Prediction of skin permeation and concentration of rhododendrol applied as finite dose from complex cosmetic vehicles

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ABSTRACT

Finite dose experiments represent clinical use wherein depletion of dose, evaporation of excipients, and gradual change in vehicle composition may occur. In the present study, we attempted a mathematical approach for predicting skin permeation and concentration of a cosmetic active, rhododendrol (RD), from complex vehicle-based formulations applied in finite dose. *In vitro* skin permeation and concentration studies of RD were conducted from formulations containing water and polyols with concentrations ranging from 10 – 100% under infinite and finite dose conditions using vertical Franz diffusion cells. Observed data for skin permeation and the viable epidermis and dermis (VED) concentration of RD were estimated by the differential equations under Fick's second law of diffusion together with water evaporation kinetics and changes in the partition coefficient from vehicles to the stratum corneum. As a result, a goodness-of-fit was observed allowing accurate estimation of skin permeation and VED concentration of RD. This mathematical approach could become a useful tool to estimate the skin permeation and concentration of actives from topical formulation applied in finite dose conditions likened in actual use.

Keywords: skin permeation, skin concentration, finite dose, cosmetic ingredients, Fick's second law of diffusion, residual formulation

1. INTRODUCTION

A number of cosmetic formulations are made of quasi-drugs (active compounds) effectively dissolved in complex vehicle systems. These formulations may contain components that enhance or decrease the penetration of active compound or other components. In addition, vehicle composition may change after topical application due to low amount of formulation applied. Therefore, the permeation of chemicals from a small amount of topically applied formulation in its in-use conditions is difficult to simulate experimentally. The finite dose experiment is supposed to best represent its clinical use (i.e, in-use conditions) wherein depletion of dose and evaporation of the excipients may occur. Investigating the percutaneous absorption of chemicals, under its in-use conditions, has been presented with huge challenges associated with incomplete recovery of the applied formulation, low extraction ratio of compounds from the skin, and inter-run variabilities for key parameters (e.g., skin permeability, partition coefficient from vehicle to skin) in such experiments (Selzer et al., 2013). To date, no definitive method has been established to address these challenges.

On the other hand, the penetration of chemicals from aqueous vehicles in infinite dose models under steady-state conditions (i.e., non-depleting dose) can generally be predicted based on their physicochemical properties (Magnusson et al., 2004; Uchida et al., 2015). However, steady-state conditions are typically unattainable in finite dose experiments where dose depletion takes place. The assumption of steady-state conditions does not apply to finite dose experiments since a high evaporation rate of applied solvents occurs after application. Generally, rapid evaporation of solvents occurs, which significantly alters the effective diffusion area of the applied formulation and the composition of the resulting residual formulation after formulations are applied on the skin (Arce, et al., 2019; Otto, et al., 2008; Poulsen, 1973). In contrast, the majority of studies done to asses this phenomenon were performed with infinite dose conditions, whereas only a limited number of studies have been

conducted for finite dose conditions. Hence, caution must be considered in extrapolating data derived from infinite dose experiments or experiments in which exposure occurs via simple aqueous vehicles, because these do not necessarily reflect the complexities of most formulations used in practice. In addition, few studies have been conducted to predict skin permeation in finite dose settings with the use of actual cosmetic formulations. Appropriate alternatives in modeling this phenomenon must then be adopted to enhance this point and better predict skin permeation for in-use conditions. Predicting skin permeation of cosmetic active compounds in finite dose settings will not only provide insights on local toxicity but also allow prediction of its systemic absorption.

In our previous work, we demonstrated the impact of in-use conditions such as layered application, evaporation in formulations, and sequential and concurrent application of polyols with cosmetic formulation in the skin permeation of cosmetic active compounds. Layered application of cosmetics and concurrent application of polyols dramatically reduced the skin permeation of active compounds (Arce et al., 2019). Findings from various reports had diverging claims on the roles of solubility in the skin permeation of chemicals under finite dose conditions (Karadzovska et al., 2013; Wiechers et al., 2012; Lane et al., 2012). Several studies have focused on estimating the amount of chemical permeating through the skin based on the physicochemical properties of permeants and formulations, yet they neglected the essential role of evaporation in the actual permeation of chemicals (Potts and Guy, 1992; Dias et al., 2007; Uchida et al., 2015). Furthermore, little is known about what governs the skin permeation and concentration of chemicals applied as a finite dose.

The efficacy and safety of cosmetics and locally acting drugs applied on skin are determined by their distribution into its intended site of action, most likely the viable epidermis and dermis (VED), and not the stratum corneum (SC). Skin whitening agents from cosmetics or steroids and antimicrobials from topical medications must be studied for their distribution

and concentration in the VED (Sugibayashi et al., 2010). The epidermal layers being the primary site of action for these products offer direct insights for safety assessments or product optimization. The importance of the concentration in the VED is greater for cosmetics and topical medications that are capable of causing skin irritation and inflammation (Oshizaka et al., 2014). In recent times, the toxicity of cosmetic active compounds may be represented well by reports on rhododendrol (RD)– related leukoderma. In this case, evaluation methods for dermatological products based upon appropriate skin models and in-use conditions are important to confirm dose-dependent toxicity of compounds at the site of action. Determining the distribution of chemicals in the VED is of great significance for cosmetic formulations, where they are expected to maintain their effective concentrations. Quantification of permeant concentration in the skin allows a high precision in predicting their efficacies or toxicities.

Establishing mathematical models aimed at predicting skin or VED concentration of chemicals entails understanding of the factors that influence skin permeation. Therefore, this investigation probed the possible role of evaporation and the composition of residual formulations on the skin permeation and concentration in finite dose conditions. The actual impact of vehicle on the skin permeation and concentration of the penetrant can be realistically clarified by simulating the residual formulation based on evaporation kinetics from applied formulations. We employed various polyols commonly used as solvents in cosmetics and simulated residual formulations composed of high polyol proportions to reveal its role in the skin permeation of active compounds. Here, we propose a method that allows investigation of the permeant disposition from residual formulations encompassing evaporation, which is a natural process during use. This is an extension of our inquiry on the fate of cosmetic active compounds from complex formulations in actual product in-use conditions (e.g., layered application, finite dose conditions). Experiments in steady-state conditions for simulated residual formulations were conducted to allow surrogate estimation of skin permeation

parameters in finite dose exposures. In the present study, we attempted to establish a mathematical method in predicting skin permeation and the concentration of cosmetic active compounds applied in finite dose from a complex vehicle-based formulation.

2. MATERIALS AND METHODS

2.1. Materials

RD (CAS no. 501-96-2, ≥ 99%) was supplied by Kanebo Cosmetics, Inc. (Tokyo, Japan). Methylparaben and glycerin were purchased from Fujifilm Wako Pure Chemicals Industries, Ltd. (Osaka, Japan). Sorbitol, trichloroacetic acid and 1,3-butylene glycol (BG) were purchased from Tokyo Chemical Industry, Co. Ltd (Tokyo, Japan) while dipropylene glycol (DPG) was purchased from Sigma Aldrich Chimie (Saint-Quentin-Fallavier, France).

The complex vehicle, a recalled lotion of RD, was supplied by Kanebo Cosmetics, Inc. It was primarily composed of water and a mixture of polyols (DPG, glycerin, BG, and sorbitol; each concentration is shown in 2.2).

2.2. Preparation of RD formulations

Aqueous formulation of RD (1%) (Table 1) was prepared by dissolving RD in a sufficient amount of purified water in a volumetric flask. An RD concentration of 1% was selected instead of 2% due to its limited solubility with water.

The polyol mixture was composed of DPG (46.15%), glycerin (23.08%), BG (20.51%), and sorbitol (10.26%) identical to that of the recalled formulation. A prepared lotion formulation (2% RD) containing identical total polyol concentration, 19.5% and water, of the recalled lotion, was also prepared (Table 1).

To reflect formulation conditions in the residual phase, formulations depicting polyol concentration following evaporation were developed. Simulated residual formulations of RD

(2%) lotion were designed to reflect varying degrees of evaporation from the formulation hence, polyol concentrations of 40%, 61.8%, and 100% (Table 1) were adopted. These polyol concentrations were particularly selected to reflect low, middle, and high degrees of water evaporation from the residual phase. These formulations were prepared by addition of a sufficient amount of purified water with its corresponding polyol proportions in a volumetric flask.

2.3. *In vitro* skin permeation experiment

Frozen porcine ears (Central Institute for Feed and Livestock; JA Zen-Noh, Tsukuba, Ibaraki, Japan) were thawed with warm water and rinsed with purified water. Hairs were trimmed and shaved, and subcutaneous fats were excised off the skin. Skin was harvested from the central dorsal region of the ears. Before excision, visual inspection was performed to ensure the integrity of the skin. Only intact and damage-free skin was excised. For stripped skin, adhesive tape was applied on the SC side and stripped 20 times prior to excision. Isolated porcine skin was set on vertical type Franz diffusion cells (effective diffusion area of 1.77 cm^2). Skin surface temperature was maintained at 32°C throughout the experiment. The receiver compartment was filled with 6.0 mL of purified water. Prior to the application of doses, the skin was applied with purified water (1.0 mL) to facilitate equilibration for 1 h. Water was then carefully removed and skin surface was blotted with a cotton swab to remove excess water. Using a positive displacement micropipette, RD formulations (1% RD aqueous, 2% RD in 19.5% polyol, 2% RD in 40% polyol, 2% RD in 61.8% polyol, 2% RD in 100% polyol) were applied as either finite (17.7 μL/ 1.77 cm^2) or infinite dose (1.0 mL/1.77 cm^2). At a predetermined schedule, an aliquot (500 μL) was withdrawn from the receiver solution. Permeation experiments were performed for 0 – 4 h or 0 – 8 h.

2.4. Skin concentration experiment

The skin concentration of RD was determined using identical experimental conditions as the skin permeation experiment using both intact and stripped skin. Formulations were applied in infinite and finite doses. Skins were demounted from the diffusion cells and adhering formulations were removed at 4 h and 8 h after the start of skin permeation experiment. Skins were rinsed thrice on both sides with purified water and blotted dry with tissue paper. Tape-stripping (20 times) was performed on the intact skin to isolate the VED. A sample (0.05 g) of the VED was reduced in size using a pair of scissors. Then, water was added and the skin was homogenized using a Polytron PT 1200E (Kinematica, Inc., Luzern, Switzerland) for 5 min. Samples were deproteinized by the addition of 16% trichloroacetic acid. The samples were agitated using a vortex mixer for 15 min, followed by centrifugation (15,000 rpm, 4°C) for 5 min. The supernatant liquid was prepared for quantification.

2.5. Water evaporation from formulation experiment

Evaporation of water from the recalled formulation was determined gravimetrically by monitoring weight loss of the applied solvent/formulation over time. The weight of an empty glass-bottom dish was first measured using an analytical balance (AUW220D; Shimadzu, Kyoto, Japan). Balance reading was deemed stable when differing readings are less than ± 0.0001 g within 3 min. A finite dose (17.7 μL) of lotion was evenly applied using a micropipette and the initial weight of the applied formulation was recorded. The set-up was placed on a thermostatically (32°C) maintained heating plate (AS ONE, Osaka, Japan). Surface temperature was monitored (32 ± 1°C) using probe and infrared thermometers throughout the experiment. Water loss (weight of the setup) was recorded over time at intervals of 1 min until constant weight was attained.

2.6. Solubility of RD in residual formulations

The solubility of RD was performed in a wide range of polyol concentrations (10.0%, 19.5%, 40.0%, 61.8%, and 100%) simulating various stages of evaporation in the residual formulations based on our previous work (Arce et al., 2019). The excess amount of RD was stirred inside a capped vial immersed in a thermostatically controlled water bath (32°C) for 48 h. This approximated the solubility of RD in the residual formulation on skin. Dissolved RD in solvents/simulated residual formulations were filtered and analyzed using high-performance liquid chromatography (HPLC).

2.7. Quantification method for RD

RD was analyzed using HPLC as described previously (Arce et al., 2019). Briefly, 100 µL of samples were added with an equal amount of internal standard (methylparaben) and centrifuged at 4°C for 5 min. Samples were injected into an HPLC system and analyzed for RD concentration at 280 nm.

2.8. Theoretical

2.8.1. Concentration-distance profile of a penetrant in SC and VED

Skin diffusion model of a penetrant is generally expressed in its concentration-distance profile as shown in Figure 1. As such, a two-layered diffusion model can be used for penetrant diffusion through the full-thickness skin (SC + VED double membrane) while one-layered diffusion model is sufficient for SC-stripped skin (VED single membrane).

2.8.2. Fick's second law of diffusion and related initial and boundary conditions

In the case of a two-layered diffusion model under infinite dose condition, SC and VED concentration of a penetrant (C_{sc}, and C_{ved}) at position, x, and time, t, can be described by the

following Fick's second law of diffusion described in our previous papers (Hada et al., 2005; Sugibayashi et al., 2010; Ishii et al., 2010; Oshizaka et al., 2014).

$$\frac{\partial C_{sc}}{\partial t} = D_{sc}\frac{\partial^2 C_{sc}}{\partial x^2} \quad (1)$$

$$\frac{\partial C_{ved}}{\partial t} = D_{ved}\frac{\partial^2 C_{ved}}{\partial x^2} \quad (2)$$

where D_{sc} and D_{ved} are the effective diffusion coefficients of a penetrant in SC and VED, respectively.

Initial and boundary conditions for penetrant concentration in infinite dose system were as follows:

t=0	$-L_{sc} < x < 0$	$C_{sc} = 0$	(3)
	$0 < x < L_{ved}$	$C_{ved} = 0$	
t>0	$x = -L_{sc}$	$C_{sc} = K_{sc} \cdot C_v$	(4)
	$x = 0$	$C_{ved} = K_{ved} \cdot C_{sc}$ and $D_{sc}\frac{dC_{sc}}{dx} = D_{ved}\frac{dC_{ved}}{dx}$	
	$x = L_{ved}$	$C_{ved} = 0$	

where L_{sc} and L_{ved} are the thicknesses of SC and VED, respectively; K_{sc} and K_{ved} are the partition coefficients of the penetrant from the donor (vehicle) to SC and VED, respectively; C_v is penetrant concentration in the applied formulation (donor or vehicle). In the present RD permeation experiments through excised porcine ear skin, L_{sc} and L_{ved} were set to be 20 μm and 1480 μm, respectively.

Against Eq. (4) for the infinite dose system, the boundary condition only at $x = 0$ in the finite dose system becomes,

$t > 0$	$x = -L_{sc}$	$C_{sc} = K_{sc} \cdot C_v$	(4')
$x = 0$	$V_v\frac{dC_v}{dt} = D_{sc}\frac{dC_{sc}}{dx}$		
$x = L_{ved}$	$C_{ved} = 0$		

where V_v is the volume of the vehicle (donor solution). The equation in the second line in Eq. (4') means that the decreased flux of the penetrant in the donor compartment is the same to the increased flux at x = 0 in the SC. When the amount of the penetrant permeated in the finite dose through membrane is very low, Eq. (4) can be used instead of Eq. (4'). Only a low percentage or less amount of RD permeated though skin in the case of the present RD skin permeation experiment, suggesting that Eq. (4) can be used for Eq. (4') even at finite dose.

2.8.3. *Equations to determine the skin permeation rate and amount of a penetrant*

The skin permeation rate of penetrant per unit area, J, is expressed by Eq. (5) using Fick's first law of diffusion. The cumulative amount of the penetrant permeated per unit area, Q, is determined by integrating Eq. (5). Q is expressed by Eq. (6).

$$J = -D_{ved}\left(\frac{dC_{ved}}{dx}\right)_{x=L_{ved}} \quad (5)$$

$$Q = -D_{ved}\int_0^t \left(\frac{dC_{ved}}{dx}\right)_{x=L_{ved}} dt \quad (6)$$

These equations can be applied to both the infinite and finite dose systems.

2.8.4. Determination of D_{ved}, D_{sc}, K_{ved} and K_{sc}

The K_{ved} and D_{ved} can be obtained from permeation experiment using SC-stripped skin (VED single membrane) in the infinite dose system (Hada et al., 2005). (Details are shown in 2.3.6). Then, K_{sc} and D_{sc} are determined by the permeation experiment using full-thickness skin (SC + VED double membrane) in the infinite dose system. The obtained K_{ved} and D_{ved} values were fixed for calculating K_{sc} and D_{sc}.

2.8.5. Determination of $C_v(t)$ and $K_{sc}(t)$

RD formulations consisted of water and polyol mixture (Table 1) were applied on skin in the present study. Water evaporated from the formulation whereas polyols remained on the skin in the present finite dose experiments. Thus, C_v and K_{sc} must be expressed as a function

of time as in $C_v(t)$ and $K_{sc}(t)$. Then, the C_v of RD in different concentrations of polyol vehicles (19.5%, 40%, 61.8% and 100%) was determined, and in each concentration of polyols, C_v was calculated using spline interpolation. Time course of the polyol concentration was determined by the water evaporation data from formulation (see 2.5 in detail). Finally, the time course of $C_v(t)$ was obtained.

In addition, K_{sc} of RD from vehicles composed of 19.5, 40, 61.8 and 100% polyols were experimentally determined by the permeation experiment through full-thickness skin using infinite dose conditions. Permeation experiments through stripped skin were also done as mentioned above. The K_{sc} of RD from each concentration of polyols in the formulation to SC was then calculated using spline interpolation. The time course of the polyol concentration was determined by the water evaporation data as above. Thus, the time course of $K_{sc}(t)$ was obtained as like in $C_v(t)$.

2.8.6. *Differential equations to obtain C_{sc} and C_{ved} at any time and any position*

Differential equations describing Fick's second law of diffusion are as follows:

$$\frac{dC_{i,j}}{dt} = \frac{1}{\Delta t}\left(C_{i,j+1} - C_{i,j}\right) \quad (7)$$

$$\frac{d^2C_{i,j}}{dx^2} = \frac{1}{\Delta x^2}\left(C_{i-1,j} - 2C_{i,j} + C_{i+1,j}\right) \quad (8)$$

where $C_{i,j}$ shows concentration of penetrant in SC or VED at an i-th skin position and a j-th time after starting the skin permeation experiment (both i and j are natural numbers), and Δx is $x_{i+1} - x_i$ and Δt is $t_{j+1} - t_j$. Fick's second law of diffusion (Eqs. (1) and (2)) is expressed using the following differential equations, Eqs. (7) and (8). The following, Eq. (9), was obtained from Eqs. (7) and (8).

$$C_{i,j+1} = rDC_{i-1,j} + (1 - 2rD)C_{i,j} + rDC_{i+,j} \quad (9)$$

where $r = \Delta t/\Delta x^2$. Eqs. (5) and (6) can be expressed using these differential equations as follows:

$$J_j = -D_{ved}\frac{C_{n+1,j}-C_{n,j}}{\Delta x} \quad (10)$$

$$Q_j = Q_{j-1} + J_j \Delta t \quad (11)$$

where n is the number of divisions of SC or VED.

2.8.7. Determination of J_j and Q_j

J_j and Q_j were calculated using a spreadsheet, Microsoft® Excel by setting n = 10 both for SC and VED. In this calculation, Δt was set to be less than 0.5 for $D_{sc}\cdot r$ or $D_{ved}\cdot r$, because the solution will diverse at 0.5 or more for $D_{sc}\cdot r$ or $D_{ved}\cdot r$. Q_j was calculated from J_j using Eq. (11). First, experimentally observed Q values (Q_j) at every sampling time point in the infinite dose system were fitted by the least-squares method calculated using a quasi-Newtonian method in Microsoft® Excel Solver (Sato et al., 2001). Permeation parameters such as partition coefficients K_{sc}, K_{ved}, diffusion coefficients D_{sc}, D_{ved}, and permeability coefficient (K_p) were calculated using the analytical method described in our previous work (Hada et al., 2005).

C_{sc}, at any t, $C_{sc}(t)$, was calculated by the following equation:

$$C_{sc}(t) = K_{sc}(t) \cdot C_V(t) \quad (12)$$

where $K_{sc}(t)$ and $C_v(t)$ are obtained as shown in 2.8.4. We inputted $C_{sc}(t)$ in the spreadsheet in the present calculation. This was a kind of sequential approach to derive the calculation method.

2.8.8. Diagram of calculation method for C_{sc} and C_{ved}

In this work, permeation parameters, K_{sc}, K_{ved}, D_{sc} and D_{ved}, from 1% RD aqueous solution through intact and stripped skin were initially determined in the infinite dose system. Figure 2 presents a detailed flow diagram to obtain $K_{sc}(t)$and $C_v(t)$.

3. RESULTS

3.1. Evaporation of water from applied formulation

Water evaporation was evaluated from a recalled lotion formulation of RD solubilized in a complex mixture of polyols (DPG, glycerin, BG, and sorbitol). Evaporation kinetics from

the applied formulation was measured gravimetrically. The use of a glass-bottom dish allowed accurate measurement of water evaporation from the formulation applied as opposed to the use of isolated skin where intrinsic water loss may lead to overestimation. Observed data for water evaporation was in agreement with previous study (Arce et al., 2019) where ~60% of total water content evaporated within the first 10 min (Fig. 3). The amount of water detected (96.3%) at the end of the experiment corresponded closely to the actual water content of the recalled formulation. Exhaustive evaporation of water from the applied formulation was observed in this study. The evaporation rate from the formulation exhibited first-order kinetics and the percent water loss, y, was calculated using the following equation, $y = 103 \times e^{-0.093t}$, where t is the time after the start of experiment (Fig. 3).

3.2. Skin permeation of RD from aqueous formulation

Figure 4 presents the time course of the cumulative amount of RD permeated through intact and stripped skin. RD permeation was 13-fold higher through stripped skin from 1% RD aqueous solution compared with intact skin. Permeation parameters, diffusion coefficients (D_{sc}, D_{ved}) and partition coefficients (K_{sc}, K_{ved}) were obtained by curve-fitting the cumulative amounts of RD that permeated through intact and stripped skin to the theoretical values using a least-squares method. Table 2 shows the calculated values of the permeation parameters.

3.3. Skin permeation profiles of RD from infinite dose experiments

Figure 5A shows the cumulative amount of RD permeated through intact skin from lotion with different polyol concentrations (19.5% - 100%). Recalled lotion and prepared lotions, having identical proportions (19.5%) of polyols, resulted in similar skin permeation profiles with negligible variances. The K_{ved}, D_{sc}, D_{ved} values obtained from skin permeation experiments using 1% RD aqueous solution were fixed to estimate K_{sc} of RD formulations with

varying polyol concentrations (Table 3). Formulations with high polyol concentrations resulted in low K_{sc} values.

3.4. Relationship between polyol concentration and RD permeation

Figure 5B presents the correlation between the cumulative amount of RD that permeated through porcine skin and polyol concentration. When the polyol concentration increased from 19.5 to 40%, 61.8 and 100%, the skin permeation of RD was reduced by 1.8-, 3.8-, and 28.8-fold, respectively. The skin permeation of RD exhibited a positive inverse correlation (r^2 = 0.98) against the polyol concentration in formulation, suggesting that a high polyol concentration would yield lower skin permeation of RD.

3.5. Solubility of RD in simulated residual formulations

Solubility of RD in the simulated residual formulations revealed a positive linear correlation (r^2 = 0.99) with the polyol concentration in the formulations (Fig. 5C). High solubility of RD was observed in residual formulations containing high polyol concentrations (90.44 to 100%) and likewise low solubility at lower polyol concentrations (19.5 – 40%) (Fig. 5C). In residual formulation containing 61.8% polyols, wherein its water concentration was about half of its original concentration in the recalled lotion, yielded a 3-fold increase in RD solubility.

Water evaporation from formulation increased polyol concentration in the residual phase induced changes in the K_{sc} (Table 3). A high polyol concentration in the formulation was correlated with lower K_{sc} values (r^2 = 0.96; $K_{sc} = 0.54e^{-0.052x}$, where x is the concentration of polyol in the formulation).

3.6. Prediction of skin permeation and concentration of RD from complex cosmetic formulations

Figure 6 presents the time course of the cumulative amount of RD that permeated through skin and the concentration in the VED from recalled lotion. C_v was obtained from water evaporation in the formulations and the decrease in the amount of RD in the formulation by permeation through skin over time. The actual experimental data were plotted against the predicted values and well-fitting lines were observed in both skin permeation and concentration.

4. DISCUSSION

In the present study, we assumed that RD solubilized in complex polyol vehicles penetrate the shallow segment of the SC. Hence, K_{ved}, D_{sc}, D_{ved} were fixed and used in estimating K_{sc} of RD solubilized in polyol vehicles. This phenomenon is mainly influenced by two factors; high polyol concentration and water evaporation from formulation on the skin surface. These factors alter the drug partitioning into the SC and consequently regulate the amount of the permeants in and through the skin.

Evaporation of volatile components from applied formulations occurs particularly in finite dose conditions and clinical applications. This highlights the fact that the actual permeation of chemicals through skin is best manifested by simulating the conditions of the residual formulations wherein complete evaporation occurs in the residual phase. The rate of evaporation in the residual phase of the formulation determines its effective area of diffusion. The increase in polyol concentration in the residual phase caused by water evaporation is thus a major determinant in the skin permeation of active compounds. By using a broad range of polyol concentrations in simulating the residual formulations, a mechanistic approach can be provided to investigate the impact of evaporation in the skin disposition of RD.

Permeation of RD through intact and stripped porcine ear skin under infinite dose conditions was determined to evaluate the partition and diffusion parameters of RD. The well-

fitting line was obtained for RD allowing estimation of the effective diffusion coefficient in the VED by considering evaporation kinetics (Fig. 3) and the related changes in the C_v and K_{sc} (Fig. 5C). The same observation was reported by Potts and Guy in predicting the permeability of chemicals through skin from aqueous solutions (Potts and Guy, 1992). However, this was not observed in the case of a finite dose since the predicted parameters yielded poor-fitting line and thus, imprecise estimation of RD concentration in the VED (data not shown).

For infinite dose conditions, the formulation dynamics are maintained throughout the experiment with the concentration gradient favoring passive diffusion, a condition obeying Fick's first law. However, in a finite dose setup, the permeation environment is abruptly altered after application of the formulation. This 'new' environment, residual formulation, therefore dictates how chemicals permeate through the skin in finite dose exposures. Otto et al. (2008) stressed the need to understand the impact of evaporation on the formulations and the consequent transformations it undergoes after application onto the skin taking into consideration that the actual permeation occurs after complete evaporation from the residual formulation. This is a factor largely ignored despite the fact that the residual formulation differs considerably from the original formulation prior to application (Poulsen, 1973). In the present study, the prediction of skin permeation and VED distribution was greatly improved upon incorporating evaporation rates of concerned formulations. A goodness-of-fit was observed for the RD permeation through porcine skin from the lotion formulation (Fig. 6).

Generally, evaporation from the residual formulation affects the permeation parameters. These parameters are determined by the interactions between the permeant and the formulation. It was clear in the present study that water evaporation altered the solubility of RD in the residual polyol vehicles. The effect of water evaporation on the skin permeation of RD from residual formulations was confirmed in infinite dose experiments, where a decrease in the skin permeation of RD was noted with an increase in polyol concentration in the vehicles (Fig. 5A

and 5B). Estimating the skin permeation parameters from a residual formulation applied as a finite dose has not been realistically achieved because formulations tend to evaporate completely leading to incomplete recovery of formulation for quantification. By simulating the composition of a residual formulation and performing permeation experiments under infinite dose conditions, it mimics permeation in the residual phase and allows a reliable estimation of parameters.

Ideally, to increase the penetration of chemicals into the SC, the solubility of the permeating chemical with the SC must be enhanced by increasing its partition coefficient, K_{sc} or by reducing its solubility in the vehicle (Wiechers et al., 2004). In the present study, we found that the solubility of RD in the residual formulation, with very high polyol concentration, proportionally reduced K_{sc} through solvent evaporation. RD formulations in polyol had significantly lower K_{sc} values relative to the aqueous formulation (Fig. 5C). RD permeation decreased when the amount of polyol (19.5% versus polyol concentrations ≥ 40%) in the residual formulation was more than the required amount to dissolve RD. Permeation and consequential distribution of RD in the VED appeared to be closely related to the thermodynamic activity of RD in the vehicle, as manifested by its partition coefficient.

In our steady-state experiments, RD fluxes of simulated formulations with high polyol concentrations were shown to be reduced as the polyol concentration was increased. An inverse relationship existed between the flux and high polyol concentration (Table 3). Calculated permeation parameters further revealed that an increase in polyol concentration decreased flux and the permeability coefficient. The decrease in partition coefficient for RD in the residual formulation supported the observed lower permeability coefficient in relation to polyol concentration leading to a reduction in flux and consequently RD permeation.

The unintended retention of RD on the skin surface instead of benefiting from a ‘solvent drag effect’ with the use of polyols, typically employed in cosmetics as solvents, can be

explained by its solubility in the residual formulation. In this experiment, RD was found to be highly soluble with specific polyols (e.g., BG, DPG) as well as increasing solubility with higher polyol concentration in the residual formulation. Enhancement of solubility of the permeating active compounds in the formulation (i.e., residual formulation) more than that in the SC resulted in reduced partitioning into the skin. The increase in its solubility will reduce its thermodynamic activity, thereby creating a weaker driving force for diffusion. Complete evaporation of volatile components from the formulation means the chemical in the residual phase has the same thermodynamic activity as in the simulated residual formulation composed of 100% polyol (Oliveira et al., 2012). In fact, the polyols involved in this investigation possess similar polarities and thus lack the ability to limit the solubility of RD. Further evidence on the influence of formulation polarity is the low permeability of RD through stripped skin (Fig. 4). Lotion formulation is presumably lipophilic and the absence of the lipophilic barrier, SC, generates a non-ideal diffusion interaction with the hydrophilic VED. These conditions had unfavorable effects on the formulation where the thermodynamic activity of RD was reduced and partitioning into the skin was hampered. Hence, RD penetrates poorly.

Interestingly, unrealistic similarities in the skin permeation of RD were observed in infinite dose experiments for aqueous and prepared lotion (19.5% polyol) (Fig. 4). This affirmed the effect of steady-state conditions in possibly overestimating key parameters. The extent of impact of solvent evaporation and enhanced polyol concentration on RD disposition, however, were revealed in data obtained from residual formulations with high polyol concentrations (40%, 61.8%, 100%) where significantly lower values were observed. For formulators, seemingly acceptable permeation may be observed with formulations containing already desirable proportions of polyols (i.e., 19.5%) although permeation of active compounds from the residual formulation is indeed in its altered (evaporated) state.

Although the present study established a practical approach in estimating skin permeation and the concentration of RD from complex vehicles under finite dose conditions, other factors such as saturated formulations, other types of formulations, solvents, and cosmetic excipients that may affect skin permeation and concentration must be investigated further. The assumption in this study is applicable to a two-layered model where active compounds predominantly permeate through the SC. Hence, the contribution of the hair follicle pathway in the permeation of hydrophilic active compounds must be recognized in future studies.

5. CONCLUSION

In conclusion, we investigated the skin permeation and concentration of an compound in cosmetics, RD, from a complex vehicle as how it would perform in finite dose exposure. Incorporating evaporation kinetics and vehicle-permeant dependent parameters (K_{sc}, K_{ved}, D_{sc}, D_{ved}) may dramatically improve the precision of mathematical models in predicting the permeation and distribution of active compounds in the skin. Predicting these parameters from a complex vehicle made up of actual cosmetic solvents was previously unattainable due to the fact that steady states were not possible in finite dose models. The use of residual formulations simulating the conditions (changes in polyol composition) of the applied finite dose under infinite experiment conditions have paved the way in the calculation of parameters and significantly enhanced estimation of permeant disposition. This method can be applied in the development and optimization of dermatological preparations aimed at enhancing delivery to the skin. Investigating realistic permeant-residual formulation interactions may further our understanding on the cutaneous and systemic absorption of drugs (e.g., steroids, antibiotics) repeatedly applied on skin, cosmetics applied in layers, and dermatological preparations (e.g., sunscreen and insect repellant) applied concurrently. Safety assessments of permeating

chemicals from complex vehicles in clinical and finite dose exposures may now be sufficiently evaluated for their distribution in the VED using simulated residual formulations.

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TABLES

Table 1. Composition of RD formulations

	Recalled formulation	***Prepared formulations***				
Components (%)	Recalled lotion	1% RD Aqueous	Prepared lotion	2% RD Lotion (40% Polyol)	2% RD Lotion (61.8% Polyol)	2% RD Lotion (100% Polyol)
Rhododendrol	2	1	2	2	2	2
Polyols	19.5	-	19.5	40	61.8	q.s. 100
Water	q.s. 100	q.s. 100	q.s. 100	q.s. 100	q.s. 100	-

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Table 2. Permeation parameters from RD formulations in various polyol concentration

	Formulations	
Parameters	1% RD Aqueous	Recalled lotion
D_{sc} *(cm²/h)*	9.0×10^{-6}	4.6×10^{-6}
D_{ved}*(cm²/h)*	1.8×10^{-3}	1.8×10^{-3}
K_{sc}	0.50	0.14
K_{ved}	0.56	0.56

600 Table 3. Calculated permeation parameters of RD through intact porcine skin

	Polyol concentration (%)				
Parameters	0	19.5	40	61.8	100
J (μg/cm²/h)	8.31 ± 1.17	4.60 ± 0.74	3.37 ± 0.59	1.51± 0.12	0.21 ± 0.03
P (cm/s)	(6.55 ± 1.75) x 10^{-07}	(7.25 ± 1.16) x 10^{-07}	(4.09 ± 0.72) x 10^{-08}	(1.41 ± 0.13) x 10^{-08}	(1.56 ± 0.23) x 10^{-09}
K_{sc}	0.5	0.14	0.10	0.04	0.002

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FIGURES

Legends to Figures

615 Figure 1. General concentration-distance profile of a penetrant in two-layered membrane diffusion model. C_v, C_{sc}, C_{ved} refers to the penetrant concentration in the vehicle, SC and VED, respectively; K_{sc} and K_{ved} refer to partition coefficients from the donor (vehicle) to SC and VED, respectively; D_{sc} and D_{ved} are diffusion coefficients in the SC and VED, respectively. L_{sc} and L_{ved} refer to thicknesses of the SC and VED, respectively; and t refers to time after starting 620 the permeation experiment.

Figure 2. Flow diagram for time course of $K_{sc}(t)$ and $C_v(t)$. C_v, C_{sc}, C_{ved} refers to the penetrant concentration in the vehicle, SC and VED, respectively; K_{sc} and K_{ved} refer to partition coefficients to SC and VED, respectively; D_{sc} and D_{ved} are diffusion coefficients in the SC and 625 VED, respectively. L_{sc} and L_{ved} refer to thicknesses of the SC and VED, respectively; t refers to time after starting the permeation experiment.

Figure 3. Percent water loss from applied formulation. Water evaporation is equal to 102.6 x $e^{-0.093t}$ (r^2 = 0.98). Each point represents the mean ± S.E. (n=4).

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Figure 4. Time course of the cumulative amount of RD permeated through skin under infinite dose conditions. Permeation profiles from 1% aqueous RD through intact skin (●); 1% aqueous RD through stripped skin (○); 2% RD lotion through intact skin (■); and 2%RD lotion through stripped skin (□); line represents the predicted profiles of RD. Each point represents the mean 635 ± S.E. (n=4). Significant difference ($^*p$<0.05) between 1% aqueous RD and 2% RD in 19.5% polyol through stripped skin.

Figure 5. Relationship of polyol concentration, RD solubility and permeation. (A) Cumulative RD permeation from lotion formulations through intact skin under infinite conditions; Prepared lotion (○); recalled lotion (□); 2% RD in 40% polyol (◆); 2% RD in 61.8% polyol (▲); 2% RD in 100% polyol (×). Significant difference (*p<0.05) between 2% RD in 100% polyol and 1% aqueous RD, 2% RD in 19.5% polyol, or 2% RD in 40% polyol. (B) Relationship between polyol concentration and cumulative amount of RD permeated (r^2 = 0.98). 1% Aqueous RD (●); 2% RD in 19.5% polyol (■); 2% RD in 40% polyol (◆); 2% RD in 61.8% polyol (▲); 2% RD in 100% polyol (×). (C) Relationship between polyol concentration with RD solubility (r^2 = 0.99) and partition coefficient (r^2 = 0.96; y = $0.54e^{-0.052x}$). 1% aqueous RD (●); 2% RD in 10% polyol (○); 2% RD in 19.5% polyol (□); 2% RD in 40% polyol (◆); 2% RD in 61.8% polyol (▲); 2% RD in 100% polyol (×) Each point represents the mean ± S.E. (n=4).

Figure 6. Time course of the cumulative amount permeated through skin (A) and concentration in VED (B) of RD recalled lotion under finite dose conditions. Unfilled circles (○) represent experimental data while lines represent the predicted profiles of RD. Each point represents the mean ± S.E. (n=4).

Figure 1

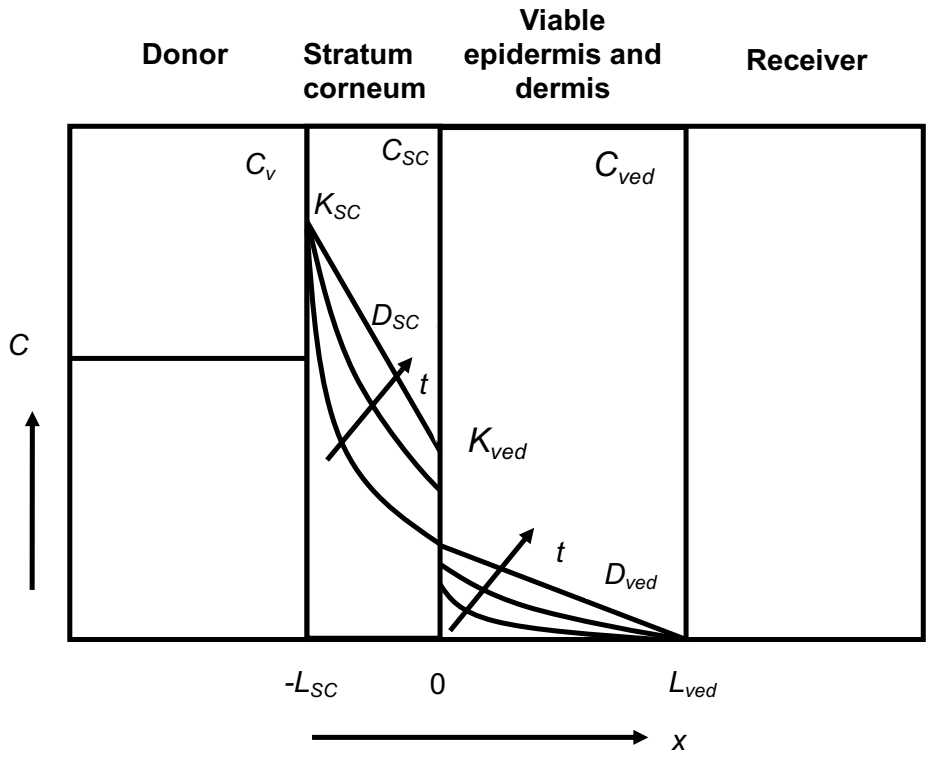

Donor
Stratum corneum
Viable epidermis and dermis
Receiver
C_v
C_{SC}
C_{ved}
K_{SC}
D_{SC}
C
t
K_{ved}
t
D_{ved}
$-L_{SC}$
0
L_{ved}
x

Figure 2

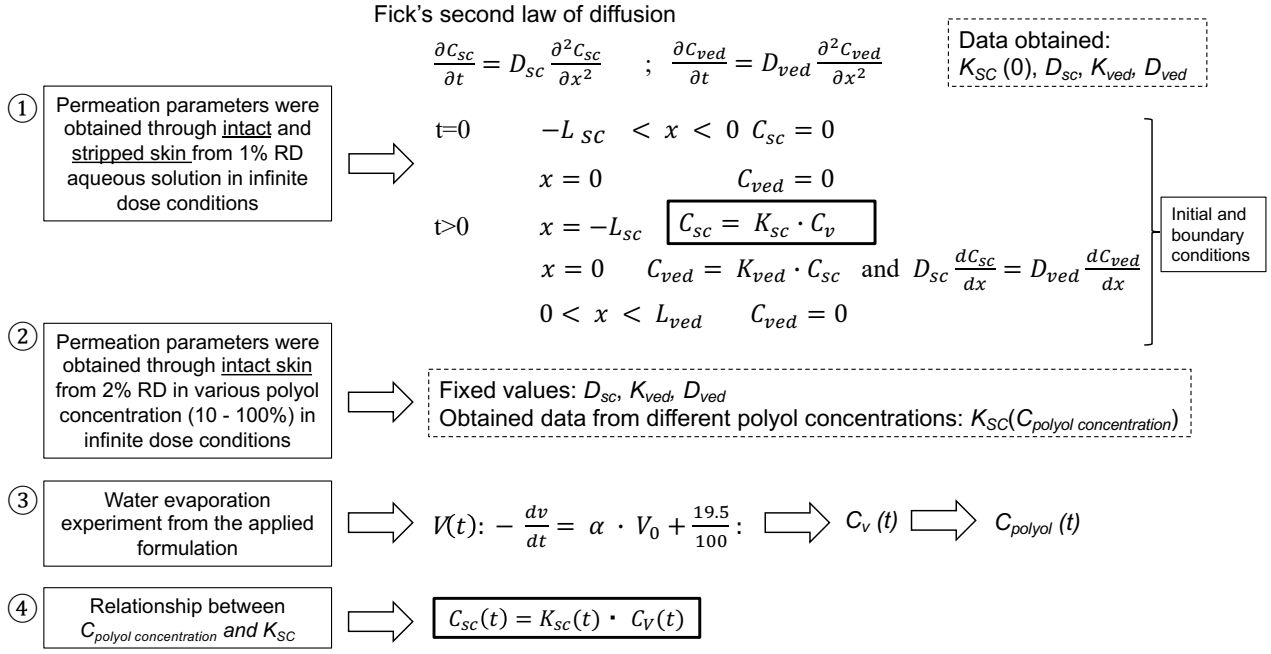

Fick's second law of diffusion
$\frac{\partial C_{sc}}{\partial t} = D_{sc}\frac{\partial^2 C_{sc}}{\partial x^2}$; $\frac{\partial C_{ved}}{\partial t} = D_{ved}\frac{\partial^2 C_{ved}}{\partial x^2}$
Data obtained:
K_{SC} (0), D_{sc}, K_{ved}, D_{ved}
① Permeation parameters were obtained through intact and stripped skin from 1% RD aqueous solution in infinite dose conditions
t=0 $-L_{sc} < x < 0$ $C_{sc} = 0$
$x = 0$ $C_{ved} = 0$
t>0 $x = -L_{sc}$ $C_{sc} = K_{sc} \cdot C_v$
$x = 0$ $C_{ved} = K_{ved} \cdot C_{sc}$ and $D_{sc}\frac{dC_{sc}}{dx} = D_{ved}\frac{dC_{ved}}{dx}$
$0 < x < L_{ved}$ $C_{ved} = 0$
Initial and boundary conditions
② Permeation parameters were obtained through intact skin from 2% RD in various polyol concentration (10 - 100%) in infinite dose conditions
Fixed values: D_{sc}, K_{ved}, D_{ved}
Obtained data from different polyol concentrations: $K_{SC}(C_{polyol\ concentration})$
③ Water evaporation experiment from the applied formulation
$V(t)$: $-\frac{dv}{dt} = \alpha \cdot V_0 + \frac{19.5}{100}$:
C_v (t)
C_{polyol} (t)
④ Relationship between $C_{polyol\ concentration}$ and K_{SC}
$C_{sc}(t) = K_{sc}(t) \cdot C_V(t)$

Figure 3

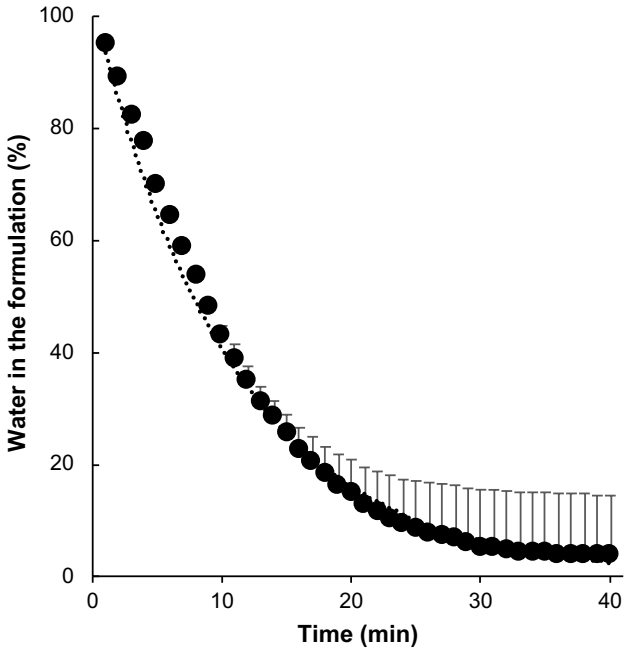


Figure 4

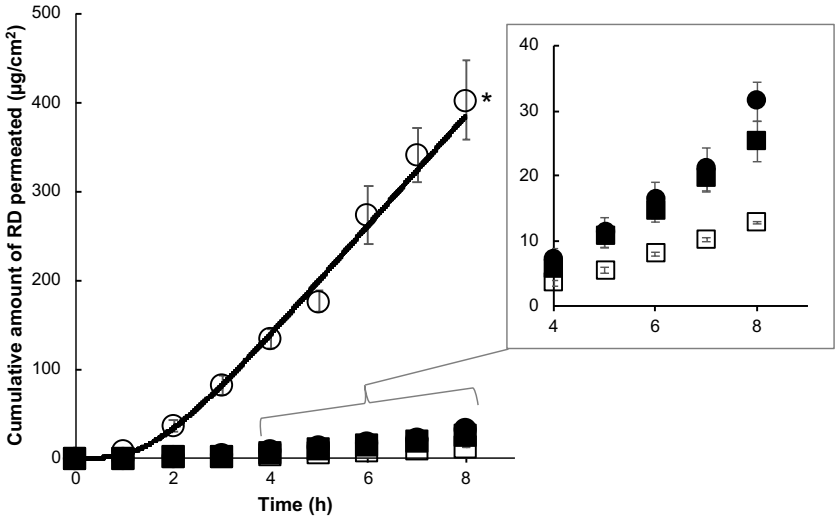

Cumulative amount of RD permeated (µg/cm²)
500
400
300
200
100
0
0
2
4
6
8
Time (h)
*
40
30
20
10
0
4
6
8

Figure 5

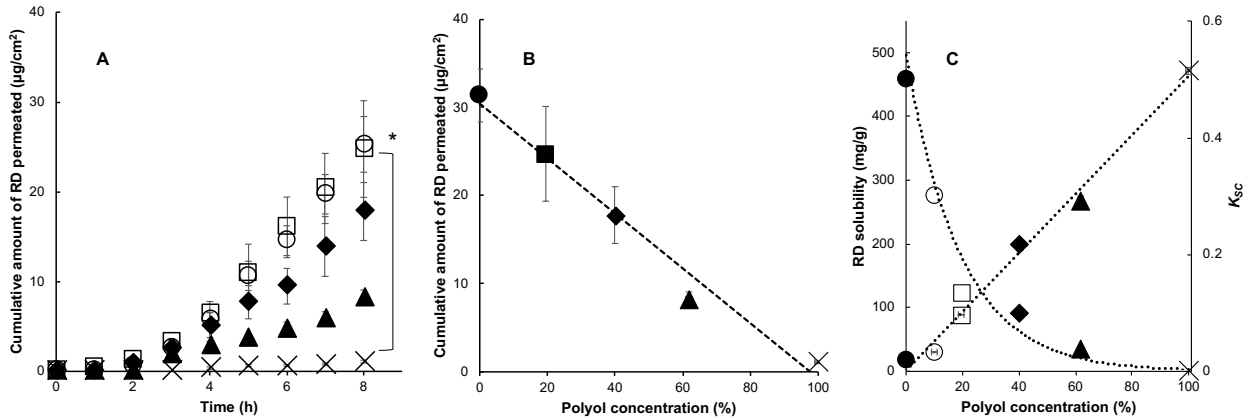
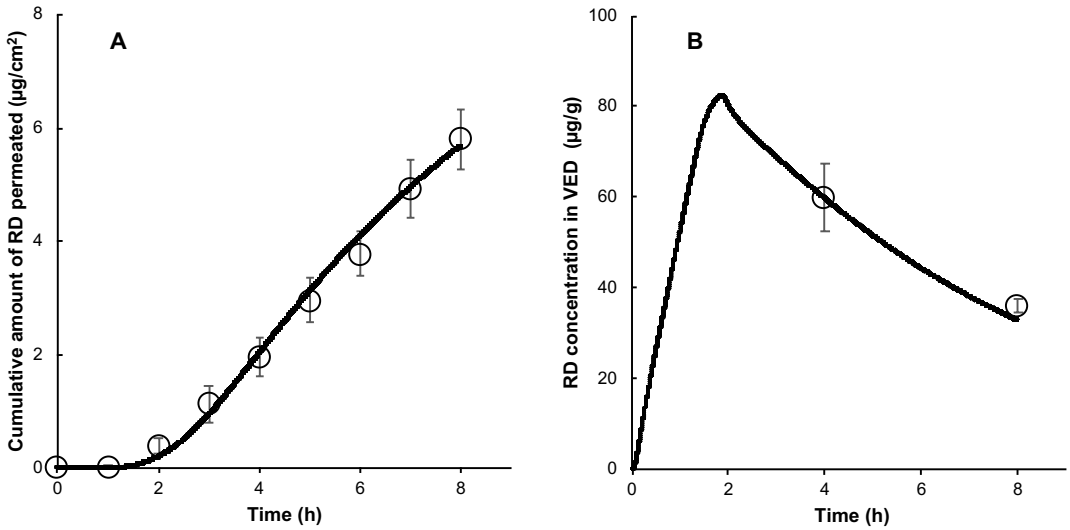

A
Cumulative amount of RD permeated (μg/cm²)
Time (h)
*
B
Cumulative amount of RD permeated (μg/cm²)
Polyol concentration (%)
C
RD solubility (mg/g)
K_{SC}
Polyol concentration (%)

Figure 6


A
Cumulative amount of RD permeated (µg/cm²)
Time (h)
B
RD concentration in VED (µg/g)
Time (h)