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Research Paper

Mathematical Model to Predict Skin Concentration of Drugs: Toward Utilization of Silicone Membrane to Predict Skin Concentration of Drugs as an Animal Testing Alternative

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Purpose. To calculate the skin concentration of active ingredients in cosmetics and topical pharmaceuticals using silicone membrane permeation.
Methods. A series of parabens were used as model ingredients. Skin concentration of parabens was calculated using silicone membrane permeability. Their partition coefficient from formulations to the silicone membrane was determined by the membrane permeation profiles, and used to calculate their silicone membrane concentration, under an assumption that the membrane is one homogenous diffusion layer. The same procedure was applied for hairless rat skin.
Results. The calculated concentration of parabens in silicone membrane was very close to their observed values. However, the skin concentration calculated by skin permeability was not similar to the observed concentration. Re-calculation was performed under the assumption that the skin consists of two diffusion layers. This modification using permeation data through full-thickness and stripped skin enabled precise prediction of the skin concentration of parabens. In addition, the partition coefficient to the silicone membrane was useful to estimate their skin concentration.
Conclusions. Ingredient concentration in skin can be precisely predicted using diffusion equations and partition coefficients through permeation experiments using a silicone membrane. The calculated in-skin concentration is useful for formulation studies of cosmetics and topical pharmaceuticals.

KEY WORDS: hairless rat skin; membrane permeation; paraben; silicone membrane; skin concentration.

INTRODUCTION

Skin has been the focus as an application site of cosmetics and therapeutic drugs. Many transdermal drug delivery systems and topical drug formulations, as well as cosmetics, are on the market, and determining the percutaneous absorption of drugs and cosmetic ingredients is important for developing good topical formulations; however, percutaneous absorption or skin permeation itself is not always important for topical formulations (1). In other words, maintaining drug and active ingredient concentrations at their sites of action is more important for most topical drug and cosmetic formulations (2,3). For example, a lack of skin distribution and permeation are necessary for sunscreens, which mostly act on the skin surface, from a safety point of view. On the other hand, skin whitening agents must be studied for their skin distribution and concentration in the viable epidermis, which is their primary site of action. High skin permeation of whitening agents, however, is not expected, because the possibility of systemic side effects is increased. Of course, efficient percutaneous absorption or skin permeation of drugs is necessary for transdermal drug delivery systems to achieve systemic pharmacological actions. Thus, we have to distinguish the skin distribution or skin concentration from percutaneous absorption or skin permeation of drugs and cosmetic ingredients. This is very important to evaluating therapeutic drug formulations and cosmetics.

Unfortunately, little investigation has been performed of the skin distribution or skin concentration of chemical compounds compared with percutaneous absorption and skin permeation, especially for cosmetics. Epidermal layers frequently serve as a site of action for ingredients in cosmetics and topical drug formulations. Active ingredients and drugs in the formulations must distribute from the formulations to the epidermal tissues and maintain efficient concentrations in the tissues to achieve their effectiveness as cosmetics or topical drug formulations. In addition, the determination of skin concentration is very important for evaluating cosmetics or topical drug formulations, as well as for developing new cosmetic and drug products.

Recently, criticism against animal experiments has greatly increased from the viewpoint of animal welfare. In the EU, animal experiments to the production and import of cosmetic formulations are banned from 2009 to 2013 (4–7). Scientists have to be aware of the spirit of the 3Rs for animal

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experiments: "Reduction" refers to methods that enable researchers to obtain comparable levels of information from fewer animals or to obtain more information from the same number of animals; "Refinement" refers to methods that alleviate or minimize potential pain, suffering or distress, and enhance the welfare of the animals used; and "Replacement" refers to the preferred use of non-animal methods over animal methods whenever it is possible to achieve the same scientific aims.

We therefore used silicone membrane as an alternative to skin membrane to determine the skin concentration of cosmetic ingredients or drugs, from the viewpoints of the great necessity of estimating their concentration in the skin in the development of cosmetics or topical formulations, and as an animal experiment alternative. Silicone membrane has been used as an alternative to skin membrane, and the permeation of cosmetic ingredients and drugs through the silicone membranes was compared with that through human or animal skin (8–11). Few experiments have been performed, however, on their skin or membrane concentration. In the present study, a method for estimating skin concentration was established using silicone membrane permeability. A series of parabens (methyl, ethyl, *n*-propyl, *n*-butyl esters) were used as model penetrants, since they have very different lipophilicities (*i.e.*, n-octanol/water partition coefficient) in spite of having a similar molecular weight (152–194 Da). Table I summarizes the physicochemical properties of parabens used in this experiment (12).

THEORETICAL (13,14)

One-Layered Diffusion Model (15)

Determination of Membrane Concentration

The diffusion of chemical compounds in the membrane is expressed theoretically by Fick's second law of diffusion, $\frac{\partial C}{\partial t} = D\frac{\partial^2 C}{\partial x^2}$, under the assumption that the membrane is a homogeneous single layer, where C is the penetrant concentration in the membrane at position, x, and time, t. When a sink condition is assumed on the receiver side of the membrane, *i.e.*, x = L, a set of initial conditions (C=0 at t=0 and 0<x<L)) and boundary conditions (C = KC_v at x=0 and C=0 at $x = L$, where K is the partition coefficient of the penetrant from the vehicle to membrane and C_v is the penetrant concentration in the vehicle) are obtained (see Fig. 1a).

Then, membrane concentration, C, and its steady-state level at an infinite time, C_{ss}, are expressed as follows (12):

$$C = KC_v\left\{\left(1-\frac{x}{L}\right)-\frac{2}{\pi^2}\sum_{n=1}^{\infty}\sin\frac{nx\pi}{L}\exp\left(-\frac{Dn^2\pi^2}{L^2}t\right)\right\} \quad (1)$$

$$C_{ss} = KC_v\left(1-\frac{x}{L}\right) \quad (2)$$

Further, the mean membrane concentrations of penetrant, $\overline{C}$ and $\overline{C}_{ss}$, are obtained by integrating C in Eqs. 1and 2 from x=0 to L:

$$\overline{C} = \frac{KC_v}{2}\left\{1-\frac{8}{\pi^2}\sum_{m=1}^{\infty}\frac{1}{(2m-1)^2}\exp\left(-\frac{D(2m-1)^2\pi^2}{L^2}t\right)\right\} \quad (3)$$

$$\overline{C}_{ss} = \frac{KC_v}{2} \quad (4)$$

As shown in Eq. 4, the mean membrane concentration of the penetrant can be determined by K and C_v, not by D. Because C_v is a known parameter, a parameter, K, is the only determinant of the mean membrane concentration of the penetrant.

Determination of Membrane Permeation

Since the silicone membrane can be supposed to be homogenous in one layer, the permeation profiles of parabens throughout the membrane were analyzed using a one-layered diffusion model (16,17). Under the initial and boundary conditions shown above, the amount of drug permeating the unit area of the silicone membrane at time t, Q, can be represented as

$$Q = KLC_v\left[\frac{D}{L^2}t-\frac{1}{6}-\frac{2}{\pi^2}\sum_{n=1}^{\infty}\frac{(-1)^n}{n^2}\exp\left(-\frac{D}{L^2}n^2\pi^2t\right)\right] \quad (5)$$

The partition parameter ($K{\cdot}L$) and diffusion parameter (D/L^2) were obtained by curve fitting the obtained data to Eq. 5 using the least squares method. In the calculation of D and K, the thickness of the silicone membrane was fixed at 68 μm. Permeability coefficient, P, was calculated by an equation, $P = \frac{KD}{L}$.

Table I. Physicochemical Parameters of Parabens

	Methyl paraben (MP)	Ethyl paraben (EP)	Propyl paraben (PP)	Butyl paraben (BP)
M.W.	152.12	166.18	180.20	194.23
Log K_{ow} [a)]	0.940	1.93	2.27	3.53
clog P [b)]	1.98	2.51	3.04	3.57
Solubility[c)](mM)	19.7	8.82	2.27	1.60

[a)] *n*-Octanol / water partition coefficient at 37°C
[b)] Calculation by software, Chem Draw (CambridgeSoft)
[c)] In water at 32°C

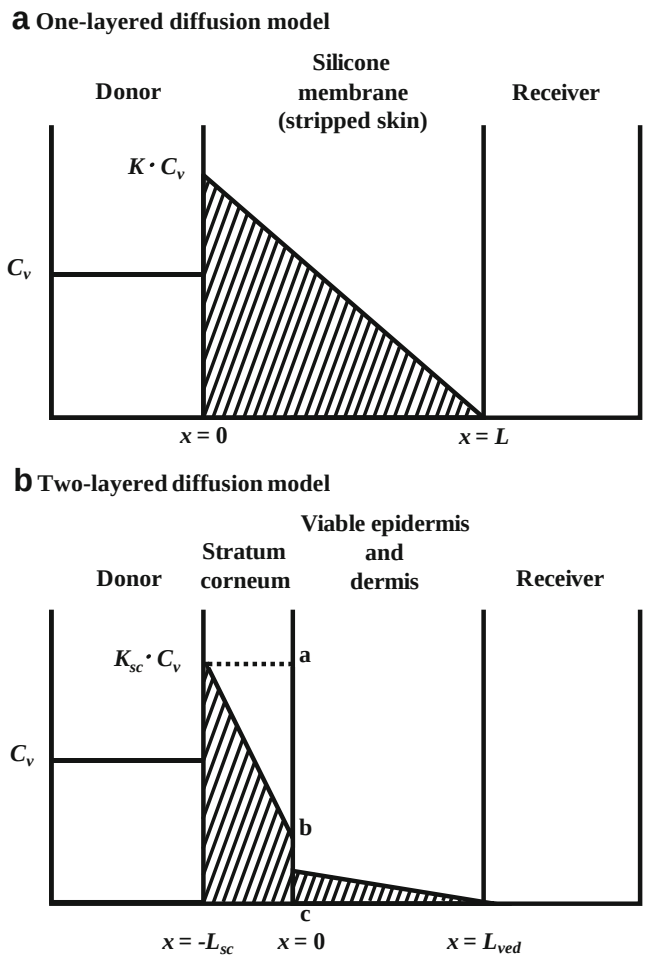

Fig. 1. Schematic diagram of concentration-distance profile in one- and two-layered diffusion membrane models in membrane permeation experiments. Membrane thickness is L and $L_{sc}+L_{ved}$ for one- and two-layered diffusion membranes models, respectively. C_v is the donor concentration of parabens, and K and K_{sc} are partition coefficients to membranes.

Stripped hairless rat skin was treated as a homogeneous layer, the same as the silicone membrane.

Two-Layered Diffusion Model

Determination of Membrane Concentration Using Two-Layered Diffusion Model

Fig. 1b shows a typical two-layered diffusion model of skin. Penetrant concentration in the first layer of skin, the stratum corneum, C_{sc}, and that in the second layer, the viable epidermis and dermis of skin, C_{ved}, can be expressed by Fick's second law of diffusion. Similarly, initial conditions and boundary conditions are represented. Boundary condition between the first and second layer is $C_{ved} = K_{sc}K_{ved}C_{sc}$ and $D_{sc}\frac{dCsc}{dx} = D_{ved}\frac{dCved}{dx}$, where K_{sc} and K_{ved} are partition coefficients of the penetrant from the vehicle to stratum corneum and to the viable epidermis and dermis, and D_{sc} and D_{ved} are diffusion coefficients in the stratum corneum and viable epidermis and dermis, respectively.

In the two-layered diffusion model, the overall permeability coefficient, P_{tot}, can be expressed by that in the stratum corneum, P_{sc}, and in the viable epidermis and dermis, P_{ved} , as follows, in Eq. 6. (18):

$$\frac{1}{P_{tot}} = \frac{1}{P_{sc}} + \frac{1}{P_{ved}} \tag{6}$$

In addition, the reciprocal of the permeability coefficient can be replaced with permeation resistant, R, and the following equation can be derived, as Eq. 7:

$$R_{tot} = R_{sc} + R_{ved} \tag{7}$$

The permeation resistance of the penetrants can be represented as in the electric circuit. Two resistances, R_{sc} and R_{ved}, exist in the skin membrane, as shown in Fig. 1b. The ratio of the Line segment ab against bc at the surface between the stratum corneum and viable epidermis, x=0, should be the ratio of R_{sc} against R_{ved}. Thus, the penetrant concentration at point b, C_b, can be represented by

$$C_b = K_{sc}C_v\frac{R_{ved}}{R_{tot}} \tag{8}$$

The amount of penetrant in the unit area of the stratum corneum, M_{sc}, can be represented using Eq. 8 as Eq. 9.

$$M_{sc} = \frac{K_{sc}C_vL_{sc}\left(1+\frac{R_{ved}}{R_{tot}}\right)}{2} \tag{9}$$

Since the partition coefficient of the penetrant from the stratum corneum to the viable epidermis and dermis is represented as K_{ved}/K_{sc}, the amount of penetrant in the unit area of the viable epidermis and dermis, M_{ved}, can be represented as

$$M_{ved} = \frac{K_{ved}C_vL_{ved}\frac{R_{ved}}{R_{tot}}}{2} \tag{10}$$

By summation of Eqs. 9 and 10, M_{tot}, the drug amount in the unit area of the skin can be represented as follows:

$$M_{tot} = \frac{C_v}{2}\left\{K_{sc}L_{sc}\left(1+\frac{R_{ved}}{R_{tot}}\right) + K_{ved}L_{ved}\frac{R_{ved}}{R_{tot}}\right\} \tag{11}$$

By dividing M_{tot} in Eq. 11 by skin thickness, L_{tot}, the average drug concentration in the skin, $\overline{C}_{ss}$, is

$$\overline{C}_{ss} = \frac{C_v}{2L_{tot}}\left\{K_{sc}L_{sc}\left(1+\frac{R_{ved}}{R_{tot}}\right) + K_{ved}L_{ved}\frac{R_{ved}}{R_{tot}}\right\} \tag{12}$$

When resistances, R, are changed to permeability coefficients, P, finally Eq. 13 is derived,

$$\overline{C}_{ss} = \frac{C_v}{2L_{tot}}\left\{K_{sc}L_{sc}\left(1+\frac{P_{tot}}{P_{ved}}\right) + K_{ved}L_{ved}\frac{P_{tot}}{P_{ved}}\right\} \tag{13}$$

Determination of Membrane Permeation Using Two-Layered Diffusion Model

Two diffusion coefficients, D_{sc} and D_{ved}, and two partition coefficients, K_{sc} and K_{ved}, were obtained by curve fitting the cumulative amount of parabens that permeated through the full-thickness skin and stripped skin to the

theoretical values using the least squares method, where theoretical values were expressed by two diffusion equations (Fick's second law) showing the diffusion profiles in the stratum corneum and viable epidermis and dermis. Differential equations describing Fick's second law are as follows (14):

$$\frac{dC_{i,j}}{dt} = \frac{1}{\Delta t}\left(C_{i,j+1} - C_{i,j}\right) \tag{14}$$

$$\frac{d^2C_{i,j}}{dx^2} = \frac{1}{\Delta x^2}\left(C_{i-1,j} - 2C_{i,j} + C_{i+1,j}\right) \tag{15}$$

Mathematical treatment for determining the skin permeation using two-layered diffusion model was the same as in our previous method (14).

Calculation of Permeation Parameters

Generally, most resistance against drug permeation throughout hairless rat skin is in the stratum corneum (17). Rat skin was also treated as a single-layered membrane, the same as the silicone membrane. Parameters D and K were calculated under the assumption that the stratum corneum is a homogeneous membrane with a thickness of 15 μm.

Calculation of Theoretical Membrane Concentration of Parabens

The theoretical membrane concentration of parabens was calculated by Eq. 6 and the K value obtained from the membrane permeation study.

EXPERIMENT

Reagents and Materials

Parabens: methyl paraben (MP), ethyl paraben (EP), *n*-propyl paraben (PP), and *n*-butyl paraben (BP) were obtained from Tokyo Kasei Chemical Co., Ltd. (Tokyo, Japan). An esterase inhibitor, diisopropyl fluorophosphate (DFP), and a deproteinization agent, trichloroacetic acid (TCA), were obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Other reagents and solvents were liquid chromatograph and special grade chemicals.

The silicone membrane (Dow Corning 7-4107) was a gift from Nagase & Co., Ltd. (Tokyo, Japan).

Experimental Animals

Male hairless rats (WBM/ILA-Ht, 230–280 g) were obtained from the Life Science Research Center, Josai University (Sakado, Saitama, Japan) or Ishikawa Experimental Animal Laboratories (Fukaya, Saitama, Japan). All animal experiments were performed according to the ethics committee of Josai University.

Membrane Permeation Experiments of Parabens

The silicone membrane was set on a Franz-type diffusion cell (receiver cell volume: 6.0 mL, effective diffusion area: 1.77 cm^2) using cyanoacrylate glue (19–22). Phosphate-buffered saline (pH 7.4, PBS) was applied to the receiver cell and maintained at 32°C for 15 min. Aliquots (0.5 mL) of different concentrations of parabens (MP 10 mM, EP 5 mM, PP 1 mM, BP 0.5 mM) in PBS were applied to the donor cell to start the permeation experiment. The experimental setup is shown in Fig. 2. The receiver solution was stirred on a magnetic stirrer with a bar; the experiment was performed at 32°C. At predetermined intervals, 400 μL of the receiver solution was sampled, and the same volume of PBS was added to the receiver cell to keep the volume constant. Paraben concentration was determined by HPLC.

Abdominal skin (full-thickness skin or stripped skin) was excised from hairless rats under pentobarbital (25 mg/kg, *i.p.*) anesthesia, and debris and excess fat were trimmed off the excised skin. Stripped skin was made by tape-stripping the stratum corneum 20 times before excising from rats (23). The obtained skin piece was then set on the Franz-type diffusion cell, as above. DFP (2.7 μmol/mL in PBS) was applied to the receiver cell for half an hour (12,24–26). After rinsing off the reagent, paraben solution (0.5 mL) in PBS and DFP in PBS (0.54 μmol/mL, 6.0 mL) were added to the donor and receiver cells, respectively, to start the skin permeation experiment. Other procedures were consistent with the silicone membrane permeation experiments.

Determination of Extraction Ratio of Parabens

Silicone Membrane. Silicone membrane was loaded with parabens in chloroform and dried. Fresh chloroform (1 mL) was then applied to the silicone membrane piece. After agitating for 15 min, the resulting chloroform was sampled, and fresh chloroform was again added for the second extraction of parabens. Total chloroform containing parabens was evaporated to dryness, and the sample was reconstituted with 1.0 mL acetonitrile. The sample was injected onto HPLC to determine the paraben concentration. The extraction ratio of parabens from silicone membrane was almost 1.0.

Hairless Rat Skin. Hairless rat skin was loaded with parabens in water, and the skin piece was minced with scissors and homogenized at 12,000 rpm using a homogenizer (Polytron PT-MR 3000; Kinematica Inc., Littau, Switzerland) for 5 min at 4°C. The homogenate was incubated for 1 h at 32°C. The same volume of 16% TCA was added to the skin homogenate and agitated for 15 min (27–29). The supernatant after centrifugation (15,000 rpm, 5 min, 4°C) was injected onto HPLC. Parabens were extracted from the skin homogenate using chloroform, as in the silicone membrane experiment. The extraction ratio of parabens was almost 1.0.

Determination of Paraben Concentration in Silicone Membrane and Hairless Rat Skin

Silicone Membrane. After the permeation experiment, the donor solution was removed, and the silicone membrane was washed with PBS (1 mL). Chloroform (1 mL) was added to the silicone membrane in the Franz-type diffusion cell (permeation area: 1.77 cm^2) and agitated for 15 min to extract parabens from the membrane. The subsequent procedure was consistent with Determination of Extraction Ratio of Parabens.

Hairless Rat Skin. After the permeation experiment, the donor solution was removed, and the stratum corneum side

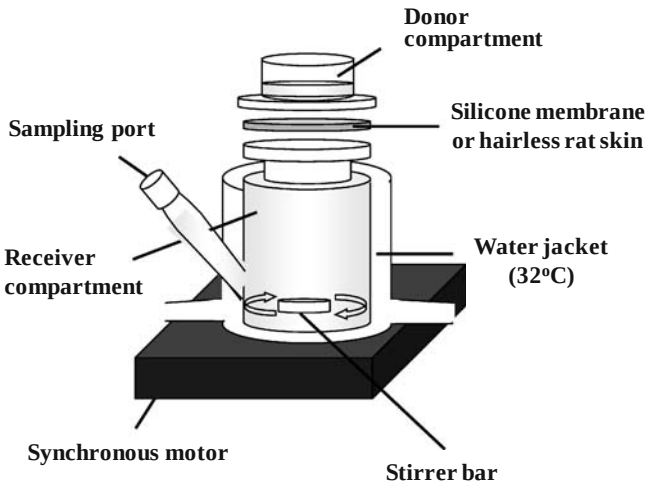

Fig. 2. Schematic representation of permeation experiment using silicone membrane and hairless rat skin.

of hairless rat skin was washed two times with PBS (1 mL). The rat skin was taken from the diffusion cell, and the permeation area of the skin (1.77 cm^2) was kept in a freezer (-15°C) before determining the skin concentration. This frozen skin was minced with scissors, and PBS (1 mL) was added to homogenize the minced skin (12,000 rpm, 5 min, 4°C). The subsequent procedure was consistent with Determination of Extraction Ratio of Parabens.

Determination Methods of Parabens

The same volume of acetonitrile containing an internal standard (another paraben) was added to the paraben samples. After slight mixing, the sample was injected onto HPLC. The HPLC system consists of a pump (LC-10 AD; Shimadzu, Kyoto, Japan), Chromatopac (C-R6A; Shimadzu), UV detector (SPD-6A; Shimadzu), system controller (SCL-6B; Shimadzu) and an auto-injector (SIL-7A; Shimadzu). The column was LiChroCART®250-4 (KGaA·64271; Merck, Darmstadt, Germany) kept at 40°C during the eluting mobile phase, 0.1% phosphoric acid : acetonitrile=75 : 25 for MP and EP and 0.1% phosphoric acid : acetonitrile=55 : 45 for PP and BP. The flow rate was 1.0 mL/min. The injection volume was 20μL, and detection was performed at 260 nm.

RESULTS AND DISCUSSIONS

Partition Coefficient and Concentration of Parabens into and in the Silicone Membrane

Since drug distribution in the skin membrane is a physical phenomenon, it can be evaluated using artificial membranes as well as human and animal skin. A silicone membrane was used in this experiment due to its cost and easy availability.

The direct measurement of drug concentration in the membrane has several problems. Generally, only one data point is obtained from one membrane after drug application. In addition, controlling the removal of the drug formulation from the membrane surface is very difficult. Hard cleaning of the membrane surface decreases the membrane concentration, whereas inadequate cleaning may leave the drug formulation on the membrane. We first performed the membrane permeation experiment, and permeation parameters were obtained. The membrane concentration can be calculated using the partition coefficient, K, of the applied drug from the vehicle to the membrane, as shown in Eq. 6. Next, the calculated values were compared with the directly observed membrane concentration. The membrane was obtained after the membrane permeation experiments.

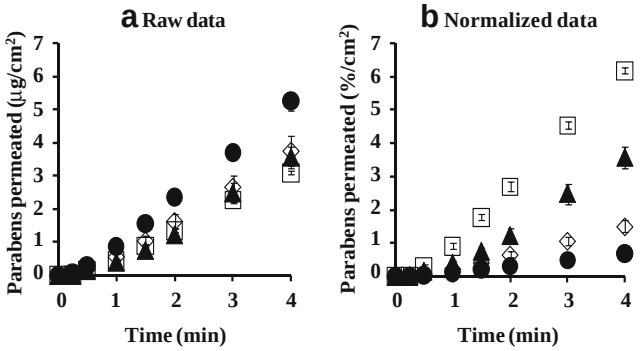

Fig. 3. Time course of the cumulative amount of parabens that permeated the silicone membrane. (**a**) raw data, (**b**) normalized data. Symbols: ●: 10 mM MP, ◊: 5 mM EP, ▲: 1 mM PP, □: 0.5 mM BP. Each data point represents the mean±S.E. (n=4–8).

Fig. 3 shows the cumulative amount of parabens that permeated the silicone membrane. Fig. 3a and b show raw and normalized data (raw data divided by application concentration of parabens (19,30)), respectively. The permeation ratio of BP against the application amount was highest, followed by PP, EP and MP. The increase in the lipophilicity of parabens increased the permeability, as shown in Fig. 3b (31).

Generally, high permeability and solubility of chemicals through and in the membrane are observed when the solubility parameter of chemicals is close to that of membrane. The solubility parameter of BP, 10.9 $(cal/cm^3)^{1/2}$, is closest to that of the silicone membrane (7.3–7.5 $(cal/cm^3)^{1/2}$) among the parabens (MP: 11.5 $(cal/cm^3)^{1/2}$, EP: 11.3 $(cal/cm^3)^{1/2}$, PP: 11.1 $(cal/cm^3)^{1/2}$) used in this experiment (32–34).

Fig. 4a and b show the observed concentration and normalized concentration corrected using the application concentration of parabens in the silicone membrane, respectively. Each concentration is that at the steady state after

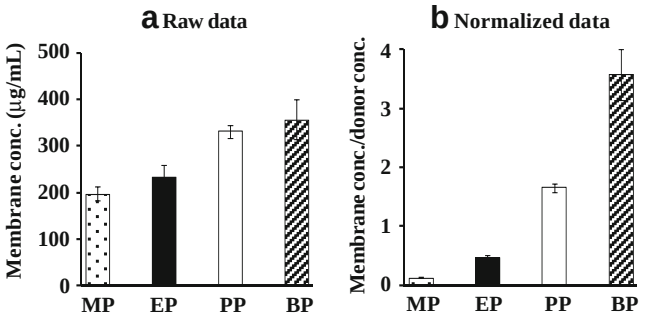

Fig. 4. Raw data (**a**) and normalized date [(silicone membrane concentration)/(donor concentration)] (**b**) for steady-state silicone membrane concentration of parabens. Each column represents the mean ± S.E. (n=4–8).

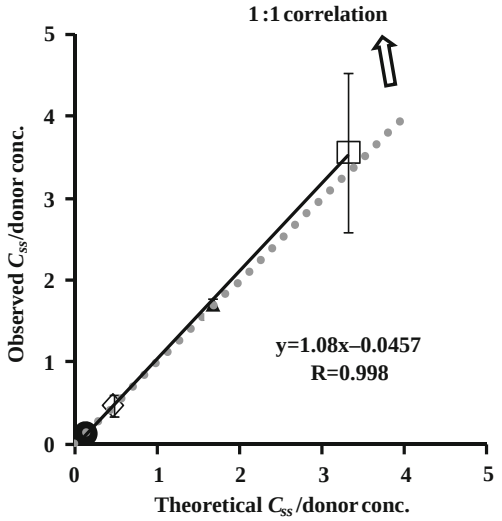

Fig. 5. Relationship between theoretical and observed steady-state silicone membrane concentration of parabens. Normalized data were used (see Fig. 4). One-layered diffusion membrane model was used to obtain theoretical steady-state membrane concentration of parabens. Symbols: see Fig. 3. The observed data represent the mean ± S.D. (n=4–8). Almost 1:1 correlation was found. K, D and P were as follows (D :×10^{-7}cm^2/s, P :×10^{-5}cm/s): MP (K=0.256, D=4.07, P=1.53), EP (K=0.907, D=2.60, P=3.47), PP (K=3.34, D=1.78, P=8.76), and BP (K=6.64, D=1.65, P=16.1).

starting the permeation experiments. An increase in $K_{o/w}$ of parabens increases the membrane concentration.

Fig. 5 shows the relationship between the theoretical and observed values of paraben concentration in the silicone membrane. This figure shows almost a 1:1 relationship between them, suggesting that the silicone membrane can be assumed to be one homogenous layer and that the membrane concentration of parabens can be theoretically determined by C_v and K. These results also suggest that the permeation experiment is useful to determine the membrane concentration. The figure also contains the permeation parameters of parabens in the legend. The permeation parameters were obtained by curve-fitting the permeation profiles to Fick's law of diffusion using the nonlinear least squares method, under the assumption that the silicone membrane is one homogenous layer. The permeability coefficient, P, of MP and BP was lowest and highest among the parabens used in this experiment. The diffusion coefficient, D, of these parabens in the silicone membranes was not so different (only 2–3 times different), whereas partition coefficient, K, was very different among these parabens (26 times different between MP and BP). A very different permeability coefficient, P, is closely related to the K of parabens. Since the P-value of parabens throughout the silicone membrane was very high (about 10^{-5}cm/s), the permeation profiles can be evaluated in a short experimental period. The theoretical membrane concentration of parabens calculated from the K-value was very close to the observed membrane concentration.

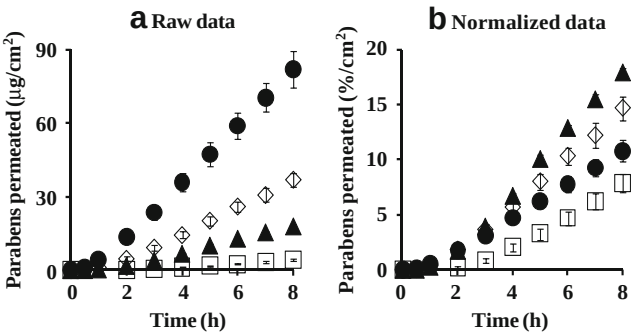

Fig. 6. Time course of the cumulative amount of parabens that permeated hairless rat intact skin. (**a**) raw data, (**b**) normalized data Symbols: see Fig. 3. Each data point represents the mean ± S.E. (n=5–11).

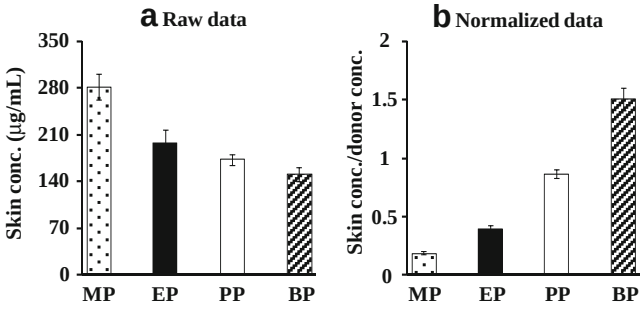

Fig. 7. Raw data (a) and normalized date [(skin concentration)/(donor concentration)] for steady-state hairless rat skin concentration of parabens. Each column represents the mean ± S.E. (n=5–11).

Membrane Permeation and Concentration: Comparison Between Silicone Membrane and Animal Skin

The theoretical concentration of parabens in silicone membrane, which was calculated from partition coefficient, K, was close to the observed concentration. A similar trial was carried out for the paraben concentration in hairless rat skin. Partition and skin concentration of parabens in rat skin were compared to those in the silicone membrane.

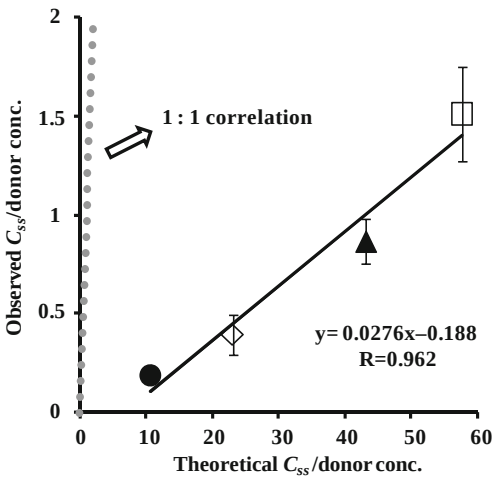

Fig. 8. Relationship between theoretical and observed steady-state hairless rat skin concentration of parabens. Normalized data were used (see Fig. 7). One-layered diffusion membrane model was used to obtain theoretical steady-state membrane concentration of parabens. Symbols: see Fig. 3. The observed data represent the mean ± S.D. (n=5–11). The obtained line was very different from 1:1 correlation. K, D and P were as follows (D :×10^{-11}cm^2/s, P :×10^{-6}cm/s): MP (K=21.3, D=14.2, P=2.03), EP (K=46.1, D=10.2, P=3.15), PP (K=86.3, D=6.67, P=3.84), and BP (K=115, D=1.63, P=1.26).

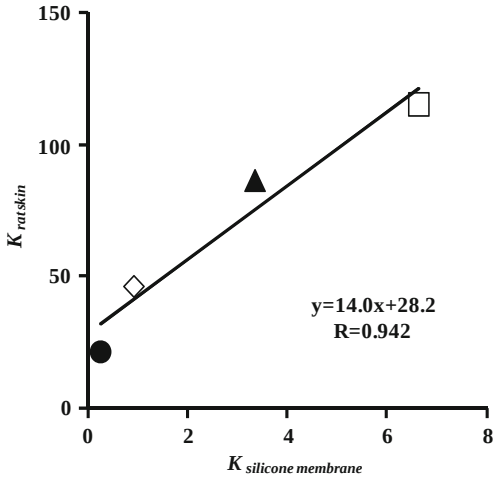

Fig. 9. Relationship between K to silicone membrane and K to rat skin (one-layered diffusion model). Symbols: see Fig. 3. Each data point represents the mean ± S.E. (n=4–8 in silicone membrane data and 5–11 in rat skin data).

Fig. 6 shows the cumulative amount of parabens that permeated the excised hairless rat skin. Fig. 6a and b show the mean raw data and normalized permeation data. The latter was normalized by the application amount of parabens. The raw skin permeation data of parabens, as shown in Fig. 6a, are similar to silicone membrane permeation; however, the permeability coefficient ratio of BP against MP in hairless rat skin was much larger than that in the silicone membrane. This may have been due to the different permeation pathways between the silicone membrane and rat skin. The silicone membrane is a homogeneous membrane, whereas hairless rat skin has appendages, such as hair follicles, as well as the stratum corneum pathway. Interestingly, BP permeation through the silicone membrane was highest in the normalized data (Fig. 3b) among the parabens used in the present study, whereas PP permeation through rat skin was highest (Fig. 6b). These data are probably due to similar solubility parameter of BP or PP to that of the silicone membrane or rat skin, respectively.

Fig. 7a and b show the observed and normalized concentrations of parabens corrected using their application concentrations in hairless rat skin, respectively. Each paraben concentration in rat skin was that at the steady state after

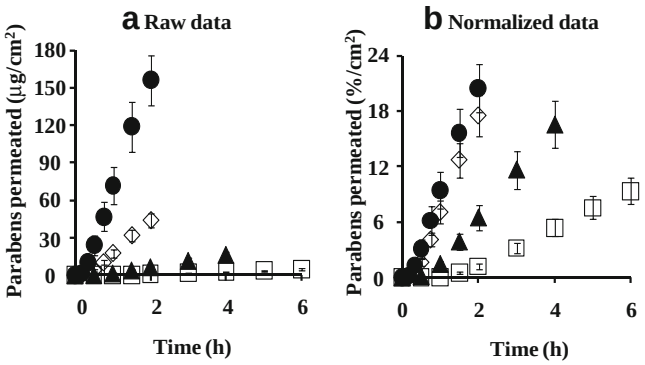

Fig. 10. Time course of the cumulative amount of parabens that permeated hairless rat stripped skin. (**a**) raw data, (**b**) normalized data. Symbols: see Fig. 3. Each data point represents the mean ± S.E. (n=3).

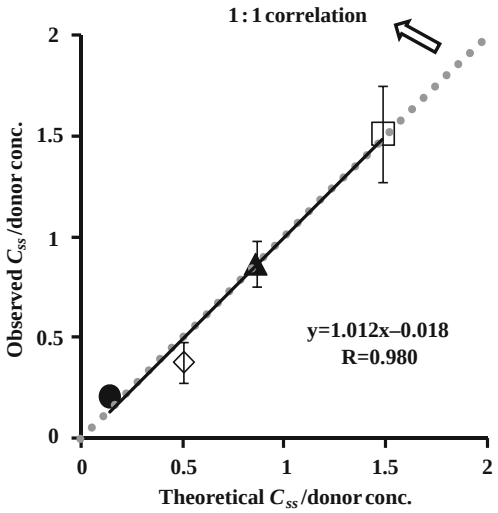

Fig. 11. Relationship between theoretical and observed C_{ss} in hairless rat skin. Normalized data were used (see Fig. 7). Two-layered diffusion membrane model was used to obtain theoretical steady-state skin concentration of parabens. Symbols: see Fig. 3. The observed data represent the mean±S.D. (n=3–8). Almost 1:1 correlation was found. K, D and P were as follows (D_{sc} :× 10^{-10}cm^2/s, P_{tot} :×10^{-6}cm/s, D_{ved} :×10^{-7}cm^2/s, P_{ved} :×10^{-5}cm/s): MP (K_{sc}=4.55, D_{sc}=9.38, P_{tot}=2.46, K_{ved}=2.95, D_{ved}=3.66, P_{ved}= 1.85), EP (K_{sc}=9.18, D_{sc} = 9.36, P_{tot}=5.73, K_{ved}=5.23, D_{ved}=2.19, P_{ved}=1.96), PP (K_{sc}=48.6, D_{sc}=5.72, P_{tot}=0.859, K_{ved}=2.57, D_{ved}= 1.78, P_{ved}=0.628), and BP (K_{sc}=42.3, D_{sc}=9.73, P_{tot}=1.25, K_{ved}= 4.58, D_{ved}=0.325, P_{ved}=0.348).

starting the skin permeation experiment. As in the silicone membrane, the increase in partition coefficient, K, of parabens increased the skin concentration. The increment, however, was marked in the silicone membrane concentration, not in rat skin, except MP. This is due to the similar solubility parameter of parabens to that in the silicone membrane.

Fig. 8 shows the relation between the theoretical and observed skin concentrations of parabens. Although no 1:1 relationship was observed, a linear relation was found. When skin permeation data of a series of compounds are obtained,

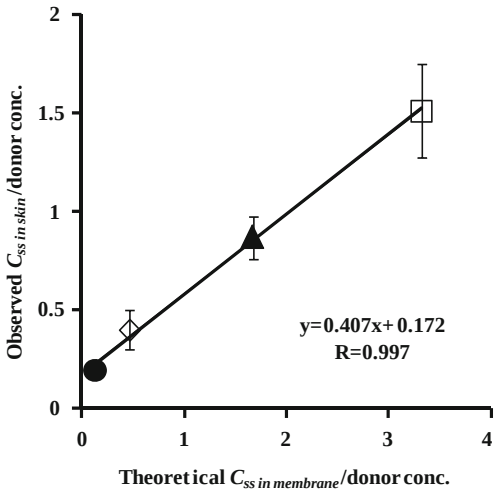

Fig. 12. Relationship between theoretical C_{ss} in silicone membrane/donor concentration and observed C_{ss} in rat skin/donor concentration. Symbols: see Fig. 3. Each data point represents the mean ± S.D. (n=3–8).

the skin concentration of the compounds may be theoretically calculated. The figure also contains the obtained permeation parameters of parabens in the figure legend, under the assumption that the skin membrane is one homogenous layer. Compared to the silicone membrane, lower P and D and higher K were observed in the rat skin. Unfortunately, the theoretical skin concentration of parabens was much higher than the observed skin concentration. This lack of a 1:1 relationship is due to the assumption that the skin is one homogenous layer.

Fig. 9 illustrates the relationship between K and silicone membrane and rat skin (11). The two K values have a linear relation. These results suggest that the skin concentration of parabens cannot be easily predicted by calculating the skin permeation profiles because of the simple assumption about the skin membrane.

Simulation of Skin Concentration of Parabens

We then assumed that skin consists of two diffusion layers, of which the first layer is the stratum corneum and the second layer is the viable epidermis and dermis. The partition coefficient from the vehicle to the stratum corneum and that to the viable epidermis and dermis was obtained from permeation data through full-thickness and stripped skin.

First, the stripped-skin permeability of parabens was measured. Fig. 10a and b show raw and normalized permeation profiles through stripped skin. Tape stripping of the stratum corneum increased the skin permeation of parabens, especially hydrophilic parabens such as MP.

Fig. 11 shows the relation between the observed and calculated skin concentrations. The figure also summarizes the obtained permeation parameters of parabens in the legend. The theoretical values are close to the observed values, although little difference was found, which may be dependent on the process of washing the skin surface. The two-layered model predicts the skin concentration of parabens from skin permeation experiments much better than the one-layered model of hairless rat skin. Human and animal skins have appendages, such as hair follicles and sweat ducts, as additional permeation pathways to the primary permeation pathway, the stratum corneum (21). The hydrophilic pathway and the lipophillic pathway play a role in the overall skin permeation of several compounds. The contribution of appendages and the hydrophilic pathway must be taken into account to better predict the skin concentration of materials from skin permeation profiles.

Fig. 12 shows the relationship between the theoretical concentration of parabens in the silicone membrane and the normalized observed concentration of parabens in hairless rat skin. The very high correlation coefficient, 0.997, between them suggests the high predictability of the skin concentration of parabens using silicone membrane permeation experiments.

We supposed a homogenous one-layered model for the silicone membrane and two-layered model consisted of stratum corneum and the following layer for the rat skin. Although these membrane models were different, their concentration-distance profiles and theoretical concentrations of drug or cosmetic ingredient in both the membranes can be expressed only by physical diffusion model. Thus, membrane concentration in the two-layered diffusion model can be easily replaced by that in the one-layered model using a mathematical approach. This is a reason why diffusion profile through silicone membrane is useful to predict the skin concentration of drugs or cosmetic ingredients.

In the near future, we plan to use broad compounds other than parabens, which are a simple series of compounds. We also plan to use several topical formulations, such as creams, ointments and patches. A silicone membrane permeation study using broad compounds from several formulations will produce a monogram of how to estimate the skin concentration of materials. All topical drugs have different target sites in skin tissues. Distribution of the skin concentration must be clarified from the shallow to deep layer in the near future. Since this is an alternative method to human and animal studies, it can be easily used by pharmaceutical and cosmetic companies to estimate the skin concentration after applying topical drug formulations and cosmetics.

CONCLUSION

The drug concentration in the silicone membrane and animal or human skin can be easily predicted using diffusion equations and membrane permeation data. This method can be applied to the design of cosmetic and topical pharmaceutical formulations. A silicone membrane can be used as an alternative membrane to human and animal skin.

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