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# Diffusion properties of model compounds in artificial sebum

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**Abstract**

Sebaceous glands secrete an oily sebum into the hair follicle. Hence, it is necessary to understand the drug partition and diffusion properties in the sebum for the targeted delivery of therapeutic agents into the sebum-filled hair follicle. A new method was developed and used for determination of sebum flux of topical therapeutic agents and other model compounds. The drug transport through artificial sebum was conducted using sebum loaded filter (Transwell®) as a membrane, drug suspensions as donor phases and HP-β-CD buffer solution as a receiver phase. The experiment was performed at 37 °C for 2 h. The results of the drug transport studies indicate that the flux ($J_{sebum}$) through the artificial sebum is compound dependent and a bell-shaped curve was observed when $\log J_s$ versus alkyl side chain length of the compounds that proved to be different from the curves obtained upon plotting $\log J$ skin versus *c*log *P* for the same compounds, indicating the possibility to select appropriate compounds for sebum targeted delivery based on the differences in the skin flux and sebum transport profiles of the molecules.


*Keywords:* Artificial sebum; Flux; Permeability coefficient; Sebaceous gland; Hair follicle; Targeted delivery

## 1. Introduction

In the treatment of skin diseases and disorders, there are primarily two potential delivery mechanisms for topically applied drugs—transepidermal and transfollicular. In transepidermal delivery, transport of drugs occurs across the stratum corneum whereas in the transfollicular route, drug absorption/transport occurs through hair follicles and sebaceous glands. A number of studies have demonstrated that the hair follicles and the sebaceous glands contribute to the penetration of drugs across the skin (Bertolino et al., 1993; Lauer et al., 1995; Illel, 1997; Grams and Bouwstra, 2002; Vogt et al., 2005). Targeting drug delivery to the pilosebaceous unit (the hair follicle, sebaceous glands and the hair shaft) may allow increased deposition of active compounds into hair follicular ducts, while at the same time, retarding transepidermal transport. This could lead to better control of drug systemic exposure and improve the overall efficacy/safety margins. However, the main obstacles to access these sites are the structure of the hair follicle itself and the physicochemical environment present in the pilosebaceous unit. The keratinous layers of the inner and outer root sheaths and the glassy membrane surrounding the entire follicle may restrict passage of molecules deep within the follicle. To date, the transport details of drug molecules in hair follicle ducts and sebaceous glands are yet to be fully understood. The possible applications of such targeted follicular delivery include the treatment of hair growth abnormalities, as well as hair follicle-associated diseases, such as acne. Targeted drug delivery to the hair follicle can be managed by two quite different ways—the first being a *formulation approach* and the second being a *molecule modification approach* (Grams and Bouwstra, 2002). Several researchers using the formulation approach have established that improved localized delivery of drugs to the hair follicle can be achieved by varying the compositions of applied formulations (Meidan et al., 2005). In one case, the gains in localized delivery were achieved through the application of a system containing a particulate carrier (Toll et al., 2004) and in the second case by using sebum miscible excipients in the topical preparation (Motwani et al., 2004). In contrast, the molecule modification approach involves a tailoring of the physicochemical properties of a drug molecule, such as its size, polarity (lipophilicity), polar surface area, solubility parameter and/or charge, any of which has a potential to modulate delivery into the hair follicle (Lauer et al., 1995; Illel, 1997). Several recently published reviews concisely capture the experience and advances in this area (Hueber et al., 1994a,b; Lauer et al., 1997; Meidan et al., 2005; Vogt et al.,

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2005). In most of the studies performed, the role of sebum (an oily secretion produced by the sebaceous gland into the hair follicle) in follicular targeted delivery is poorly understood, except for a study on the use of differential scanning calorimetry to understand the nature of artificial sebum–excipient interactions (Motwani et al., 2001, 2002). Hence, an efficient drug delivery into the sebum-filled hair follicle and sebaceous gland would greatly depend on the partition/diffusion of the drug molecule in sebum. Until now, there were no reports available on the drug partition and diffusion in the sebum. In lieu of performing studies with human sebum samples, it is desirable to study drug partition and diffusion in a controlled environment by using artificial sebum which has similar chemical and physical properties as human sebum. Previously, we studied the partition of model compounds in artificial sebum/water in which we demonstrated that the partition coefficient of model drugs in artificial sebum is a primary function of chemical structure and lipophilicity of the molecule (Valiveti et al., 2007). Drug diffusion through the artificial sebum gives information about the mobility of the molecule in and out of the sebum from aqueous solution or suspensions. In this study, we have investigated the diffusion properties of model drugs in the artificial sebum and its relationship with the sebum partition coefficient, $c\log P$, and alkyl chain length.

## 2. Materials and methods

### 2.1. Materials

Salicylic acid (purity 99%), cholesterol, octyl 4-hydroxybenzoate, methyl 4-hydroxybenzoate, ethyl 4-hydroxy benzoate, butyl-4-hydroxybenzoate and heptyl 4-hydroxybenzoate were obtained from Lancaster Synthesis, Inc. (Pelham, NH). 3,4-Dihydroxy benzoic acid (purity 98%), tretinoin, oxalic acid (purity >99%), *o*-phosphoric acid, paraffin wax (melting point, 58–62 °C), oleic acid, hexadecyl 4-hydroxybenzoate and 4-hydroxybenzoic acid were obtained from Aldrich Chemical Company, Inc. (Milwaukee, WI). Ketoconazole, minoxidil, cottonseed oil, palmitoleic acid, squalene and octanol were obtained from M.P. Biomedical, LLC (Aurora, OH). Lidocaine base (purity >98%), trifluroacetic acid, acetyl salicylic acid, methyl 5-acetyl salicylate, lidocaine HCl, prednisolone, hydrocortisone 21-caprylate, hydrocortisone 17-butyrate, hydrocortisone 17-valerate, hydrocortisone 17-acetate and propyl 4-hydroxybenzoate were obtained from Sigma Chemical Company, Inc. (St. Louis, MO). Coconut oil was obtained from Aldon Corporation (Avon, NY). Olive oil, hydrocortisone and palmitic acid were obtained from EMD Chemicals (Gibbstown, NJ). Spermaceti wax and cholesterol oleate were obtained from Sargent-Welch (Buffalo, IL) and Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan), respectively. Amyl 4-hydroxybenzoate, hexyl 4-hydroxybenzoate, phenyl 4-hydroxybenzoate, betamethasone, dexamethasone, nonyl 4-hydroxybenzoate and ethyhexyl 4-hydrxybenzoate were obtained from TCI America (Portland, OR). Acetonitrile, methanol, water for HPLC, tetrahydrofurone, acetic acid, potassium dihydrogen phosphate, sodium dihydrogen phosphate, disodium hydrogen phosphate and ammonium hydroxide were obtained from Mallinckrodt Baker, Inc. (Philipsburg, NJ). Adapalene and dodecyl 4-hydroxybenzoate were obtained from ChemPacific (Baltimore, MD) and Alfa Aesar (Ward Hill, MA), respectively.

### 2.2. Instruments

Equipment used consisted of a 1100 series high-pressure liquid chromatography (HPLC) instrument with an Agilent 1100 series autosampler and a Diode Array detector model 785A (Agilent Technologies, Inc., Palo Alto, CA) and an Innova® 4000 series incubator and shaker (New Brunswick Scientific Co., Inc., Edison, NJ).

### 2.3. Preparation of artificial sebum

The main components of the sebum are triglycerides, wax esters, squalene, cholesterol and cholesterol esters (Strauss et al., 1976). The chemical composition of the artificial sebum has been chosen based on the human sebum chemical composition reported in the literature (Walters and Roberts, 2002; Rosenthal, 1964; Greene et al., 1970; Nordstrom et al., 1986).

The chemical composition (%, w/w) of the artificial sebum is shown in Table 1. The ingredients were weighed out (%, w/w) in a glass beaker and heated at 60 °C with intermittent stirring until all the solids became a clear liquid (10 min). This was done to ensure uniform mixing of the model sebum lipids. The mixture was allowed to cool down at room temperature. All components of the artificial sebum were miscible at 60 °C and there were no visual indications of separation of sebum lipids. Moreover, the variation in the in vitro partition and diffusion data from different lots of the artificial sebum was less than 20% indicating a good reproducibility of preparation and uniformity of the artificial sebum prepared.

### 2.4. Drug transport through the artificial sebum

The drug transport through the artificial sebum is carried out in 24-well format (Transwell®, Corning Incorporated, NY). The supporting membrane (polycarbonate membrane, pore size of 0.4 μm) of each insert was loaded with $2.1 \pm 0.2$ mg of the artificial sebum (previously heated at 50–55 °C). A 150 μL aliquot of aqueous suspension of model drug (10 mg/mL in citrate–phosphate buffer (CPB, pH 5.5) equilibrated overnight on a shaker) was applied onto the insert and 1 mL of preheated (37 °C) 10% HP-β-cyclodextrin in the CPB was used as receiver solution. The entire study was carried out in an incubator at 37 °C and 125 rpm. The sampling interval was every 10 min for 2 h. At each sampling time, the entire receiver solution was replaced with fresh buffer. The withdrawn receiver solutions were analyzed for drug content using reported HPLC methods in the literature with or without modification. The cumulative quantity of drug in the receiver compartment was plotted as a function of time. The flux value for a given experiment was obtained from the linear slope (steady-state portion) of the cumulative amount of drug permeated versus time curve. The aqueous solubility of each compound was determined by centrifugation of the suspen-

Table 1
Chemical composition of the human sebum and artificial sebum

| Composition | Human sebum-I[a] (lumen/surface) (%) | Human sebum-II[b] (%) | Human sebum-III[c] (%) | Human sebum-IV[d] (%) | Artificial sebum (%) |
|---|---|---|---|---|---|
| Squalene | 15/15 | 13 | 12 | 19.9 | 15 |
| Wax esters | 25/25 | 26 | 26 | 25.3 | |
| Paraffin wax | | | | | 10 |
| Spermaceti wax | | | | | 15 |
| Triglycerides | 57/42 | 32 | 57.5 | 16.1 | |
| Olive oil ($C_{16}$–$C_{18}$) | | | | | 10 |
| Cotton seed oil ($C_{16}$–$C_{18}$) | | | | | 25 |
| Coconut oil ($C_{12}$–$C_{16}$) | | | | | 10 |
| Fatty acids | 0/15 (C16) | 23 | – | 33.0 | |
| Oleic acid | | | | | 1.4 |
| Palmitic acid | | | | | 5 |
| Palmitoleic acid | | | | | 5 |
| Cholesterol | 1/1 | 1.6 | 1.5 | 3.8 | 1.2 |
| Cholesteryl esters | 2/2 | 3.5 | 2.0 | 2.0 | |
| Cholesterol oleate | | | | | 2.4 |

[a] Walters and Roberts (2002)
[b] Rosenthal (1964).
[c] Greene et al. (1970).
[d] Nordstrom et al. (1986).

sion used for the donor phase and analysis of the supernatant with HPLC. The permeability coefficients were calculated from the steady-state flux and the drug concentration (solubility) in the vehicle. The diffusion coefficients were calculated using permeability coefficients, the thickness of sebum layer and the sebum partition coefficient generated in the previous study (Valiveti et al., 2007).

## 3. Results and discussion

Human sebum flows outward from the sebaceous gland to skin surface and may hinder the passage of drug into the hair follicle. Hence, an effective drug delivery to the hair follicle is a function of partition and diffusion of a therapeutic agent in the sebum, while balancing and counteracting the outward flow of sebum as well as drug elimination from hair follicles to the surrounding tissues and blood circulation.

This can be understood by the following equations, assuming that drug transport through the skin is primarily through transepidermal (SC is primary barrier) and follicular pathways (Valiveti et al., 2007).

$$J_{\text{total}} = J_{\text{sebum}} + J_{\text{sc}} = APC \tag{1}$$

where $J_{\text{total}}$ is the total flux, and '$J_{\text{sebum}}$' and '$J_{\text{sc}}$' are fluxes through the independent pathways. $A$ is the total permeation area, $P$ the permeability coefficient and $C$ is the concentration of drug in the application. It follows that

$$J_{\text{total}} = A\left[f_{\text{sebum}}\frac{D_{\text{sebum}}K_{\text{sebum}}}{h_{\text{sebum}}} + f_{\text{sc}}\frac{D_{\text{sc}}K_{\text{sc}}}{h_{\text{sc}}}\right]C$$
$$= \left[A_{\text{sebum}}\frac{D_{\text{sebum}}K_{\text{sebum}}C}{h_{\text{sebum}}}\right] + \left[A_{\text{sc}}\frac{D_{\text{sc}}K_{\text{sc}}C}{h_{\text{sc}}}\right] \tag{2}$$

In these equations, $f_{\text{sebum}}$ and $f_{\text{sc}}$ are the fractional areas of the transfollicular and transepidermal routes, respectively, and '$A_{\text{sebum}}$' and '$A_{\text{sc}}$' are the actual areas of the sebum and stratum corneum routes. '$D_{\text{sebum}}$ and $D_{\text{sc}}$' are the functional diffusion coefficients for the drug in question through sebum and the stratum corneum, while '$K_{\text{sebum}}$' and '$K_{\text{sc}}$' are the drug's partition coefficients in sebum and stratum corneum, respectively. The terms, '$h_{\text{sebum}}$' and '$h_{\text{sc}}$', are the functional thicknesses of the sebum and stratum corneum. In these equations the partition coefficients exhibit the greatest variability between compounds within a family and thus are the parameters most likely to differentiate the mechanism (Flynn, 1996). Partition coefficient is a thermodynamic parameter measured at equilibrium whereas the permeability coefficient reflects both thermodynamic and kinetic properties and the diffusion coefficient represents primarily the kinetic properties of molecules. Therefore, when $K_{\text{sebum}} \gg K_{\text{sc}}$, drug molecules are more likely to transport into sebum-rich hair follicles and sebaceous glands. When the reverse is true, that is, when $K_{\text{sebum}} \ll K_{\text{sc}}$, the transfollicular pathway may play a minimal role in topical drug delivery. Therefore, the ratio of $K_{\text{sebum}}/K_{\text{sc}}$, and similarly, the ratio of $D_{\text{sebum}}/D_{\text{sc}}$ provides a useful parameter for assessment of the potential pathway of topically applied agents.

In the previous study (Valiveti et al., 2007), we investigated the partitioning between artificial sebum and water ($K_{\text{sebum}}$), and between human stratum corneum and water ($K_{\text{sc}}$). These studies demonstrate that $K_{\text{sebum}}$ is different from $K_{\text{sc}}$ and also $P_{\text{o/w}}$, attesting to the fact that these three media have fundamentally different capacities to dissolve organic compounds. In the present study, we further developed an in vitro sebum diffusion model to understand the kinetic behavior of various molecules in artificial sebum.

As shown in Table 1, an artificial sebum developed in our lab was used for our previous and present studies. The chemical composition of the artificial sebum is similar to the chemical composition of human sebum (Table 1). Paraffin wax and spermaceti wax were the source of wax esters. Olive oil, cotton seed oil and coconut oil served as the triglycerides in the artificial sebum based on the similar carbon chain length of the fatty esters with the chemical compositions of human sebum. The percentage of fatty acids in human sebum samples varies greatly and depends on the degree of metabolism of triglycerides while sebum secretes from the sebaceous glands (no fatty acids) to the skin surface (up to 45% fatty acids). Considering the sebum of interest is in the upper duct of hair follicles, a relatively low percentage of fatty acid (∼11%) was added in the artificial sebum. The artificial sebum showed very good correlation with human sebum in terms of chemical ($^1$H NMR) and physical (DSC, partition and diffusion) properties (unpublished data).

Transwell® 3413 inserts are widely used for drug permeation through cell layers. Polycarbonate filter membrane is compatible with cultured cells and the relevant excipients. Using information from the supplier (Corning, NY), the effective permeation area and the thickness of the artificial sebum membrane loaded on the filter were calculated for the estimation of sebum flux and diffusion coefficient. According to the pore size of 0.4 μm, the insert diameter of 6.5 mm, and the pore density of $1 \times 10^8$ pores/cm$^2$, the effective permeation area was estimated as 4.17 mm$^2$/insert, which is 12.6% of the total area of the insert. Approximately $2.1 \pm 0.25$ mg of the artificial sebum was loaded on the filter of each insert, and the loading reproducibility was ensured by weighing the insert before and after loading. The variation of the sebum loading on the inserts was less than 15%. The density of the sebum was determined as $0.886 \pm 0.0005$ g/mL for conversion of weight and volume. Using the volume of the sebum loaded and the area of the filter, the thickness of the sebum membrane formed on the filter was estimated as 80 μm (the filter was not taken into account). Although the artificial sebum melts at temperatures >36 °C, the sebum membrane on the filter appeared to be undisturbed under the testing conditions (37 °C), even with mild agitation. Since HP-β-CD has been widely used to increase aqueous solubility of poorly soluble drugs, a 10% of HP-β-CD buffer solution (pH 5.5) was used as the receiver solution, to maintain sink conditions. We have evaluated the effect of HP-β-CD on the permeability of drug across the artificial sebum layer using lidocaine as a model compound. The results of this study show that there is no significant difference in the permeability of lidocaine with the receiver solution containing 5 and 10% HP-β-CD in citrate–phosphate buffer compared to the receiver solution containing citrate–phosphate buffer alone. It appears that HP-β-CD in the receiver solution has no significant affect on the sebum integrity under the tested conditions, which is in agreement with the result that HP-β-CD has minimal effect on the phase transitions of model sebum reported by Motwani et al. (2002). Initially drug transport through artificial sebum was carried out for 6 h. Since the suspensions were used as donor phases, the fluxes quickly reached the steady state within <30 min. Therefore, a 2-h sampling period was selected for the study.

Compounds with great diversity in terms of lipophilicity, charge, acidity and molecular weight were tested with this novel method, and the results are shown in Tables 2 and 3. The variation of sebum flux is generally less than 20% ($n = 4$–6). The developed model is very sensitive to small changes of molecular structure including the molecular steric orientation. Therefore, for the first time, a simple, reliable and rapid method has been developed, which makes the measurement of drug transport through sebum at 37 °C or other temperatures possible.

Fig. 1 shows the typical profiles of cumulative amount of drug permeated across the artificial sebum from an aqueous drug suspension. The sebum permeation of tested compounds

Table 2
Mean (±S.D.) steady-state flux, permeability coefficient and diffusion coefficient of model drugs with different chemical structures through the artificial sebum ($n = 6$)

| Drug | $c \log P$ | Sebum flux, $J_{Sebum}$ (μg/cm$^2$/min) | Solubility (μg/mL) | Permeability coefficient (cm/s) × $10^{-3}$ |
|---|---|---|---|---|
| Ketoconazole | 2.88 | 16.9 ± 1.78 | 22.17 | 12.7 |
| Minoxidil | 0.69 | 14.8 ± 2.87 | 2483 | 0.10 |
| Lidocaine | 2.4 | 1913 ± 181 | 23,318 | 1.37 |
| Lidocaine HCl | 2.4 | 103 ± 12.6 | 484,919 | 0.0036 |
| Salicylic acid | 2.2 | 309 ± 16.1 | 6972 | 0.74 |
| Acetyl salicylic acid | 1.1 | 72.1 ± 5.58 | 8359 | 0.14 |
| Methyl 5-acety salicylic acid | 2.45 | 159 ± 12.4 | 546.3 | 4.87 |
| Hydrocortisone | 1.43 | 0.91 ± 0.10 | 283.4 | 0.05 |
| Prednisolone | 1.5 | 0.60 ± 0.10 | 166.6 | 0.06 |
| Betamethasone | 1.87 | 0.03 ± 0.01 | 60.40 | 0.83 |
| Dexamethasone | 1.87 | 0.31 ± 0.03 | 74.57 | 0.07 |
| Hydrocortisone 21-caprylate | 5.7 | 17.6 ± 3.45 | 0.091 | 3220 |
| Hydrocortisone 17-butyrate | 2.81 | 6.89 ± 0.65 | 42.50 | 2.70 |
| Hydrocortisone 17-valerate | 3.34 | 8.89 ± 1.81 | 18.82 | 7.87 |
| Hydrocortisone 17-acetate | 2.5 | 0.67 ± 0.05 | 7.82 | 1.43 |
| 2,5-Dihydroxyl benzoic acid | −0.67 | 55.0 ± 5.58 | 23,816 | 0.039 |
| Hexadecyl 4-hydroxybenzoate | 13 | BDL | | |
| Ethyhexyl 4-hydroxybenzoate | 5.4 | 221 ± 19.1 | 3.68 | 1000 |
| Phenyl 4-hydroxybenzoate | 3.5 | 79.8 ± 5.24 | 23.19 | 57.4 |

Table 3
Mean (±S.D.) steady-state flux, permeability coefficient and diffusion coefficient of model drugs with similar chemical structure through the artificial sebum ($n = 6$)

| Drug | $c \log P$ | Sebum flux ($\mu g/cm^2/min$) | Solubility ($\mu g/mL$) | Permeability coefficient (cm/s) $\times 10^{-3}$ | Sebum partition coefficient, $K_{sebum}$ | Diffusion coefficient ($cm^2/s$) $\times 10^{-6}$ |
|---|---|---|---|---|---|---|
| 4-Hydroxybenzoic acid | 1.42 | 25.9 ± 3.13 | 6531 | 0.07 | 2.38 | 0.222 |
| Methyl 4-hydroxybenzoate | 1.86 | 106 ± 9.45 | 1641 | 1.08 | 5.74 | 1.51 |
| Ethyl 4-hydrooxybenzoate | 2.4 | 143 ± 20.4 | 640.1 | 3.71 | 13.66 | 2.175 |
| Propyl 4-hydroxybenzoate | 2.93 | 157 ± 24.1 | 245.0 | 10.7 | 51.96 | 1.644 |
| Butyl 4-hydroxybenzoate | 3.46 | 195 ± 14.8 | 177.2 | 18.3 | 151.5 | 0.967 |
| Amyl 4-hydroxybenzoate | 3.99 | 319 ± 21.4 | 103.1 | 51.5 | 471.2 | 0.875 |
| Hexyl 4-hydroxybenzoate | 4.5 | 265 ± 29.3 | 24.29 | 182 | 1768 | 0.823 |
| Heptyl 4-hydroxybenzoate | 5.05 | 268 ± 9.99 | 11.45 | 390 | 3818 | 0.817 |
| Octyl 4-hydroxybenzoate | 5.58 | 160 ± 9.15 | 0.93 | 2815 | 11,078 | 2.033 |
| Nonyl 4-hydroxybenzoate | 6.12 | 108 ± 9.29 | 0.11 | 16,294 | 25,100 | 5.193 |
| Dodecyl 4-hydroxybenzoate | 7.71 | 18.6 ± 3.59 | 0.02 | 16,150 | 55,012 | 2.349 |

reached the steady state within <30 min. Downing and Strauss (1982) estimated that it took approximately 14 h for sebum to transport from sebaceous glands to the skin surface with a distance of 200–500 μm. In the present study, the thickness of the sebum layer is ~80 μm. Therefore, the transport rates of the molecules through artificial sebum are likely much faster than the discharge rate of sebum in human subjects. Table 2 shows the measured sebum flux ($J_s$), aqueous solubility and calculated permeability coefficient ($P_s$) of model compounds with different chemical structure through the artificial sebum. Among the tested compounds, lidocaine, lidocaine HCl, salicylic acid and ethylhexyl 4-hydroxybenzoate showed higher sebum flux (>100 $\mu g/cm^2/min$) while hydrocortisone, prednisolone, betamethasone, dexamethasone and hydrocortisone 17-acetate transport through sebum was much slower than others ($J_s < 1$ $\mu g/cm^2/min$). The sebum flux from the aqueous suspension of hexadecyl 4-hydroxybenzoate was below the detection limit, possibly due to the extreme lipophilic nature ($c \log P$, 13.0) of the molecule. It is very interesting to note that lidocaine permeated through the sebum layer much faster than its HCl salt even though the aqueous solubility of lidocaine was only 5% of the salt. The results indicated that the diffusion of the charged molecules in sebum was much slower than the neutral molecules. Therefore, it is not surprising that the calculated permeability of lidocaine was 380 times higher than that of lidocaine HCl. In addition, the interaction between basic lidocaine and acidic sebum could be substantially favorable for the partition of the non-ionized form compared to the ionized form. However, effect of basic compounds on sebum partition and diffusion needs to be further investigated. In the case of hydrocortisone and its esters, the mean flux (Table 2) of hydrocortisone 21-caprylate (17.58 $\mu g/cm^2/min$), hydrocortisone 17-butyrate (6.89 $\mu g/cm^2/min$) and hydrocortisone 17-valerate (8.89 $\mu g/cm^2/min$) were significantly ($p < 0.001$) higher than that of hydrocortisone (0.91 $\mu g/cm^2/min$) and hydrocortisone 17-acetate (0.67 $\mu g/cm^2/min$), indicating that the lipophilicity of molecules plays a critical role in the transport rate through the artificial sebum by controlling drug partition/diffusion process. Surprisingly, the flux of dexamethasone (0.31 $\mu g/cm^2/min$) was 10-fold higher than that of betamethasone (0.03 $\mu g/cm^2/min$) although the $c \log P$s of these compounds were identical, and there was small difference in the solubility. Therefore, in addition to lipophilicity and molecular weight, the molecular orientation which is associated with molecular volume also significantly affects the sebum flux, primarily by the alteration of diffusion coefficient. By plotting the data in Table 3, poor correlation between $c \log P$ and log sebum flux ($r^2 = 0.04$) was obtained. However, a good linear relationship between $c \log P$ and log permeability coefficient ($r^2 = 0.702$) was observed, because of the removal of the solubility factor from the equation.

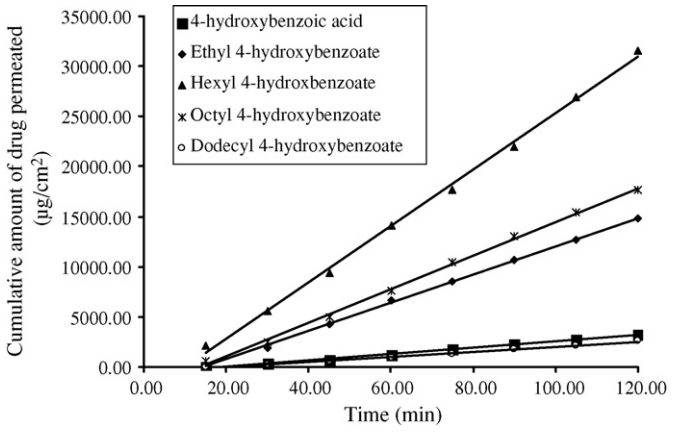


Fig. 1. Typical permeation profiles of 4-hydroxybenzoic esters across the artificial sebum from the aqueous drug suspensions (n=6).

In order to further understand the effect of molecular structure on sebum flux, a group of compounds having similar chemical structure was selected for investigation. Table 3 shows measured sebum flux ($J_s$) and aqueous solubility, as well as the calculated permeability coefficient ($P_s$) and sebum diffusion coefficient ($D_s$) of homologous series of 4-hydroxybenzoic acid ester compounds. It was observed that the sebum flux of 4-hydroxybenzoate series compounds increased with increasing carbon side chain length from $C_0$ to $C_5$ (25.88–318.54 $\mu g/cm^2/min$). However, the sebum flux decreased with further increase in the side chain length ($C_7$–$C_{12}$), heptyl 4-hydroxybenzoate (267.89 $\mu g/cm^2/min$) to dodecyl 4-hydroxybenzoate (18.64 $\mu g/cm^2/min$). The sebum flux versus chain length profile (Fig. 3) from the present study

is different from the sebum partition coefficient versus chain length from the previous study (Valiveti et al., 2007), in which the sebum partition coefficients of 4-hydroxybenzoic acid esters linearly increases with the addition of alkyl side chain length over the range of $C_0$–$C_{12}$. As shown in Table 2, the aqueous solubility of the compounds substantially decreased with the increase of carbon chain length. The diffusion coefficients of these compounds varied within a relatively small range ($0.222 \times 10^{-6}$ to $5.193 \times 10^{-6}$ $cm^2/s$), while the partition coefficient and the solubility values covered a range more than 5 orders of magnitude. Therefore, the partition coefficient and solubility appeared to be primary factors contributing to the sebum flux. It is reasonable to understand that the partition of the compounds into sebum dominated the sebum flux at the range of the alkyl side chain less than 4. With further increase of the chain length, the negative impact of the solubility on sebum flux gradually offset and then surpassed the positive contribution from the partition coefficient to sebum flux. The results of the present study revealed that the transport of drugs through the artificial sebum is a function of aqueous solubility, partition coefficient and diffusion coefficient.

Fig. 2 demonstrates the correlations between $c \log P$ and sebum permeability of two homologous series of compounds, hydrocortisone esters and 4-hydroxybenzoic acid esters. There was a clear trend that sebum permeability is a linear function of $c \log P$ ($r^2 = 0.945$ and 0.997). Compared to the data in Table 2, the homologous series of compounds showed better linear correlations than the compounds with diverse structures. These results indicated the possibility to predict the sebum permeability of homologous compounds from a linear curve. Hydrocortisone esters and 4-hydroxybenoic acid esters are two sets of the compounds with different chemical structures, and it is anticipatable that the influence of carbon chain on sebum permeability depended on molecular properties. The slopes of the linear curves may provide information in the degree of influence on the sebum permeability by altering carbon side chain length. However, more data are needed to develop a mathematic model for prediction of the sebum permeability based on carbon chain length as well as the lipophilicity or other molecular parameters.

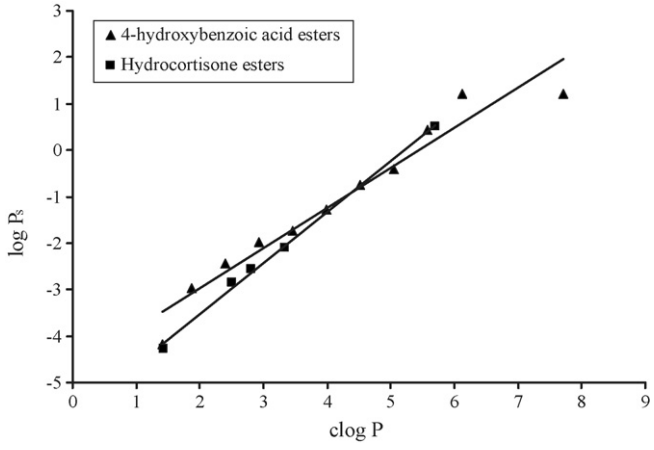


Fig. 2. Plot of correlation for clog P vs. $P_s$ (sebum permeability coefficient) of 4-hydroxybenzoic acid ester series compounds ($R^2$=0.945) and hydrocortisone esters compounds ($R^2$=0.997).

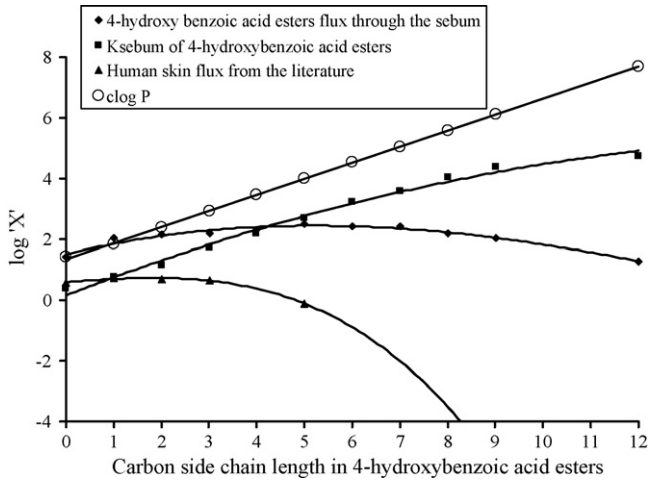


Fig. 3. Plot showing the relationship of carbon side chain length of 4-hydroxbenzoic acid esters with log $J_s$ (Sebum flux), log $K_{sebum}$ (sebum partition coefficient), clog P (calculated octanol-water partition coefficient) and log $J_{hs}$ (human skin flux from the literature by *Pozzo and Pastori, 1996*).

Fig. 3 shows the relationship between $\log J_s$ and alkyl side chain length of the 4-hydroxybenzoic acid esters. A bell-shaped curve was observed when $\log J_s$ versus alkyl side chain length of the compounds was plotted, which proved to be different from the curves obtained upon plotting $\log J$ skin versus $c \log P$ for the same compounds (Pozzo and Pastori, 1996). Pozzo and Pastori (1996) reported the absorption of six 4-hydroxybenzotaes through the human skin from aqueous solutions. It is interesting to note that the transport profile (Fig. 3) of a homologous series of 4-hydroxybenzoic acid ester compounds through the artificial sebum is different from that of bulk skin. As shown in Fig. 3, the maximum skin flux was observed for ethyl 4-hydroxybenzoate and the skin flux decreased with increasing lipophilic character of the molecule, while skin flux from octyl 4-hydroxybenzoate was undetectable. Whereas the sebum flux decreased from octyl 4-hydroxybenzoate with further increase in the lipophilicity of molecule. The penetration of a drug through the skin is a function of its solubility within the vehicle and its partition into and diffusion through the stratum corneum (Idson, 1983). Therefore, the extremely low skin flux for highly liphophilic compounds is contributed to poor aqueous solubility. The same trend was observed in sebum flux and skin flux profiles as shown in Fig. 3.

Another possible reason for decreasing sebum flux with increasing alkyl side chain length from $C_6$ is the increase in lipophilicity, molecular weight, and possibly the size of the higher chain 4-benzoates that limits their movement through the artificial sebum. Overall reduction in diffusivity with increasing chain length is indicative of either increased steric hindrance due to the increased molecular size, molecular interaction with the artificial sebum, or binding. Furthermore, these results are substantiated by the Cross et al. (2003) study, which shows a relationship between solute lipophilicity and skin penetration using a series of homologous alcohols ($C_2$–$C_{10}$), where the permeability coefficient increased with increasing lipophilicity to alcohols $C_8$ (octanol) with no further increase for $C_{10}$ (decanol).

It is well documented that molecules at $c\log P$ of 2–3 are desirable for transdermal delivery because skin flux decreases at higher $\log P$s. However, the decrease of sebum flux with increased lipophilicity occurred in a more lipophilic range compared to that of skin flux. In case of the 4-hydroxybenzoic acid ester compounds, the decline of sebum flux was shown at $c\log P > 4$ or the alkyl side chain > $C_6$. Fig. 3 demonstrates a window between hexyl 4-hydroxybenzoate and nonyl 4-hydroxybenzoate ($C_6$–$C_9$) in which the skin flux of these compounds are extremely low but the sebum flux and sebum partition remains high. Molecules falling into this window would be ideal candidates for sebum or follicular targeted delivery due to lower systemic exposure and higher localization in the pilosebaceous unit. However, the clinical indications of the sebum flux, sebum permeability and diffusion coefficient are yet to be fully understood. For example, the retention of therapeutic agents in sebum may be preferred for the treatment of acne, so that a high partition coefficient and low diffusion coefficient is desirable. High sebum permeability is likely to be beneficial for the treatment of hair conditions due to the fact that the target sites of the therapeutic agents are deeper than sebaceous glands.

## 4. Conclusions

A simple, reliable and rapid in vitro method was developed for the determination of sebum flux of therapeutic agents. The sebum flux study was conducted using sebum loaded Transwell® inserts both for diverse and homologous molecules. The variation of sebum fluxes obtained was generally less than 20%. The results indicates that the flux through the artificial sebum is primarily a function of lipophilicity and solubility while acidity, charge, molecular weight (or volume) and molecular orientation also contribute to the transport across artificial sebum. Interestingly, a bell-shaped curve from the series compounds was observed when plotting $\log J_{sebum}$ versus carbon side chain length which was different from the $\log J_{skin}$ versus $c\log P$ for the same compounds, indicating the possibility to select appropriate compounds for sebum targeted delivery.

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## References

Bertolino, A.P., Klein, L.M., Freedberg, I.M., 1993. Biology of hair follicles. In: Fitzpatrick, T.B., Eisen, A.Z., Wolff, K., Freedberg, I.M., Austen, K.F. (Eds.), Dermatology in General Medicine, fourth ed. McGraw-Hill, New York, pp. 289–291.

Cross, S.E., Magnusson, B.M., Winckle, G., Anissimov, Y., Roberts, M.S., 2003. Determination of the effect of lipophilicty on the in vitro permeability and tissue reservoir characteristics of topically applied solutes in human skin layers. J. Invest. Dermatol. 120, 759–764.

Downing, D.T., Strauss, J.S., 1982. On the mechanism of sebaceous secretion. Arch Dermatol Res. 272, 343–349.

Flynn, G.L., 1996. Cutaneous and transdermal delivery. In: Banker, G.S., Rhodes, C.T. (Eds.), Modern Pharmaceutics, third ed. Marcel Dekker Inc., New York, pp. 262–269.

Grams, Y.Y., Bouwstra, J.A., 2002. Penetration and distribution of three lipophilic probes in vitro in human skin focusing on the hair follicle. J. Control Release 83, 253–262.

Greene, R.S., Downing, D.T., Pochi, P.E., Strauss, J.S., 1970. Anatomical variation in the amount and composition of human skin surface lipid. J. Invest. Dermatol. 54, 240–247.

Hueber, F., Schaefer, H., Wepierre, J., 1994a. Role of transepidermal and transfollicular routes in percutaneous absorption of steroids: in vitro studies on human skin. Skin Pharmacol. 7, 237–244.

Hueber, F., Besnard, M., Schaefer, H., Wepierre, J., 1994b. Percutaneous absorption of estradiol and progesterone in normal and appendage-free skin of the hairless rat: lack of importance of nutritional blood flow. Skin Pharmacol. 7, 245–256.

Idson, B., 1983. Vehicle effects in percutaneous absorption. Drug Metab. Rev. 14, 207–222.

Illel, B., 1997. Formulations for transfollicular drug administration: some recent advances. Crit. Rev. Ther. Drug Carrier Syst. 14, 207–219.

Lauer, A.C., Lieb, L.M., Ramachandran, C., Flynn, G.L., Weiner, N.D., 1995. Transfollicular drug delivery. Pharm. Res. 12, 179–186.

Lauer, A.C., Elder, J.T., Weiner, N.D., 1997. Evaluation of the hairless rat as a model for in vivo percutaneous absorption. J. Pharm. Sci. 86, 13–18.

Meidan, V.M., Bonner, M.C., Michniak, B., 2005. Transfollicular drug delivery—is it a reality? Int. J. Pharm. 306, 1–14.

Motwani, M.R., Rhein, L.D., Zatz, J.L., 2004. Deposition of salicylic acid into hamster sebaceous. J. Cosmet Sci. 55, 519–531.

Motwani, M.R., Rhein, L.D., Zatz, J.L., 2001. Differential scanning calorimetry studies of sebum models. J. Cosmet Sci. 52, 211–224.

Motwani, M.R., Rhein, L.D., Zatz, J.L., 2002. Influence of vehicles on the phase transitions of model sebum. J. Cosmet Sci. 53, 35–42.

Nordstrom, K.M., Labows, J.N., McGinley, K.J., Leyden, J.J., 1986. Characterization of wax esters, triglycerides, and free fatty acids of follicular casts. J. Invest. Dermatol. 86, 700–705.

Pozzo, D.A., Pastori, N., 1996. Percutaneous absorption of parabens from cosmetic formulations. Int. J. Cosmet Sci. 18, 57–66.

Rosenthal, M.L., 1964. In: Libowe, I., Wells, F.V. (Eds.), Cosmetics and the Skin. Reinhold Publishing Corporation.

Strauss, J.S., Pochi, P.E., Downing, D.T., 1976. The sebaceous gland: twenty years of progress. J. Invest. Dermatol. 67, 90–97.

Toll, R., Jacobi, U., Ritcher, H., Lademan, J., Schaefer, H., Blume-Peytavi, U., 2004. Penetration profile of microspheres in follicular targeting of terminal hair follicles. J. Invest. Dermatol. 123, 168–176.

Valiveti, S., Wesley, J., Lu, G.W., 2007. Investigation of drug partition property in artificial sebum, Int. J. Pharm., doi:10.1016/j.ijpharm.2007.06.001.

Vogt, A., Mandt, N., Lademann, J., Schaefer, H., Blume-Perytavi, U., 2005. Follicular targeting—a promising tool in selective dermatotherapy. J. Invest. Dermatol. Symp. Proc. 10, 252–255.

Walters, K.A., Roberts, M.S., 2002. The structure and function of skin. In: Walters, K.A. (Ed.), Dermatological and Transdermal Formulations. Marcel Dekker, New York, pp. 1–40.