Effect of Sebum and Ointment Rubbing on the Skin Permeation of Triamcinolone Acetonide from White Petrolatum Ointment

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Effect of sebum and ointment rubbing was evaluated on the permeation of a model steroidal drug, triamcinolone acetonide (TA), from white petrolatum ointment through excised hairless rat skin. Two kinds of white petrolatum ointment containing TA at a concentration of 1.0% were made using a "pre-applied ointment," which was made by applying and recovering white petrolatum on and from the rat skin, respectively, and the "original ointment." Solubility and skin permeation of TA in and from both the ointments were measured and compared. TA solubility in the pre-applied ointment was about twice to that of the original ointment. Full-thickness skin and stripped skin permeabilities of TA from pre-applied ointment were about 2.3- and 1.5-times higher than those from the original ointment. In addition, the calculated partition coefficient of TA from pre-applied ointment to full-thickness skin was 2.7-times that of the original ointment. Next, the ointment-rubbing effect was determined. Skin permeation of TA from 1.0% TA original ointment with a 30 s-rubbing was 9-times higher than that after drug-free ointment-rubbing and application of 1.0% TA original ointment. The increase in solubility and skin permeation of TA due to pre-applied ointment is probably due to dissolution of TA by skin lipids extracted from the skin surface. Ointment rubbing increases the transient decrease in skin impedance and enhanced delivery of ointment base to the skin. These results are useful for development of ointment formulations and skin penetration mechanisms from an ointment base.

Key words   skin permeation; steroid; ointment; sebum; ointment rubbing; skin impedance

It is very important to clarify the partition and diffusion of drugs into and through the skin barrier for effective therapy when using topical drug formulations. Several studies have already been carried out on the release and skin permeation of drugs from topical formulations such as ointments and creams.[1—4] We have studied the effect of sebum or relative skin lipids on the release and skin permeation of drugs from topical formulations. When a pressure sensitive adhesive (PSA) patch containing lidocaine was applied to hairless rat skin, the drug crystal was dissolved in the PSA by the sebum or skin lipids that penetrated from the skin surface to the PSA matrix.[5] In addition, release and skin permeation of a hydrophilic drug were increased from white petrolatum by addition of sebum or skin lipids.[6] Cholesterol, cholesteryl oleate and ceramide were detected in a white petrolatum which was pre-applied to hairless rat skin.[6] However, the effect of sebum or skin lipids has not yet been determined on the skin permeation of lipophilic drugs.

Semisolid formulations such as ointments and creams are applied to skin, sometimes by rubbing on the skin surface. This is a key difference of semisolid formulations compared to skin adhesive topical formulations like patches and plasters. Rubbing ointments on skin may increase the skin permeation of entrapped drugs in ointments. However, quantitative studies have not yet been done on the ointment-rubbing effect on the skin permeation of drugs.

In the present study, a broadly used steroidal drug, triamcinolone acetonide (TA) (MW; 434.5, mp; 290 °C, log P; 2.53, solubility in water at 25 °C; 22.05 ± 0.05 μg/ml, and solubility parameter; 9.45 $(cal/cm^3)^{1/2}$) was selected as a model lipophilic drug, and white petrolatum was used as an ointment base.[7] The effect of sebum or skin lipids was determined on the dissolution and skin permeation of TA in and from white petrolatum, respectively. The rubbing effect of the ointment on the skin surface was also measured on the skin permeation and skin concentration of TA.

MATERIALS AND METHODS

Materials and Experimental Animals TA powder (mean diameter; 5 μm) was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). White petrolatum was purchased from Kosakai Pharmaceutical, Ltd. (Tokyo, Japan). Other chemicals and solvents were of reagent grade or HPLC grade and used without further purification. Male hairless rats (WBM/ILA-Ht, 8—9 weeks-old, body weight: 230—260 g) were obtained from the Life Science Research Center, Josai University (Sakado, Saitama, Japan) or Ishikawa Experimental Animals (Fukaya, Saitama, Japan). The animal feeding and experiments were conducted according to the ethical committee in Josai University.

Preparation of White Petrolatum Ointment Containing Triamcinolone Acetonide White petrolatum ointments (original ointment) of TA at concentrations of 10—50 mg TA/g (1.0—5.0%) were prepared by thoroughly mixing with white petrolatum to prepare a "TA original ointment." In order to evaluate the effect of sebum extracted from hairless rat skin on the skin permeation of TA, the white petrolatum base was pretreated for 6 h on full-thickness skin (application of 300 mg base on 0.95 cm² skin) and collected according to our previous paper.[6] The obtained pre-applied white petrolatum (pre-applied ointment) was used to prepare a "TA pre-applied ointment" of TA at concentrations of 1.0—5.0%. We have already reported that cholesterol (0.52 mg/cm² skin sureface), cholesteryl oleate (4.41 mg/cm² skin surface) and nonhydroxy fatty acid ceramide (0.22 mg/cm² skin surface) were extracted from the above pre-applied ointment.[6]

***In Vitro* Skin Permeation Experiments** Male hairless rats were fixed on their back after anesthesia by intraperitoneal (i.p.) injection of sodium pentobarbital, and hair on

the abdomen was shaved using an electric shaver. The skin was excised and excess fat was trimmed off. Stripped skin in the hairless rat was prepared by 20 consecutive tape-strippings of the stratum corneum from hairless rats. In the skin permeation experiments using ointments, 1.0% TA original or pre-applied ointment (about 300 mg) was placed in a disc-shaped cell cap with an effective diffusion diameter of 1.6 cm and thickness of 2.5 mm.[6] The cell cap was applied to the epidermal side of excised abdominal skin (full-thickness skin or stripped skin) set in a diffusion cell with an effective permeation area of 0.95 cm^2, with a water jacket connected to a water bath at 32 °C, while pH 7.4 phosphate-buffered saline (PBS, 3.0 ml) was applied to the dermal side. The receiver compartment was agitated using a magnetic stirrer and a stirrer bar. At predetermined times, an aliquot (600 μl) was withdrawn from the receiver solution and the same volume of fresh PBS was added to keep the volume constant. When the effect of ointment rubbing was determined on the skin permeation of TA, 1.0% TA original ointment or TA-free original ointment (100 mg/0.95 cm^2) was applied on the excised hairless rat skin and rubbed at a pressure of 1.87—3.12 N/cm^2 by a rubber-sacked forefinger for 30 s. Then, 1.0% TA original ointment or original ointment alone was again applied to the skin surface to start the skin permeation experiment. In addition, the amount of TA retained in skin was also determined after each permeation experiment as follows. The skin sample was removed, and the skin surface was cleaned off a few times with ethanol (99.5%) and once with PBS. The obtained skin sample was kept at −20 °C until analysis. The frozen skin was finely cut and homogenized with 5 ml of PBS on ice. This skin homogenate was used to determine the TA concentration.

Determination of Triamcinolone Acetonide Each 600-μl sample from the *in vitro* skin permeation experiment or skin homogenate was mixed with the same volume of internal standard (1.0 μg/ml of methyl 4-hydroxybenxoicate in chloroform) for HPLC determination. Next, the chloroform solution was collected and evaporated by a nitrogen gas purge. The dried sample was reconstituted with 50 μl of water : acetonitrile (65 : 35). An aliquot (20 μl) was injected into an HPLC system. The HPLC system consisted of a pump (LC-20AS; Shimadzu, Kyoto, Japan), a column (CAPCELL PAK C18 UG120 5 μm, 4.6×250 mm; Shiseido, Tokyo, Japan), an auto-injector (SIL-20A; Shimadzu), a UV detector (SPD-20A; Shimadzu) and an analysis system (LC solution; Shimadzu). The mobile phase was distilled water : acetonitrile (65 : 35) and the flow rate was 1.0 ml/min. Detection was performed at UV 240 nm.

Determining the Solubility of Triamcinolone Acetonide in White Petrolatum Ointment by Difference Scanning Calorimetry Solubility of TA in the ointment was calculated from the endothermic peak area of TA crystals in the ointment by a difference scanning calorimeter (DSC8230, Rigaku Co., Tokyo, Japan), according to previous studies.[8,9] The original ointment and pre-applied ointment (10 mg) containing TA at a concentration of 0, 1, 2, 3, 4 or 5% were packed in a stainless pan (Rigaku Co.). Al$_2$O$_3$ was used as a reference. Each sample was heated from 30 °C to 350 °C at a rate of 20 °C/min.

Measurement of Skin Impedance Excised hairless rat abdominal skin, full-thickness or stripped skin, or full-thick-

ness skin pretreated by rubbing on the skin surface with a forefinger for 30 s was sandwiched between two half cells (effective diffusion area of 0.95 cm^2 and donor and receiver volume of 3.0 ml).[10] PBS (3.0 ml each) was added to the stratum corneum and dermis sides. Skin impedance was determined by an impedance meter (10 Hz alternating current, Advance, Tokyo, Japan).

Analysis of Membrane and Skin Permeation by the Difference Method According Fick's 2nd Law The permeation profile of TA through rat skin was analyzed by Fick's 2nd law of diffusion. The analytical method was shown in our pervious papers.[9,11] A one-layered model was used for stripped hairless rat skin, and a two-layered model for full-thickness hairless rat skin.

RESULTS AND DISCUSSION

Effect of Sebum or Skin Lipids on the Solubility of Triamcinolone Acetonide in White Petrolatum Ointment The effect of sebum or skin lipids was determined on the solubility of TA from white petrolatum ointment. First, pre-applied ointment and original ointment containing TA at a concentration of 0, 1, 2, 3, 4 and 5% were prepared and the endothermic peak area due to TA crystals was determined using the ointments by DSC to estimate the effect of sebum or skin lipids on the dissolution of TA in the ointments. Figure 1a shows typical DSC charts for the TA original ointment and TA pre-applied ointment. The endothermic peak for TA crystals alone was detected at 302.8 °C. On the other hand, endothermic peaks for the original ointment and pre-applied ointment were detected at 312.2 °C and 297.6 °C, respectively. Ointment alone (original and pre-applied ointments) showed no peak for TA. The high temperature of the endothermic peak for the TA crystals in the original ointment was probably due to low heat conduction in the white petrolatum. In addition, it was reported that addition of sebum into surgical tapes decreased the glass-transition temperature.[12] Therefore, sebum penetrated from the skin surface to the white petrolatum may increase the heat conduction through the white petrolatum matrix. This may be one reason why the endothermic peak temperature for TA crystals in the pre-applied ointment was similar to that for simple TA crystals. Intercellular lipids in the stratum corneum may also affect the DSC chart as well as sebum.

The endothermic peak area of TA crystals in the pre-applied ointment was less than that for original ointment. When each endothermic peak area was plotted against TA concentration in the ointment, a good correlation line was obtained (Fig. 1b). Next, the solubility of TA in the ointment was determined by the *x*-axis intercept. Solubility of TA in the pre-applied ointment was determined to be 0.60% (see solid arrow in Fig. 1b), whereas that in the original ointment was 0.33% (see open arrow), suggesting that addition of sebum or skin lipids increased TA solubility about 1.8-times in the white petrolatum ointment. Sebum or relative lipids dissolve TA crystals in white petrolatum.

Effect of Sebum or Skin Lipids on the Skin Permeation of Triamcinolone Acetonide from White Petrolatum Ointment The effect of sebum or skin lipids was determined on the permeation of TA through excised hairless rat abdominal skin (full-thickness and stripped skin) from white petrolatum.
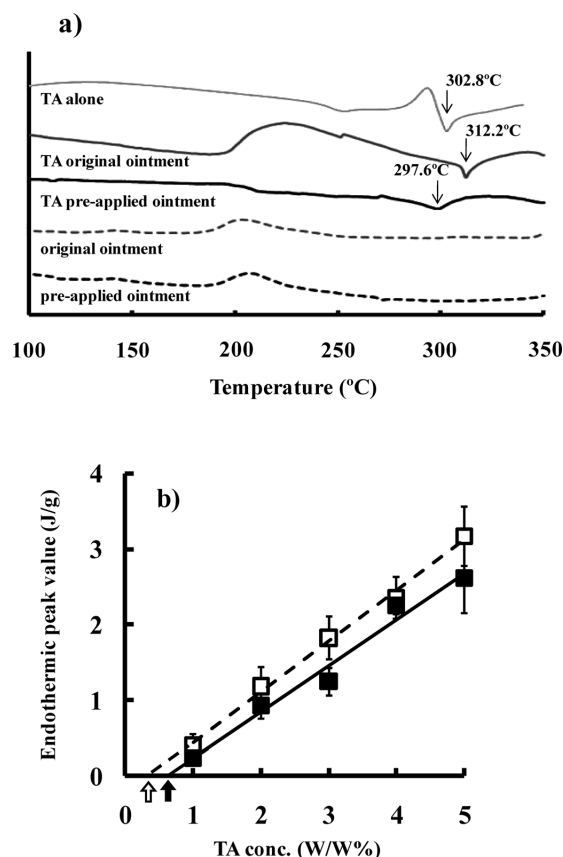
Fig. 1. DSC Chart (a) and Endothermic Peak Area-Triamcinolone Acetonide Concentration (b) for TA Original Ointment (□) or TA Pre-applied Ointment (■)

Abbreviation in Fig. 1a: TA=triamcinolone acetonide, original ointment=white petrolatum, pre-applied ointment=pre-applied white petrolatum on hairless rat skin. Each value shows the mean±S.E. (n=3).
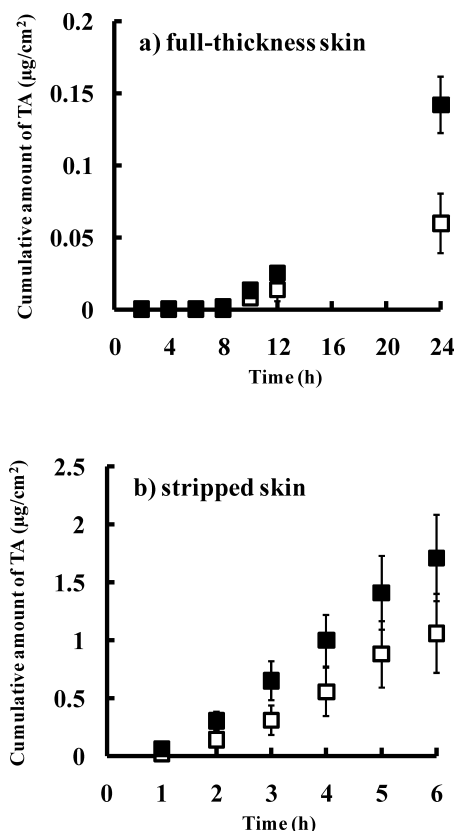

Fig. 2. Time Course of the Cumulative Amount of Triamcinolone Acetonide that Permeated through Full-Thickness Skin (a) and Stripped Skin (b) from Original Ointment (□) or Pre-applied Ointment (■) at a Concentration of 1.0% Triamcinolone Acetonide

Each value shows the mean±S.E. (n=4—6).

Figure 2 shows the time course of the cumulative amount of TA that permeated through the skin, and Table 1 summarizes the permeation parameters of TA. The cumulative amount of TA permeated through stripped skin over 6 h was 1.71 μg/cm^2 from 1.0% TA pre-applied ointment, and the value was about 1.7-times higher than that (1.06 μg/cm^2) from 1.0% TA original ointment (Fig. 2b). P_{ved} and K_{ved} for 1.0% TA pre-applied ointment were 0.74 and 0.45 times, respectively, those of P_{ved} and K_{ved} for 1.0% TA original ointment, and D_{ved} and J_{ved} for 1.0% TA pre-applied ointment were 1.62 and 1.33 times, respectively, those of 1.0% TA original ointment (Table 1).

Table 2 shows the TA concentration in the stripped skin 6 h after starting the skin permeation experiments. TA concentration in the stripped skin after application of 1.0% TA pre-applied ointment was similar to that of 1.0% TA original ointment (26.3 and 26.1 μg/g, respectively). The decrease in K_{ved} after application of pre-applied ointment may be due to the high affinity of TA for the ointment *via* incorporation of sebum or relative lipids into the ointment, since affinity of sebum-entrapping white petrolatum to the viable epidermis and dermis is lower than that to the stratum corneum. It is still unknown why the D_{ved} after application of 1.0% TA pre-applied ointment increased.

On the other hand, the cumulative amount of TA that permeated through full-thickness skin over 24 h from the 1.0%

Table 1. Parameters Calculated by Permeation Data through Full-Thickness or Stripped Hairless Rat Skin (n=4—6)

	1.0% TA original ointment	1.0% TA pre-applied ointment
C_v (mg/g)	3.3	6.0
$K_{sc} \times 10^{-2}$	3.66±0.30	9.78±2.15
$K_{ved} \times 10^{-2}$	1.91±0.17	0.86±0.19
D_{sc} (cm^2/s)×10^{-11}	1.78±0.44	1.05±0.09
D_{ved} (cm^2/s)×10^{-7}	0.72±0.13	1.17±0.04
P_{sc} (cm/s)×10^{-10}	4.27±1.09	6.62±1.02
P_{ved} (cm/s)×10^{-8}	2.35±0.51	1.73±0.04
J_{sc} (μg/cm^2/s)×10^{-6}	1.41±0.36	3.97±0.61
J_{ved} (μg/cm^2/s)×10^{-4}	0.78±0.17	1.04±0.23

TA pre-applied ointment (0.14 μg/cm^2) was about 2.3 times higher than that from the 1.0% TA original ointment (0.06 μg/cm^2) (Fig. 2a). Furthermore, K_{sc} (9.78×10^{-2}), P_{sc} (6.62× 10^{-10} cm/s) and skin concentration (13.9 μg/g) of TA for the 1.0% TA pre-applied ointment were 2.7, 1.6 and 1.4 times higher than those for the 1.0% TA original ointment (3.66× 10^{-2}, 4.27×10^{-10} cm/s and 10.2 μg/g, respectively) (Tables 1, 2). Rosado *et al.* reported that skin partition of diazepam (log P, 2.96) showed the highest value from a vehicle having a solubility parameter, δ, of 10 (cal/cm^3)$^{1/2}$ among several vehicles having δ values of 7 to 14 (cal/cm^3)$^{1/2}$.[13] The reason for the increase in K_{sc} of TA (log P, 2.53) from the 1.0% TA pre-applied ointment may be an increase in the solubility pa-

Table 2. Amount of Triamcinolone Acetonide Retained in Full-Thickness and Stripped Skin after Application of 1.0% Triamcinolone Acetonide Original Ointment or Pre-applied Ointment with or without Rubbing Original Ointment Alone or 1.0% Triamcinolone Acetonide Original Ointment on Full-Thickness Skin ($n=3$—6)

	Full-thickness skin (μg/g)	Stripped skin (μg/g)
1.0% TA original ointment	10.2±3.1	26.1±5.8
1.0% TA per-applied ointment	13.9±9.4	26.3±5.7
Rubbing original ointment alone on the skin (application: 1.0% TA original ointment)	19.2±8.0	—
Rubbing 1.0% TA original ointment on the skin (application: original ointment alone)	20.8±5.9	—
Rubbing 1.0% TA original ointment on the skin (application: 1.0% TA original ointment)	31.2±4.4	—

Fig. 4. Time Course of Changes in the Skin Impedance of Full-Thickness Skin with (○) or without (□) Rubbing on Full-Thickness Skin and Stripped Skin (△)

Each value shows the mean±S.E. ($n=3$—5).

The 24 h-permeation and skin concentration of TA after application of TA-free original ointment with a pretreatment of rubbing using 1.0% TA original ointment (0.38 μg/cm^2 and 20.8 μg/ml) were about 3.5-times greater compared with those after application of 1.0% TA original ointment with a pretreatment of rubbing using TA-free original ointment.

Figure 4 shows time course of skin impedance. No ointment was used in this case, since the ointment itself markedly increased skin impedance. Skin impedance decreased with a passage of time. When compared with and without 30 s-rubbing on skin, skin impedance for rubbing group 8 h after starting the impedance determination (1.36± 0.36 kΩ) was a third lower than that without rubbing (4.11± 0.36 kΩ). On the other hand, impedance of stratum corneum-stripped skin was 0.08±0.02 kΩ, which was one fiftieth that of full-thickness skin. This result again proved that the stratum corneum is a primary barrier against skin permeation. Basically, ointment rubbing on the skin surface increases the skin permeation of TA from the ointment. This technique can be used as a simple skin-penetration-enhancing method in hospitals and at home. Ointment rubbing may increase partition of drugs and vehicles into the stratum corneum and hair follicles, as well as reduce skin impedance.

CONCLUSION

The effects of sebum-entrapping in vehicle and ointment rubbing were evaluated on the skin concentration and permeation of TA. Use of 1.0% TA pre-applied ointment increased the TA solubility in the ointment (1.8 times) and TA permeation through the full-thickness skin (2.3 times). Skin partition of TA from pre-applied ointment was increased compared with that from original ointment, and this may be due to the dissolution properties for TA crystals caused by sebum or skin lipids entrapped in the ointment. Ointment rubbing further increased the skin partition and skin permeation of TA, and one of the reasons was a decrease in skin impedance. Since the strength, treatment period and the ointment amount of ointment rubbing on skin may strongly affect the skin permeation of TA, further consideration is necessary.

These results provide very useful information for the development and proper use of topical steroidal formulations.
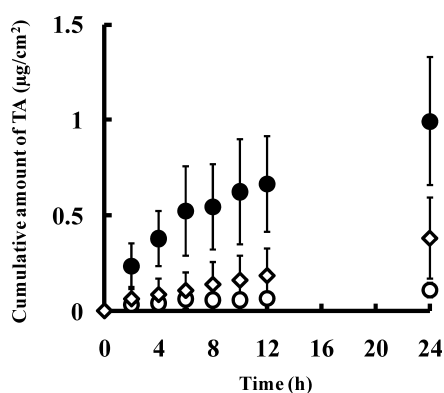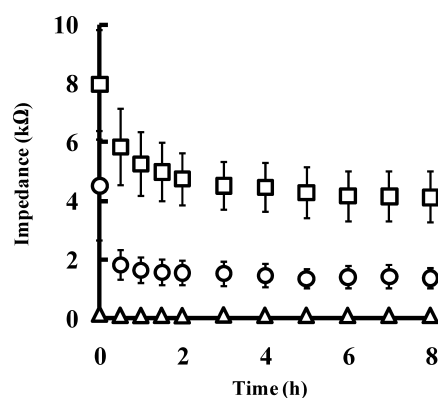

Fig. 3. Time Course of the Cumulative Amount of Triamcinolone Acetonide that Permeated through Full-Thickness Skin from the Original Ointment Alone (◇) or 1.0% Triamcinolone Acetonide Original Ointment (●) after Rubbing 1.0% Triamcinolone Acetonide Original Ointment on Full-Thickness Skin and from 1.0% Triamcinolone Acetonide Original Ointment after Rubbing Original Ointment Alone on Full-Thickness Skin (○)

Each value shows the mean±S.E. ($n=3$—4).

rameter of white petrolatum ($\delta=7.3$ (cal/cm^3)$^{1/2}$) compared to that of the stratum corneum ($\delta=9.8$ (cal/cm^3)$^{1/2}$) by entrapping cholesterol ($\delta=9.55$ (cal/cm^3)$^{1/2}$) and nonhydroxy fatty acid ceramide ($\delta=9.44$ (cal/cm^3)$^{1/2}$) into the ointment.

Rubbing Effect on the Skin Permeation of Triamcinolone Acetonide from White Petrolatum Ointment Next, the ointment-rubbing effect was determined on the skin permeation and skin concentration of TA from white petrolatum. Since ointment-rubbing on the skin surface may reduce the skin barrier function, skin impedance was also determined. Figure 3 shows the cumulative amount of TA that permeated through full-thickness skin from original ointment alone or 1.0% TA original ointment after rubbing 1.0% TA original ointment on full-thickness skin and from 1.0% TA original ointment after rubbing original ointment alone on the full-thickness skin. The cumulative amounts of TA permeated over 24 h and its skin concentration after application of 1.0% TA original ointment with a pretreatment of rubbing using 1.0% TA original ointment were 0.99 μg/cm^2 and 31.2 μg/ml, respectively, and these were 9-times and 1.6-times higher than that after application of the 1.0% TA original ointment with a pretreatment of rubbing using TA-free original ointment (0.11 μg/cm^2 and 19.2 μg/ml, respectively).

REFERENCES

1) Higuchi T., *J. Soc. Cosmetic Chemists*, **11**, 85—97 (1960).
2) Hatanaka T., Oguchi M., Sugibayashi K., Morimoto Y., *Chem. Pharm. Bull.*, **39**, 1802—1805 (1991).
3) Buhse L., Kolinski R., Westenberger B., Wokovich A., Spencer J., Chen C. W., Turujman S., Gautam-Basak M., Kang G. J., Kibbe A., Heintzelman B., Wolfgang E., *Int. J. Pharm.*, **295**, 101—112 (2005).
4) Wiedersberg S., Leopold C. S., Guy R. H., *Eur. J. Pharm. Biopharm.*, **68**, 453—466 (2008).
5) Cheng Y. H., Hosoya O., Sugibayashi K., Morimoto Y., *Biol. Pharm. Bull.*, **17**, 1640—1644 (1994).
6) Ishii H., Todo H., Terao A., Hasegawa T., Akimoto M., Oshima K., Sugibayashi K., *Drug Dev. Ind. Pharm.*, **35**, 1356—1363 (2009).
7) Rantuccio F., Scardigno A., Conte A., Sinisi D., Coviello C., *Contact Dermatitis*, **5**, 392—397 (1979).
8) Theeuwes F., Hussain A., Higuchi T., *J. Pharm. Sci.*, **63**, 427—429 (1974).
9) Sato K., Mitsui N., Hasegawa T., Sugibayashi K., Morimoto Y., *J. Controlled Release*, **73**, 269—277 (2001).
10) Okumura M., Sugibayashi K., Ogawa K., Morimoto Y., *Chem. Pharm. Bull.*, **37**, 1404—1406 (1989).
11) Hada N., Hasegawa T., Takahashi H., Ishibashi T., Sugibayashi K., *J. Controlled Release*, **108**, 341—350 (2005).
12) Andrews E. H., Khan T. A., Rieke J. K., Rudd J. F., *Clin. Mater.*, **1**, 205—213 (1986).
13) Rosado C., Cross S. E., Pugh W. J., Roberts M. S., Hadgraft J., *Pharm. Res.*, **20**, 1502—1507 (2003).