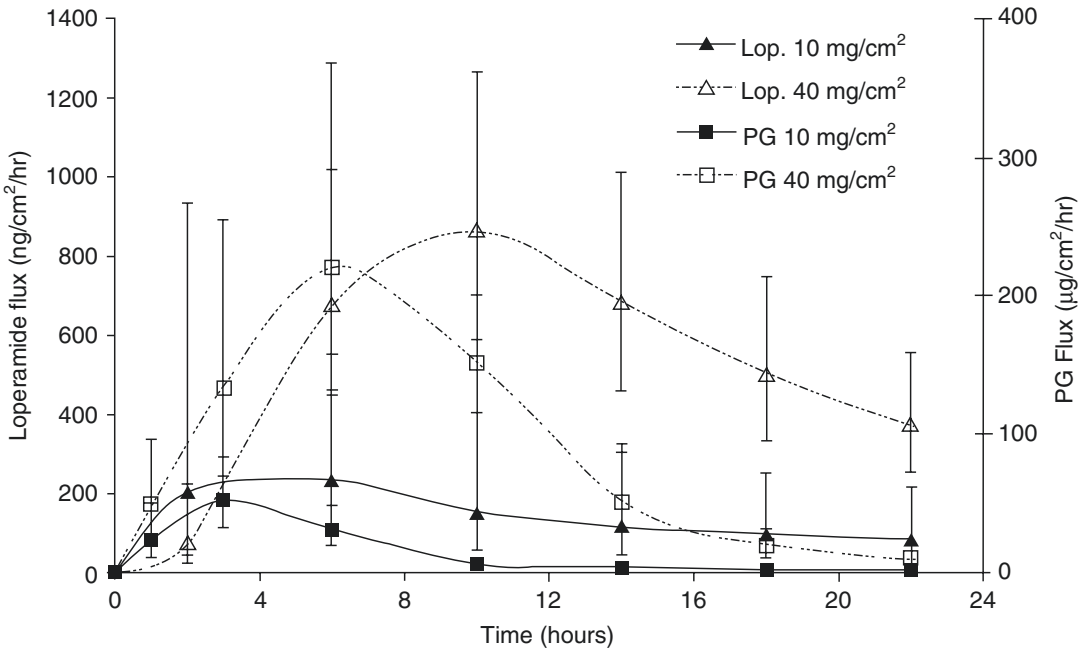

Fig. 3.4 Exemplar data showing instantaneous flux of finite doses of loperamide and propylene glycol (PG) through human skin over time (Data are from Trottet et al. (2004). Figure copyright (2012) reprinted with permission from Elsevier)

finite dose formulation evenly across a membrane in vitro can be difficult. Since the bioavailability of drugs applied to the skin in finite doses depends on the amount of drug applied per unit area (Wester and Maibach 1976; Franz et al. 1993), a homogenous drug distribution over the whole donor area is needed to minimise variation within and between experiments.

Applying semisolid formulations homogenously poses more complications than liquid dosing. Semisolids are usually applied manually, using a mechanical device such as a glass rod (Hadgraft et al. 2003; Franz et al. 1993; Brain et al. 1995; Gupta et al. 1999) or spatula (Dreher et al. 2002; Trottet et al. 2004; Youenang Piemi et al. 1998) to help distribute the formulation evenly on the skin surface. In this case, it is important that the application device is also analysed in order to determine the actual dose applied to the skin. This is commonly done by extraction (Gupta et al. 1999) of the remaining drug from the application device, or by weight difference (Walters et al. 1997; Brain et al. 1995; Vallet et al. 2007). Sometimes, the permeant is dissolved in a volatile medium to allow easy application and homogenous distribution (Kasting and Miller 2006; Southwell and Barry 1984). The volatile solvent evaporates, leaving a thin film of the drug on the skin surface. However, the volatile solvent may extract some lipids from the stratum corneum, affecting the integrity of the skin membrane. Another method used for uniform dose distribution is by massaging the formulation onto the skin surface, but this mechanical stress may influence the absorption rate in some cases (Amidouche et al. 1994; Lademann et al. 2007, 2009). To date, a very limited number of publications have investigated drug formulation distribution over the incubation area (Lademann et al. 2008); homogenous distribution is generally assumed in many reports.

The degree of occlusion of diffusion cells during experiments can affect skin hydration and, consequently, drug permeation (Akhter and Barry 1985; Zhai and Maibach 2001; Sarpotdar and Zatz 1986). Some researchers recommend that the skin be exposed to ambient conditions to simulate the in-use conditions of topically applied formulations (Wilkinson and Williams 2002; Grégoire et al. 2009). However, evaporation of

the vehicle and/or permeant can occur in non-occluded experiments which can impact significantly permeant absorption (Frasch et al. 2011). Moreover, the skin may become dry, and its barrier function can thus be affected (Selzer et al. 2013). Occlusion is also used during finite dosing to avoid drying of the skin surface due to application of small amounts of formulation, or to prevent evaporation, which could lead to a change in permeant concentration (van de Sandt et al. 2004; Koyama et al. 1994). However, care is required to avoid damage to skin integrity caused by excessive stratum corneum hydration (Zhai and Maibach 2002).

For finite dose studies, exposure times usually reflect 'in-use' scenarios to allow quantification of permeant absorption over a realistic period of time that relates to potential human exposure (OECD 2004a). Exposure times of up to 48 h are commonly used (Hadgraft et al. 2003; Gupta et al. 1999; Walters et al. 1998), but may be shorter to mimic exposure to rinse-off products (Brain et al. 1995, 2005; Okuda et al. 2011), or in studies on occupational exposure to hazardous materials (Howes et al. 1996; Moody et al. 2007). In such cases, data collection commonly continues for at least 24 h. On the other hand, if the drug formulation is for a once-a-week product, for example, the experiment design should mimic the application exposure period and thus should be performed over 7 days (Williams 2003).

Due to the limited amount of test substance used in finite dose studies, it is important that a mass balance is performed on completion of the experiment to ensure the total amount of applied permeant could be recovered. The OECD guidelines state that the mean recovery of the permeant and metabolites should be in the range of 100 ± 10 %, and reason must be sought if this is not met (OECD 2004a, b). However, under certain circumstances, the test of a volatile substance that had to be trapped in a filter, recovery can be broadened to 100 ± 20 % (OECD 2004a; European Commission 2006). The mass balance should include permeant remaining in the donor phase, that within the skin and all equipment in contact with the test substance (e.g. the donor and receptor chambers of the diffusion cell). In some cases, the recommended total recovery may not be achieved, for example, due to the limits of analytical detection or difficulties with simple extraction methods. Degradation of the permeant could also aggravate the accuracy of total recovery. For example, skin esterase has been shown to degrade permeants in situ (Lau et al. 2010, 2012). Chemical degradation could also have a significant impact on permeant stability (Kubota et al. 1995; Müller et al. 2003). The degradation products are likely to have lower permeation rates than the test compound. Thus, failure to detect degradation products using suitable analytical methods may confound experimental results.

3.2.2 Penetration Kinetics

3.2.2.1 Infinite-Dose Penetration

Although skin permeation data allow the transport parameters across skin to be determined, constructing a drug concentration/depth profile can be valuable. The tape-stripping technique is most commonly used to assess the change in permeant concentration throughout the stratum corneum (Lau et al. 2010; Thomas and Heard 2007; Weerheim and Ponec 2001; Pellett et al. 1997; Mohammed et al. 2012; Stinchcomb et al. 1999; Dreher et al. 1998). This is a method, whereby the stratum corneum is progressively removed by adhesive tape and the drug within each tape is then extracted and determined to calculate the diffusivity and solubility of the drug within the stratum corneum.

Under infinite dosing, it is possible to predict the concentration of the penetrant ($c_{(x,\ t)}$) as a function of position and time within the stratum corneum, using the appropriate solution of Fick's second law of diffusion (Anissimov et al. 2012; Moser et al. 2001; Selzer et al. 2013):

$$c_{(x,t)} = KC_0\left[\left(1-\frac{x}{h}\right)-\frac{2}{\pi}\sum_{n=1}^{\infty}\frac{1}{n}\sin\left(\frac{n\pi x}{h}\right)\exp\left(\frac{-Dn^2\pi^2 t}{h^2}\right)\right] \tag{3.11}$$

In Eq. 3.11, K is the partition coefficient between the stratum corneum and the formulation vehicle, x is the vertical position within the stratum corneum (where $0 \leq x \leq h$), t is the time at which the permeant concentration is to be determined and D/h^2 gives the characteristic diffusion parameter. If the concentration of the permeant in the stratum corneum is obtained experimentally, the experimental concentration profiles can be fitted into Eq. 3.11 to determine K and D/h^2 (Pirot et al. 1997). Again, it is assumed that the skin is a homogenous membrane and that the permeant transports across the stratum corneum by passive diffusion. In addition, the formulation should not alter the membrane integrity or act as a carrier for the test permeant. It is also assumed that the experiment is maintained under sink conditions, and the stratum corneum is the rate-limiting barrier.

3.2.2.2 Finite-Dose Penetration

Similarly, with finite dosing, it is possible to predict permeant concentration at a given position inside the stratum corneum and at a given time, using Eq. 3.12 (Kasting 2001; Selzer et al. 2013):

$$c_{(x,t)} = 2KC_{v0}\sum_{n=1}^{\infty}\frac{\beta\cos\left(\alpha_n x/h\right)-\alpha_n\sin\left(\alpha_n x/h\right)}{\beta+\beta^2+\alpha_n^2}\exp\left(-\frac{\alpha_n{}^2 Dt}{h^2}\right) \tag{3.12}$$

where C_{v0} is the initial concentration of the permeant in the donor compartment, $\beta = K(h/h_v)$, h_v being the theoretical height of the volume of donor formulation and α_n is related to β such that $\alpha_n \cdot \tan\ \alpha_n = \beta$. However, very few studies have made use of this mathematical model.

Conclusion

Finite and infinite dose regimens have different applications in transdermal delivery. Each approach presents its own advantages and challenges. Mathematical models allow us to predict, characterise and compare the skin absorption kinetics relating to finite or infinite dosing. In this respect, they are an invaluable tool for transdermal delivery. However, in applying these models, it is important to appreciate the underlying assumptions and limitations of the models.

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