Evaluation of Skin Permeability of Drugs by Newly Prepared Polymer Membranes

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Four polymeric membranes were prepared consisting of various ratios of 2-hydroxyethyl-methacrylate/polydimethylsiloxane-methacrylate copolymer. These membranes had both lipophilic and hydrophilic domains in their copolymer networks like skin barrier. From permeation studies of the aqueous solutions or suspensions of various lipophilic and hydrophilic drugs using these membranes, a membrane having a similar drug permeability to hairless rat and human skins was selected. Permeation of indomethacin through the selected polymer membrane from simple ointment, gel and a marketed cream were then measured. About 10^2 times higher permeability was found in the polymer membrane than through excised hairless rat skin, but the rank order of the permeability through the polymer membrane among the formulations was the same through the skin membranes. These higher permeations of indomethacin were probably related to physical and chemical properties of pharmaceutical additives in the topical formulations. It is concluded that these synthetic polymer membranes may be utilized as an alternative tool to predict human or rat skin permeability of various (hydrophilic or lipophilic) drugs as well as to screen drug candidates for transdermal drug delivery.

Key words synthetic polymer membrane; skin; membrane permeation; skin permeation; topical formulation; transdermal drug delivery system

Transdermal drug delivery systems (TDDSs) are known to have various advantages over conventional oral dosage forms and injections: simple dosage, prolonged and controlled action of drugs, sequestration of first pass effect of drugs, and greater improved patient compliance.[2)] One of the key TDDS shortcomings, however, is poor skin permeation. Many penetration enhancing methods have been sought to improve the skin permeation. Screening of drug candidates which have good skin permeability and high pharmacological potential with low dose application is also important for the development of potential TDDS.[3)] In such efforts, *in vitro* permeation studies using cadaver human skin would be the best method,[4)] although intra- and inter-individual variations of the skin samples must be recognized. Use of excised human skin, however, is restricted in Japan. Use of animal skin has several problems of cost, cruelty to animals and wide biological variability.[5)] Species difference is also critical. Thus, establishment of an *in vitro* method using human skin-alternative membrane is desired.

Many researchers have dreamed of estimating drug permeation through human or animal skin by an alternative (artificial) membrane. The mechanism and mathematical modeling of drug permeation through skin have been reported: (1) Barry[6)] and Flynn[7)] reported that there were two microdomains, the intercellular and transcellular paths, in the skin barrier; (2) we also proposed a parallel pathway permeation model which consists of lipid and aqueous pathways in the skin barrier,[8)] and (3) Yamashita *et al.* kinetically analyzed the permeation of drug through skin using a similar model.[9)] In these models, aqueous domain (the hydrophilic path), through which primarily hydrophilic compounds penetrate, is also included in the skin barrier, so that higher permeabilities or fluxes of drugs (especially hydrophilic drugs) are expected than those estimated by a homogeneous lipophilic membrane model. In contrast, Potts and Guy presented a drug diffusion model of skin consisting of lipophilic domain alone.[10)] They explained that higher permeability of hydrophilic compounds was due to smaller molecular volume of the drugs, and that no requirement have been considered for the existence of a hydrophilic path in the diffusion model of skin. Consensus still was not obtained, but an *in vitro* model for skin penetration should successfully mimic the barrier properties of the human stratum corneum.

Garret and Chemburke,[11)] Barry and Brace[12)] and Hadgraft and Ridout[13)] much earlier prepared alternative membranes to predict and assess drug permeation through skin. However, such prediction using these membranes was limited to either lipophilic or hydrophilic drugs. An artificial membrane was desired that could predict the permeabilities of both characteristics of drugs.

The objective in the present study was to improve the predictability of the *in vitro* skin permeations of lipophilic and hydrophilic drugs using synthetic artificial membranes. We earlier prepared a composite membrane composed of polydimethyl siloxane (pDMSA) membrane as a lipoidal region and poly 2-hydroxymethacrylate (pHEMA) membrane as a hydrophilic region, and indicated that permeability of most drugs through this membrane was similar to those through hairless rat skin.[14)] This membrane consists of two sheets of pDMSA and pHEMA membrane, and each sheet with a suitable size hole to adjust the drug permeability. In the present study, we prepared an artificial polymer membrane having both lipid and aqueous domains using synthetic graft co-

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polymers consisting of polydimethyl siloxane polymethacrylate (pDMSpMA) and pHEMA units. Drug permeations through the obtained membranes were measured to compare with those through hairless rat or human skin. The membrane was also used for a permeation study using topical formulations.

Materials and Methods

Materials 2-Hydroxymethacrylate (HEMA), polydimethyl siloxane methacrylate (pDMSMA) and azobisisobutylironitril (AIBN) for the preparation of polymer membranes were obtained from Nacalai Tesque Inc. (Kyoto, Japan), Tisso Chemical Co., Ltd. (Tokyo, Japan) and Wako Pure Chemical Ind., Ltd. (Osaka, Japan), respectively. Eleven drugs were used as penetrants in the permeation study as follows (Table 1): Aminopyrine (AMP), lidocaine (LC), *dl*-isoprenarine hydrochloride (IPH) were obtained from Wako Pure Chemical Ind., Ltd. Antipyrine (ANP), 5-fluorouracil (5-FU) and cyclobarbital (CB) were purchased from Tokyo Kasei Kogyo Co., Ltd. (Tokyo). L-Dopa (L-DP) was obtained from Sigma Chemical Co., Ltd. (St. Louis, MO, U.S.A.), and dopamine hydrochloride (DPH; JP grade) was obtained from Yodogawa Pharmaceutical Co., Ltd. (Osaka). Indomethacin (IDM), ibuprofen (IP) and isosorbide dinitrate (ISDN) were gifts from Toko Pharmaceutical Ind. Co., Ltd. (Tokyo), Nisshin Flour Milling Co., Ltd. (Tokyo) and Kyukyu Pharmaceutical Co., Ltd. (Toyama, Japan), respectively. Inteban® and Cortes® creams were obtained from Sumitomo Pharmaceuticals Co., Ltd. (Osaka) and Taisho Pharmaceuticals Co., Ltd. (Tokyo), respectively. Other chemical and solvents were of reagent grade and obtained commercially. All the drugs were used without further purification.

Synthesis of Graft Copolymer Appropriate amounts of HEMA and pDMSMA, 10 ml of ethanol and 0.1 g of AIBN as an initiator were put into a polymerization ampule made of glass. The ampule was closed under vacuum after degassing in the usual way. The mixture was continuously stirred at a reaction temperature of 60 °C for 4 h. After the reaction, the mixture was purified using excess water to remove unreacted HEMA. Four graft copolymers with different compositions of pHEMA and pDMSpMA, A, B, C and D, were obtained as shown in Table 2.

Preparation of Artificial Membrane One gram of copolymer A or B was added to 7.5 ml of ethanol, and allowed to stand at room temperature until completely dissolved. The solution was then degassed. A Teflon frame was put on a sheet of silicone coated polyethylene-terephthalate (PET) film which was laid on a glass plate. The polymer solution was then dropped into the frame and air-dried at room temperature for 24 h. Since C and D copolymers were too tender at room temperature, the copolymer was sandwiched between two sheets of membrane filter (pore size: 0.45 μm, Advantec Co., Ltd., Tokyo) so that it assumed the membrane shape. One gram of C or D copolymer was added to 7.5 ml of tetrahydrofuran and completely dissolved. A sheet of membrane filter was layered on a hard glass plate. The polymer solution was applied to and penetrated into the membrane filter, and a new sheet of membrane filter was placed atop the polymer-penetrated membrane filter. Drying procedure of the membrane was as described above.

Structural Analysis of Graft Copolymer The average number (Mn) and molecular weight (Mw) of the copolymers were determined in a gel permeation chromatography system (Tosoh, model HLC-82A) using four polystyrene-filled columns ($G5000H_6$, $G4000H_6$, $G3000H_6$, $G2000H_6$). A primary calibration curve was established using five monodispersed polystyrene samples. Attenuated total reflectance (ATR) infrared spectra of the polymers were recorded with a Fourier transformed infrared (FTIR) spectrometer. Proton nuclear magnetic resonance spectroscopy (^{1}H-NMR) (Varian EM-390, 90 MHz spectrometer, Palo Alto, CA, U.S.A.) was used to determine the composition of the synthetic polymers. Composition of the copolymers was calculated from the carbon content as a ratio of pDMSMA(m)/HEMA(n) by an elemental analyzer (Perkin Elmer 240, Norwalk, CT, U.S.A.).

Determination of Swelling Ratio of Artificial Membrane in Water Approximately 0.5 g of the artificial membrane was weighed and added to 100 ml of distilled water. The membrane was withdrawn from the water at predetermined intervals and weighed by an electric chemical balance. After equilibrium of the membrane weight, swelling ratio was measured according to the following equation:

$$\text{swelling ratio (\%)} = \frac{\text{equilibrated swelling weight}}{\text{dry weight}} \times 100$$

Measurement of Melting Point by Differential Scanning Calorimeter (DSC) A piece of polymer membrane weighing approximately 0.5 g was placed in an aluminum pan. DSC equipment (Model TAS-200 system; Rigaku Electric Co., Ltd., Tokyo) was used at a scanning rate of 10 °C/min from −160 to 120 °C.

Drug Permeation through Graft Copolymer Membrane The membrane (without pretreatment) was mounted between two diffusion half-cells (2.5 ml of volume and 0.95 cm^2 of effective diffusion area) with a water jacket connected to a water bath at 37 °C.[16] The donor compartment was filled with 2.5 ml of drug solution or suspension. The receiver compartment was filled with 2.5 ml of distilled water or 40% polyethyleneglycol 400 (PEG) solution for hydrophilic drugs or lipophilic drugs, respectively. Phosphate buffer solution (pH 7.4) was also used for IDM. The receiver and donor solutions were then stirred at 1400 rpm

Table 1. Physicochemical Parameters of Drugs Used in This Experiment

Drugs	IP	IDM	LC	ISDN	CB	AMP
Mw	206.27	357.81	234.33	236.14	236.26	231.29
C_w (mg/ml)	0.04	0.01	3.03	1.34	3.07	55.90
log K_{ow}	3.94	3.29	2.37	1.34	0.87	0.50

Drugs	5-FU	ANP	IPH	DPH	L-DP
Mw	130.08	188.23	247.70	189.64	197.00
C_w (mg/ml)	17.1	816.00	345.00	520.00	5.00
log K_{ow}	−0.86	−1.55	−2.69	−3.40	−4.70

Table 2. Preparation of pHEMA/pDMSpMA Graft Copolymers

Code	Feed of each unit		Mn	Mw	Molar ratio of unit	
	HEMA (n%)	pDMSMA (m%)			HEMA (n%)	pDMSMA (m%)
A	8	2	—	—	96.3	3.7
B	7	3	114000	182000	76.0	24.0
C	2	8	224000	783000	66.9	33.1
D	1	9	225000	610000	54.9	45.1

with a star-head bar driven by a synchronous motor. At predetermined times, 100 μl of sample was withdrawn from the receiver compartment and the same volume of fresh buffer solution was added to keep the volume constant.

Drug Permeation through Excised Hairless Rat Skin The skin was freshly excised from the abdomen of male hairless rats (WBN/ILA-Ht, Life Science Research Center, Josai University, Saitama, Japan), aged 8 weeks, after the fine soft hair have been carefully shaved away. Excised hairless rat skin or artificial polymer membrane was mounted on a modified Franz type diffusion cell (12 ml of volume in lower compartment and 7.07 cm^2 of effective diffusion area)[17] with a water jacket connected to a water bath at 32 °C. Approximately 0.5 g of gel, simple ointment or Inteban® cream each containing IDM or Cortes® cream containing hydrocortisone acetate was applied to the upper compartment facing the stratum corneum of skin. The receiver solution was then stirred at 1200 rpm. The lower compartment (receiver side) was filled with pH 7.4 phosphate buffer solution or 40% PEG 400 solution for IDM or hydrocortisone acetate, respectively. At predetermined times, 100 μl of sample was withdrawn from the receiver compartment and the same volume of fresh buffer or PEG solution was added.

Analytical Methods Drugs were assayed by a high-performance liquid chromatograph (HPLC). The HPLC system consisted of a pump (LC-6A, Shimadzu, Kyoto), a 4.6 mm × 150 mm stainless-steel column packed with Nucleosil® 5C18 (Macherey Nagel, Germany), in a column oven (CTO-6A, Shimadzu) at 40 °C, an ultraviolet detector (SPD-6A, Shimadzu) and an integrator (C-R6A, Shimadzu). Analytical conditions for LC, ISDN, AMP, CB, 5-FU, ANP and IDM were previously reported.[8] Hydrocortisone acetate was determined by the same method for IDM except an internal standard (butyl *p*-hydroxybenzoate) was used. Conditions for the other drugs were: mobile phase, acetonitrile–0.1% phosphoric acid (70 : 30); flow rate, 1.0 ml/min; detector, UV 220 nm; internal standard, *p*-hydroxybenzoic acid for IP: mobile phase, acetonitrile–0.1% phosphoric acid (60 : 40) + 5 mM sodium 1-hexanesulfonate; flow rate, 1.0 ml/min; detector, UV 280 nm; internal standard, butyl *p*-hydroxybenzoate; absolute calibration for DPH, IPH and L-DP.

Results and Discussion

Structure of Synthesized Copolymer Table 2 shows composition and molecular weight of the copolymers in the membranes measured by gel permeation chromatogram (GPC) and elemental analysis. The Mn and Mw of the synthesized copolymers B, C and D were in a range from 114 to 225 kDa and 182 to 610 kDa, respectively. The Mn of copolymer A could not be measured by GPC. The molecular vibrations of the copolymers associated with their major bands were measured by FTIR and ^{1}H-NMR, and the results are listed in Table 3. The –OH stretching vibration from pHEMA was observed at a wave number of 3340 cm^{-1}. Figure 1 shows formation and chemical structure of the copolymer identified from these results, which suggests that these membranes are made of graft copolymers constituted of different ratios of pHEMA/pDMSMA.

Physicochemical Property of Artificial Membrane Figure 2 shows swelling ratio of artificial membranes prepared using different graft copolymers in water. Water uptake into the membranes almost reached a plateau between 4 to 7 d after treatment. The swelling ratio increased with increasing pHEMA content in the membranes. This swelling phenomena is similar to that in the stratum corneum. DSC measurement of the artificial membranes was done to confirm the existence of lipophilic and hydrophilic microdomains. Phase transition of all four membranes was observed at −125 and 80 °C originating in pDMSpMA and pHEMA, respectively (data not shown), although the copolymers in the membranes have much different properties than the latter two polymers, suggesting that these membranes have a microdomain structure. It is suggested from these results that all the artificial membranes have an aqueous channel or lipoidal domain in a phase separation structure of the membranes.

Table 3. Results of FTIR and ^{1}H-NMR Analyses

FTIR		^{1}H-NMR	
Neat (cm^{-1})		ppm	
3340	–OH	0.05	Si–C″H_3'
2960		0.56	Si–C″H_2'
1730	C=O	1.00	–CH_2C(C″H_3')–
1250	Si–C	1.32	–CC″$H_2'CH_2$Si–
1100—1000	Si–O	1.62	–C″H_2'C(CH_3)–
800		3.98	–OC″H_2'C″H_2'OH, –C″$H_2'CH_2CH_2$Si–

Drug Permeation through Artificial Membranes Permeation study was performed on eleven drugs from their aqueous solutions or suspensions using these artificial membranes, and the obtained permeability was compared with that through human skin.[8] Drugs tested in this experiment have a similar Mw, but different octanol/water partition coefficients (K_{ow}) and solubility in water (C_w) as shown in Table 1.

Figure 3 shows relationships between log permeability coefficients (log *P*) of eleven drugs through the membranes A—D and log K_{ow}. log *P*s of the drugs through the membranes A and B were almost the same regardless of different log K_{ow} indicating higher drug permeations

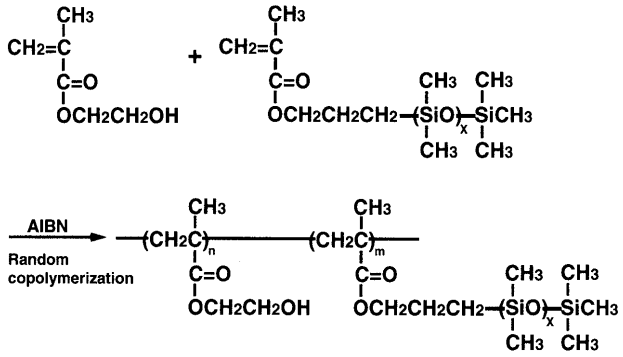

Fig. 1. Formation and Structure of pHEMA/pDMSpMA Graft Copolymer

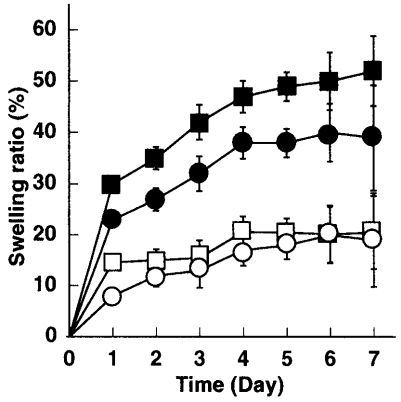

Fig. 2. Swelling Ratio of Artificial Membrane

■ membrane A; ● membrane B; □ membrane C; ○ membrane D. Each point represents the mean ± S.E. of 3 experiments.

through an aqueous region (*i.e.*, water rich pHEMA domain) than through a lipoidal region in the membranes A or B. log P of drugs through membranes C and D, on the other hand, was increased proportionally with increase in log K_{ow}. Permeability coefficients of hydrophilic drugs (log $K_{ow} < 0$) through membrane D, however, was lower than those through membrane C, and L-DP (log $K_{ow} = -4.70$) permeation through membrane D was not detected (below the detection limit). These results suggest that membrane C would have both aqueous and lipoidal regions with a suitable ratio like skin, and that aqueous region (pHEMA domain) might exist as a discontinuous phase. The membrane C, therefore, was selected and used for the following experiments.

Figure 4 shows the relationship between log Ps of drugs through human skin[17] and artificial membrane C. A good relation was observed ($r = 0.888$) and the P values for the membrane were close to those for human skin, suggesting that the drug permeations through this artificial membrane were very similar to those through human skin.

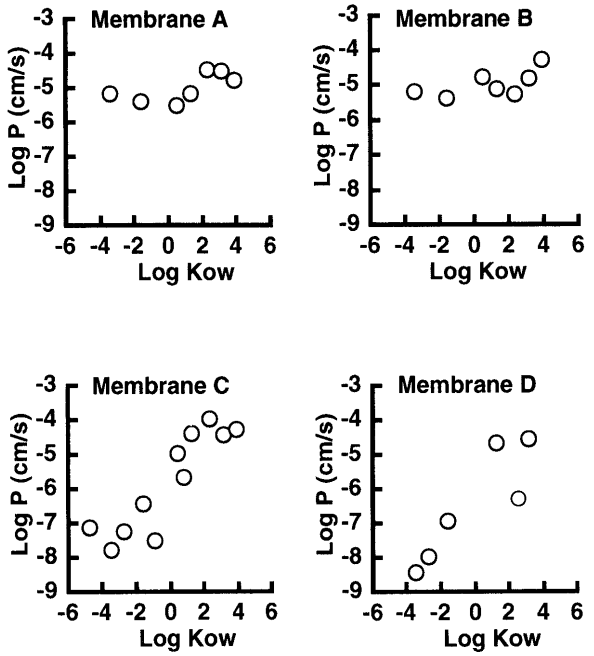

Fig. 3. Double Logarithmic Relationship between Permeability Coefficients (P) of Drugs through Artificial Membranes A, B, C, and D and Octanol/Water Partition Coefficients (K_{ow})

We have already characterized drug permeation through hairless rat and human skin by assuming a parallel pathway model, *i.e.* existence of both lipophilic and hydrophilic pathways, in the skin barrier. Drug permeation through human skin was thus expressed as follows[17]:

$$P(\text{cm/s}) = 1.17 \times 10^{-7} k_{ow}^{0.751} + 2.73 \times 10^{-8} \quad (1)$$

The P values obtained using membrane C in Fig. 3 were analyzed by Eq. 1. The minimal effect of the unstirred water layers on the P values through the membrane was confirmed by measuring their thickness.[18] The obtained simulation curve was very similar to that for human skin. The P values were expressed as a function of K_{ow} as follows:

$$P(\text{cm/s}) = 4.43 \times 10^{-8} K_{ow}^{0.779} + 4.17 \times 10^{-8} \quad (2)$$

It is concluded from these results that the polymer membrane C can be alternatively used instead of human skin to predict permeations of a drug.

Drug Permeation from Semi-solid Dosage Form through Artificial Membrane It may become feasible to predict bioavailability of a drug after application of a topical formulation using the alternative membrane C. IDM permeations through membrane C from two formulations (simple ointment and gel) and Inteban® cream were then measured and compared with those through hairless rat skin (Rat skin was used instead of human skin due to the low availability of the latter sample). Permeation of

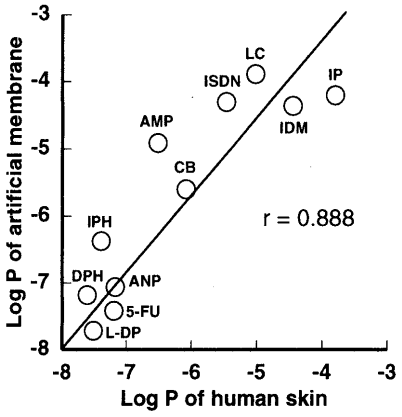

Fig. 4. Relationship between log P of Artificial Membrane and Human Skin

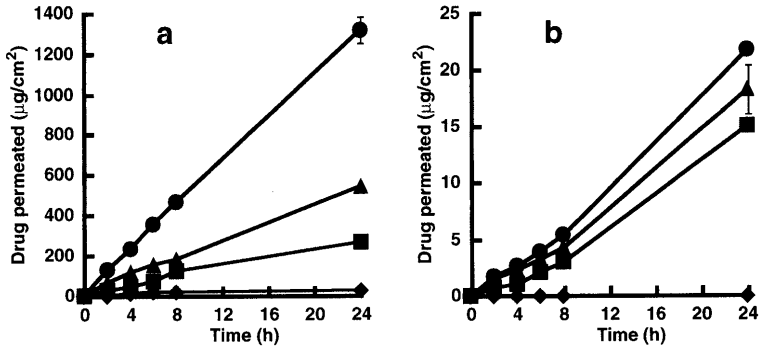

Fig. 5. Permeation Profiles of Indomethacin and Hydrocortisone Acetate from Topical Formulations through Artificial Membrane (a) and Hairless Rat Skin (b)

● Inteban cream (IDM); ▲ simple ointment (IDM); ■ gel ointment (IDM); ◆ Cortes cream (hydrocortisone acetate). Each point represents the mean ± S.E. of 3 experiments.

hydrocortisone acetate from Cortes® cream through the artificial membrane was also compared with that through hairless rat skin. Figures 5a and 5b show permeation profiles of the two drugs through membrane C and hairless rat skin, respectively. The rank order of the IDM flux (steady state permeation rate) obtained for the two membranes was the same: Inteban® cream > simple ointment > gel. The fluxes of IDM and hydrocortisone acetate through the artificial membranes from these formulations, however, were consequently about 10^2 higher than those through the rat skin, although the drug permeabilities from aqueous solutions or suspensions through membrane C were similar to those through skin (Fig. 4). Surfactants and other ingredients contained in such semi-solid dosage forms may have a greater enhancing effect on permeation of drug through the polymer membranes than through skin: These ingredients may reduce the barrier function of the polymer membranes. Since the same rank order of the fluxes was found, however, between the artificial membrane and hairless rat skin, the artificial membrane C could be applied to differentiate topical formulations and vehicles.

We developed an alternative membrane which can be used instead of human skin to evaluate the skin permeability of a drug. Drug permeation through polymer membrane C, especially, was very similar to that through human skin (Fig. 4). This polymer membrane may be utilized to measure drug permeation from different vehicles containing the same drug. However, membrane C was so tender that it needed membrane filters to support its shape. Simple membrane is better for easy handling. Further experiments on the physical and chemical interactions between pharmaceutical additives in topical dosage forms and polymer materials of the membranes may also be needed to prepare a more useful alternative membrane.

References

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2) Chien Y. W., "Transdermal Controlled Systemic Medications," ed. by Chien Y. W., Marcel Dekker, New York, 1987, pp. 1—22.
3) Guy R. H., Hadgraft J., "Transdermal Drug Delivery," ed. by Guy R. H., Hadgraft J., Marcel Dekker, New York, 1989, pp. 59—81.
4) Skelly J. P., Shah V. P., Maibach H. I., Guy R. H., Wester R. C., Flynn G., Yacobi A., *Pharm. Res.*, **4**, 265—267 (1987).
5) Bartek M. J., LaBudde J. A., Maibach H. I., *J. Invest. Derm.*, **58**, 114—123 (1972).
6) Barry B. W., *J. Controlled Release*, **15**, 237—248 (1991).
7) Flynn G. L., "Percutaneous Absorption," ed. by Bronaugh R. L., Maibach H. I., Marcel Dekker, New York, 1985, pp. 17—42.
8) Hatanaka T., Inuma M., Sugibayashi K., Morimoto Y., *Chem. Pharm. Bull.*, **38**, 3452—3459 (1990).
9) Yamashita F., Yoshioka T., Koyama Y., Okamoto H., Sezaki H., Hashida M., *Biol. Pharm. Bull.*, **16**, 690—697 (1993).
10) Potts R. O., Guy R. H., *Pharm. Res.*, **9**, 663—669 (1992).
11) Garrett E. R., Chemburke P. B., *J. Pharm. Sci.*, **57**, 944—959 (1968).
12) Barry B. W., Brace A. R., *J. Pharm. Pharmacol.*, **29**, 397—400 (1977); Barry B. W., El Eini D. I. D., *ibid.*, **28**, 219—227 (1976).
13) Hadgraft J., Ridout G., *Int. J. Pharmaceut.*, **39**, 149—156 (1987); **42**, 97—104 (1988).
14) Hatanaka T., Inuma M., Sugibayashi K., Morimoto Y., *Int. J. Pharmaceut.*, **79**, 21—28 (1992).
15) Okumura M., Sugibayashi K., Ogawa K., Morimoto Y., *Chem. Pharm. Bull.*, **37**, 1404—1406 (1989).
16) Yukawa J., Sugibayashi K., Morimoto Y., *Yakuzaigaku*, **49**, 254—262 (1989).
17) Morimoto Y., Hatanaka T., Sugibayashi K., Omiya H., *J. Pharm. Pharmacol.*, **44**, 634—639 (1992).
18) Yu C. D., Fox J. L., Ho N. F. H., Higuchi W. I., *J. Pharm. Sci.*, **68**, 1347—1357 (1979).