

## Regular Article

# Effect of Esters on the Permeation of Chemicals with Different Polarities through Synthetic Artificial Membranes Using a High-Throughput Diffusion Cell Array

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This study investigated the effects of 25 kinds of esters that are used in cosmetics on the permeation of four model compounds with different polarities (caffeine [CF], aminopyrine [AMP], benzoic acid [BA], and flurbiprofen [FP]). The amount of each model compound that permeated through two types of artificial membrane (silicone and Strat-M<sup>®</sup>) was measured and correlated with the physicochemical properties of the esters, including their solubility, viscosity, wettability, surface tension, and uptake. The amount of each model compound that permeated through the silicone membrane was not significantly correlated with the solubility of the esters but was significantly correlated with all other measured physical properties of the esters. Similar correlations were observed for the amounts of AMP, BA, and FP that passed through the Strat-M<sup>®</sup> membrane. However, the amount of CF that permeated through the Strat-M<sup>®</sup> membrane also correlated with the solubility of the esters. There was a highly significant correlation between the amount permeating through the silicone and Strat-M<sup>®</sup> membranes because the model compounds had high lipophilicity. These findings demonstrated that to control the permeation of various chemicals through artificial membranes, it is important to consider the uptake of the esters and that the solubility of the esters is also an important consideration when using a more complex membrane.

**Key words** alternative membrane; high-throughput; ester; silicone membrane; Strat-M; physical property

In order to develop transdermal drug delivery systems, permeability has been evaluated using Fick's law of diffusion.<sup>1,2)</sup> Fick's First Law indicates that the rate of mass transfer across a membrane is proportional to the concentration gradient expressed across the membrane. Synthetic artificial membranes are uniform and are designed to mimic human skin to avoid variability among tissues. They offer a simple and reproducible alternative to human and animal skins and provide key information to help understand the mechanisms of drug permeation by the evaluation of changes in a basic permeation parameter because, theoretically, skin permeation of chemicals can be expressed by physicochemical phenomena of diffusion and partition of compounds. Therefore, synthetic artificial membranes have been used in permeability evaluations.<sup>3-5)</sup> Maitani *et al.*<sup>6)</sup> evaluated the permeability of diclofenac salts and free diclofenac acid through a silicone membrane to understand the mechanism of percutaneous absorption of ionizable drugs, and the existence of the lipid pathway for diclofenac salts was the same as for diclofenac acid. Strat-M<sup>®</sup> is a commercially available multi-layered artificial membrane that is made from polyether sulfonic acid.<sup>7)</sup> We reported previously also that the permeability coefficients, partition parameters, and diffusion parameters of chemicals through a Strat-M<sup>®</sup> membrane were similar to those in human skin, and those in silicone membrane were under- or over-estimation to those in human skin, depending on the lipophilicity of the model compounds.<sup>8,9)</sup> In addition, several previous studies have assessed the effects of vehicles on the permeation of chemicals through artificial membranes.<sup>10-13)</sup> For example, Twist and Zatz<sup>10)</sup>

evaluated the effect of alcohol on the permeation rate (flux) of methyl-, ethyl-, propyl-, and butyl-parabens. Dias *et al.*<sup>12)</sup> reported that the flux values and permeability coefficients for caffeine, benzoic acid, and salicylic acid from water, polyols, and oils could not be explained by the differences in the solubility parameters of the solutes and solvents. Karadzovska and Riviere<sup>14)</sup> reported that the vehicle rank order of absorption values for diclofenac sodium, mannitol, salicylic acid, and testosterone through a Strat-M<sup>®</sup> membrane were the same as in porcine skin.

On the other hand, few studies have investigated the effects of oily vehicles on the permeation of chemicals through human skin and artificial membranes. Oily vehicles tend to be selected for topical formulations because they can impart desired sensory effects (*e.g.*, increased ability to be spread on the skin) and improve the solubility of lipophilic drugs in the formulation. The effect of fatty acid esters on the skin permeation of indomethacin and ketoprofen and the effect of diesters on the skin permeation of non-steroidal anti-inflammatory drugs has been reported.<sup>15-17)</sup> In addition, previous studies found that the permeability of haloperidol was affected by the wettability and surface tension of the formulation.<sup>18)</sup> Furthermore, the penetration of stearyl glycerethinate through Yucatan micropig skin at a finite dose was affected by the molecular weight and surface tension of the vehicles,<sup>19)</sup> and skin permeation of model compounds through porcine skin was affected by the viscosities of the vehicles.<sup>20)</sup> These physical properties could be a key parameter to enhance the skin permeation of chemicals. However, systematic evaluation with

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many kinds of esters, including triesters and tetraesters, has not been performed to determine the effect of these physical properties of esters on the skin permeation of chemicals.

*In silico* models have been utilized to estimate the skin permeation of topically applied chemicals, and most reports were able to successfully estimate chemical permeation through skin from an aqueous solution.<sup>21–23</sup> In addition, Grégoire *et al.*<sup>24</sup> reported the usefulness of an *in silico* prediction model for skin permeation of chemicals from oil-in-water (o/w) emulsion, although there were several limitations such as negligible penetration enhancement effects from the formulations, applicable to o/w emulsion only, chemical partitioning into the skin from the continuous (aqueous) phase only, amongst others. Thus, *in vitro* skin permeation experiments with real skin are more reliable in the development of topical formulations.

High-throughput screening allows numerous permeation tests to be carried out simultaneously. For example, Karande *et al.*<sup>25,26</sup> reported that a high-throughput screening cell containing an array of 100 holes each with a diameter of 3 mm could be used to conduct 100 permeation tests at the same time, and Karadzovska and Riviere<sup>14</sup> described a high-throughput apparatus with an improved Strat-M<sup>®</sup> membrane and 96-well filter plate. This would be the most effective method for evaluating the effect of vehicle on the skin permeability of chemicals for many different formulations.

In the present study, four model compounds with different polarities (caffeine [CF], aminopyrine [AMP], benzoic acid [BA], and flurbiprofen [FP]) were dissolved in 25 types of esters, and their permeation through silicone and Strat-M<sup>®</sup> membranes was investigated using a high-throughput diffusion cell array system, which had a 1-cm effective permeation diameter and allowed 48 permeation tests to be conducted simultaneously. Then, the major properties of esters to increase skin permeation of compounds were investigated by evaluating the relationships between the physical properties of the esters and the amounts of the model compounds that permeated through the silicone and Strat-M<sup>®</sup> membranes.

## Experimental

**Materials** AMP, BA, and CF were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan), and FP was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). All esters used as oily vehicles were of cosmetic ingredients grade. The molecular weights (MWs), octanol/water coefficients ( $\log K_{o/w}$ ), and Hansen's 3D solubility parameters (HSPs) of the model compounds and esters are shown in Tables 1 and 2. The MWs were calculated using ChemBioDraw Ultra 14.0 (PerkinElmer, Inc. Japan Co., Ltd., Tokyo, Japan),  $\log K_{o/w}$  values were estimated using EPI Suite<sup>™</sup> (U.S. Environmental Protection Agency, Washington, DC, U.S.A.), HSPs

were calculated using the Yamamoto-Molecular Break (Y-MB) method in HSPiP version 4.0.05, and SP distance were calculated using the following equation:

SP distance

$$= \sqrt{4(\delta_{d\text{solvent}} - \delta_{d\text{solute}})^2 + (\delta_{p\text{solvent}} - \delta_{p\text{solute}})^2 + (\delta_{h\text{solvent}} - \delta_{h\text{solute}})^2}$$

where  $\delta_d$  is the dispersion parameter,  $\delta_p$  is the polar parameter, and  $\delta_h$  is the hydrogen bonding parameter.

Silicone membranes were a kind gift from Lintec Co., Ltd. (Tokyo, Japan), and Strat-M<sup>®</sup> was purchased from Merck Millipore (Billerica, MA, U.S.A.). All other reagents were of HPLC grade and were used without further purification.

**Solubility Studies** An excess of each of the four model compounds (CF, AMP, BA, and FP) was added to 25 different esters and dissolved at 80°C in glass vials. Then, each solution was stirred using a stirrer bar in a water bath at 32°C for 48 h. The obtained suspensions were sampled and filtered through a syringe and filtration unit (25-mm polytetrafluoroethylene [PTFE] filter media device, 0.45- $\mu$ m pore size; Millipore). The resultant saturated solutions were adequately diluted with acetonitrile for determination by ultra-performance liquid chromatography (UPLC).

**High-Throughput Permeation Studies with Artificial Membranes** High-throughput permeation studies were performed using a diffusion cell array system (Ikeda Scientific Co., Ltd., Tokyo, Japan) (Fig. 1).

A silicone or Strat-M<sup>®</sup> membrane (8.5×12.5 cm) was set in the diffusion cell array system with an effective permeation area of 0.785 cm<sup>2</sup> to determine the cumulative amounts of model compounds from each oily suspension that permeated through the artificial membranes.

Phosphate-buffered saline (PBS; pH 7.4) was used as a receiver solution (1.4 mL) for CF, AMP, and BA, whereas 40% polyethylene glycol (PEG) 400/PBS was used for FP. Each solution was stirred using a stir ball and maintained at 32°C using a thermo-shaker. The model compounds were applied into the donor compartment at a concentration of 10 mg/g. In the case of CF and AMP, a suspended or saturated solution was used as the donor because the solubility of CF in the all esters and AMP in the some esters was less than 10 mg/g. In total, 0.2 mL of each donor solution was used, and the receiver solutions were then collected after 1 h for the silicone membrane and after 3 h for the Strat-M<sup>®</sup> membrane in order to satisfy the steady-state and sink conditions. The concentration of each model compound in the receiver solution was assayed using UPLC to determine the cumulative amount of chemicals that permeated through the silicone and Strat-M<sup>®</sup> membranes.

**Physical Properties of the Esters** The viscosity of the

Table 1. Physicochemical Properties of the Model Compounds Used in the Present Study

	Abbreviation	MW	$\log K_{o/w}$	HSP (MPa <sup>1/2</sup> )		
				$\delta_d$	$\delta_p$	$\delta_h$
Caffeine	CF	194.19	−0.12	20.0	12.7	8.3
Aminopyrine	AMP	231.30	1.10	18.5	3.8	6.9
Benzoic acid	BA	122.12	1.41	19.2	5.7	9.5
Flurbiprofen	FP	244.27	3.86	18.8	4.7	7.7

MW, molecular weight;  $\log K_{o/w}$ , octanol/water coefficients; HSP, Hansen's 3D solubility parameter.

Table 2. Physicochemical Properties of the Esters Used in the Present Study

	Abbreviation	MW	$\log K_{ow}$	HSP (MPa <sup>1/2</sup> )		
				$\delta_d$	$\delta_p$	$\delta_h$
Diisopropyl adipate	DIPA	230.30	3.20	16.0	3.8	4.7
Diisobutyl adipate	DIBA	258.36	4.19	15.8	3.3	4.5
Diethyl sebacate	DES	258.36	4.33	16.2	4.1	5.0
Propyleneglycol dicaprylate	PGDC	328.49	6.72	16.2	3.3	4.1
2-Ethylhexyl isononanoate	EHIN	270.46	6.99	15.7	1.2	2.3
Dioctylol carbonate	DOC	286.46	7.11	15.8	3.5	3.0
Isopropyl myristate	IPM	270.46	7.17	16.0	2.1	2.7
Isononyl isononanoate	ININ	284.48	7.37	15.3	0.7	1.7
Neopentyl glycol diethylhexanoate	NPGDEH	356.55	7.51	16.1	2.0	2.8
Propyleneglycol isostearate	PGIS	342.56	7.60	16.2	3.3	4.9
Isopropyl palmitate	IPP	298.51	8.16	15.9	2.2	2.5
Isopropyl linoleate	IPL	322.53	8.71	16.4	1.9	2.9
Glyceryl tri-2-ethylhexanoate	GTEH	470.69	8.98	16.4	2.2	3.1
Isopropyl isostearate	IPIS	326.57	9.07	16.0	1.7	2.2
Isotridecyl isononanoate	ITDIN	340.59	9.52	15.6	1.0	1.8
Neopentyl glycol dicaprate	NPGDC	412.66	9.62	17.7	2.2	3.0
Di-2-ethylhexyl sebacate	DEHS	426.68	10.08	16.2	2.1	2.8
Cetyl 2-ethylhexanoate	CEH	368.65	10.61	16.1	1.5	2.1
2-Ethylhexyl palmitate	EHP	368.65	10.61	16.0	1.7	2.2
2-Ethylhexyl hydroxystearate	EHHS	412.70	12.01	16.0	2.8	4.2
2-Octyldodecyl myristate	ODM	508.92	15.52	16.1	1.1	1.9
2-Octyldodecyl ricinoleate	ODR	579.01	15.73	16.0	2.2	3.1
Isostearyl isostearate	ISIS	536.97	16.06	15.4	0.0	0.6
Glyceryl triisostearate	GTIS	891.50	23.72	16.5	1.5	1.7
Pentaerythritol tetraisostearate	PETIS	1202.0	31.98	17.4	1.3	0.9

MW, molecular weight;  $\log K_{ow}$ , octanol/water coefficients; HSP, Hansen's 3D solubility parameter.

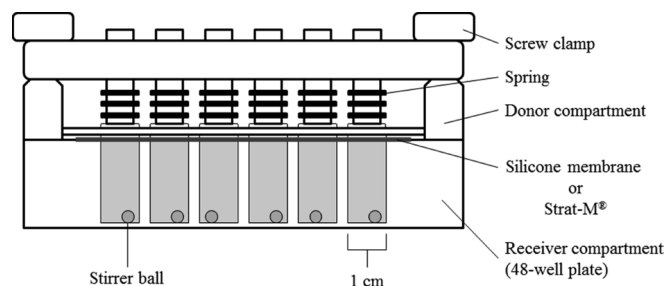


Fig. 1. Side View of the High-Throughput Diffusion Cell Array System

esters was measured using the vibration method at 32°C with three replicates, in a Viscometer VM-10A (SEKONIC, Tokyo, Japan). The surface tension and wettability of the esters were determined using a Dropmaster DM-701 (Kyowa Interface Science, Saitama, Japan). The surface tension of the esters was measured using the pendant drop method at 25°C with six replicates, and the wettability of the silicone membrane, Strat-M® membrane, and a standard acrylic plate was determined by measuring the sessile drop contact angle.

**Solvent Uptake** The solvent uptake into the single-layered silicone membranes was determined using a previously described gravimetric method.<sup>27,28)</sup> The solvent uptake into the Strat-M® was slightly modified, since it has a multi-layer structure with lipid in the two top layers.

**Silicone:** The silicone membrane (*ca.* 75  $\mu$ m thick) was wiped to remove dust. The treated membrane was then cut into 1.5-cm<sup>2</sup>, weighed accurately, and placed into glass sample vials. A 3 mL sample of each ester was added to the mem-

brane and the vials were maintained at 32°C in a thermostatic chamber. After 3 h, the membrane was removed from the solvent, blotted dry on Kimtowel® (Nippon Paper Crexia Co., Ltd., Tokyo, Japan), and carefully weighed. The relative mass uptake of each ester per gram of membrane ( $m_{upt}$ ) was calculated from the difference between the weight of the membrane before ( $m_b$ ) and after ( $m_a$ ) soaking, as described by Eq. 1:

$$m_{upt} = \frac{m_a - m_b}{m_b} \quad (1)$$

**Strat-M®:** The Strat-M® (*ca.* 320  $\mu$ m thick) membrane was weighed accurately and set in a Franz-type diffusion cell (effective area, 1.77 cm<sup>2</sup>). A 1 mL sample of each ester and 6 mL PBS were added to the donor and receiver sides, respectively. The receiver chamber was maintained at 32°C and the relative humidity of the room was kept at 35±5%. After 15 min, the applied ester was removed from the donor side, then the Strat-M® membrane was removed from the diffusion cell. The surplus solution on the surface was blotted using Kimtowel®, and each membrane was weighed carefully. The relative mass uptake was then calculated using Eq. 1.

**UPLC Analysis** The concentration of each model compound was determined using UPLC. The UPLC system consisted of a pump (LC-30ADVP; Shimadzu, Kyoto, Japan), an ultraviolet-visible detector (SPD-20A; Shimadzu), analysis software (LabSolutions, Shimadzu), a system controller (CBM-20A; Shimadzu), an autosampler (SIL-30AC; Shimadzu), and a column oven (CTO-20A; Shimadzu). A Sunniest C18-HT column (ChromaNik Technologies, Osaka,

Japan) was maintained at 40°C. Analyses of the model compounds were carried out using gradient elution systems. A solvent series that initially started with 20% acetonitrile and 80% 10mM sodium acetate (adjusted pH 4.8 using acetic acid) was changed linearly (from 1 to 3 min) to a 8:2 ratio of acetonitrile to 10mM sodium acetate and held for an additional 0.5 min. The flow rate was adjusted to 0.45 mL/min and peaks were detected at 254 nm. The system was then gradually returned to the starting condition for the next sample.

**Statistical Analysis** Statistical analysis was performed using SPSS Statistics software (version 23; IBM, Chicago, IL, U.S.A.). Spearman's rank correlation coefficients were calculated to characterize the relationship between the amounts of the model compounds that permeated through the membranes and the solubility of the model compounds in the esters and the physicochemical properties of the esters. A *p* value less than 0.05 was taken as the minimal degree of statistical significance.

## Results

**Solubility of Model Compounds in Esters** Table 3 shows the solubility of model compounds in the esters. All of the model compounds had high solubility in PGIS and di-esters such as DIPA, DIBA, and DES, which had lower  $\log K_{ow}$  values (7.60, 3.20, 4.19, and 4.33, respectively). CF and BA were the most soluble in DES, whereas FP was the most soluble in DIPA, and AMP had the highest solubility in PGIS. On the other hand, AMP, BA, and FP had lower solubility in long-chain fatty acids and long-chain alcohol monoesters, tries-

ters, and tetraesters, such as ODM, ISIS, GTIS, and PETIS, which had higher  $\log K_{ow}$  values (15.52, 16.06, 23.72, 31.98, respectively). In addition, the solubility of CF was lower in long-chain fatty acids and long-chain alcohol monoesters, such as ODM, ISIS, CEH, and EHP, which had  $\log K_{ow}$  values of 15.52, 16.06, 10.61, and 10.61, respectively.

Figure 2 shows the relationship between the solubility of the model compounds and physicochemical properties of the esters. No significant correlation was found between the solubility of each model compound and the dispersion solubility parameter (CF;  $r=0.285$ , AMP;  $r=0.132$ , BA;  $r=-0.013$ , FP;  $r=-0.068$ , Fig. 2B). In contrast, significant correlations were observed between the solubility of each model compound and the  $\log K_{ow}$  values (CF;  $r=-0.546$ , AMP;  $r=-0.721$ , BA;  $r=-0.674$ , FP;  $r=-0.632$ , Fig. 2A), the polar solubility parameters (CF;  $r=0.745$ , AMP;  $r=0.744$ , BA;  $r=0.804$ , FP;  $r=0.809$ , Fig. 2C), the hydrogen bond solubility parameters (CF;  $r=0.795$ , AMP;  $r=0.844$ , BA;  $r=0.843$ , FP;  $r=0.917$ , Fig. 2D), and the SP distances (CF;  $r=-0.788$ , AMP;  $r=-0.819$ , BA;  $r=-0.832$ , FP;  $r=-0.881$ , Fig. 2E) of the esters.

**Silicone Membrane Permeation by the Model Compounds in Esters** The amounts of model compounds that permeated through the silicone membrane are summarized in Table 3. Permeation experiments showed that the applied compounds exhibited greater permeation through the silicone membrane when they were dissolved in low MW fatty acids ester, such as ININ, EHIN, and IPM. In contrast, AMP, BA, and FP exhibited lower permeation when they were dissolved in esters containing a hydroxyl group, such as PGIS, ODR,

Table 3. Solubility of the Model Compounds and Permeated Amount of Model Compounds through Silicone and Strat-M® Membranes in Esters

	Solubility (mg/g)				Amount of permeated model compound ( $\mu\text{g}/\text{cm}^2$ )							
					Silicone membrane				Strat-M®			
	CF	AMP	BA	FP	CF	AMP	BA	FP	CF	AMP	BA	FP
DIPA	5.56	58.9	117	135	218.5	523.6	390.3	77.12	571.2	684.2	554.4	155.4
DIBA	5.23	48.8	104	115	176.6	387.5	306.6	67.56	579.1	527.0	681.4	197.2
DES	6.68	62.9	133	133	94.07	262.3	148.7	32.52	424.0	629.5	405.5	80.34
PGDC	2.08	32.9	66.9	64.5	122.1	429.2	332.0	89.64	319.2	562.8	747.6	259.6
EHIN	0.946	16.2	48.8	38.6	240.2	1146	711.0	356.3	229.9	1011	1034	334.9
DOC	1.45	19.0	63.9	37.4	116.0	887.3	611.3	130.4	160.8	663.3	629.6	273.9
IPM	0.973	18.6	59.9	43.3	160.4	1118	867.9	308.9	242.2	953.5	913.9	382.0
ININ	1.67	15.9	59.0	31.9	201.9	1201	1075	359.6	187.7	996.6	960.7	367.0
NPGDEH	1.83	23.5	65.1	52.3	124.2	832.7	505.2	182.5	200.0	639.7	709.7	218.2
PGIS	6.24	70.0	83.5	77.7	32.53	62.07	50.03	6.493	306.0	197.9	248.2	44.67
IPP	0.860	15.0	56.0	34.9	144.7	529.9	494.8	203.7	218.9	912.4	833.4	345.4
IPL	1.19	21.7	54.5	40.2	136.0	720.3	552.2	168.0	255.2	881.4	837.7	285.0
GTEH	1.67	23.9	48.8	39.2	70.88	467.6	306.3	91.21	64.39	232.1	225.3	140.4
IPIS	0.848	14.8	50.7	29.8	97.07	945.5	592.5	238.3	149.3	776.9	792.2	361.4
ITDIN	0.663	9.57	39.9	23.3	105.0	710.9	598.6	226.5	86.44	632.0	629.6	309.8
NPGDC	1.82	20.2	64.9	46.3	65.59	463.8	234.6	59.67	91.50	321.6	377.0	149.4
DEHS	1.71	21.4	71.7	58.5	52.61	425.2	217.1	40.50	94.72	246.1	196.9	87.44
CEH	0.592	9.28	41.3	18.2	48.00	697.5	256.5	63.12	57.38	544.1	454.9	212.6
EHP	0.630	9.23	43.3	23.9	65.54	748.1	401.1	134.7	50.79	430.5	377.1	277.2
EHHS	1.49	7.97	57.1	55.4	22.31	159.4	55.25	7.184	19.17	97.56	44.91	34.46
ODM	0.408	5.40	31.8	11.5	28.12	559.6	309.5	74.24	11.74	248.7	171.4	135.0
ODR	1.79	26.1	67.0	65.0	21.91	166.7	58.64	6.579	22.69	56.47	31.96	34.97
ISIS	0.628	6.58	35.8	18.6	39.29	337.4	184.4	62.43	22.65	227.1	177.9	54.76
GTIS	1.04	6.38	15.7	10.8	22.05	264.6	195.5	35.67	8.420	90.90	76.31	46.08
PETIS	1.04	1.55	14.7	10.3	20.69	230.1	160.5	35.09	4.750	45.68	39.92	8.020

CF, caffeine; AMP, aminopyrine; BA, benzoic acid; FP, flurbiprofen.

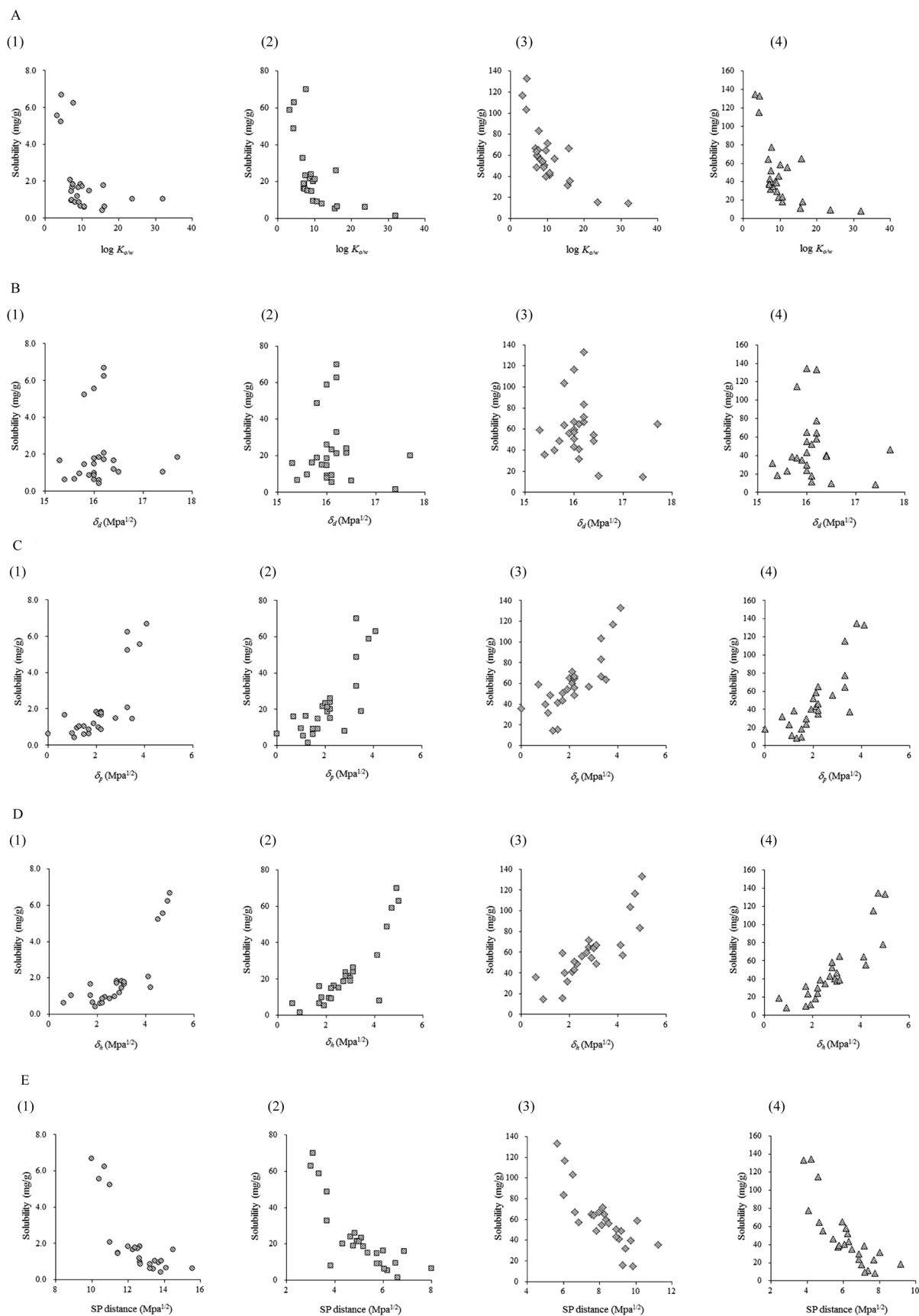


Fig. 2. (A) Relationships between the Octanol/Water Coefficients ( $\log K_{ow}$ ) of Esters and the Solubility of the Model Compounds; (B) Relationships between the Dispersion Solubility Parameter ( $\delta_d$ ) of the Esters and the Solubility of the Model Compounds; (C) Relationships between the Polar Solubility Parameter ( $\delta_p$ ) of the Esters and the Solubility of the Model Compounds; (D) Relationships between the Hydrogen Bond Solubility Parameter ( $\delta_h$ ) of the Esters and the Solubility of the Model Compounds; (E) Relationships between the Solubility Parameter SP Distance of the Esters and the Solubility of the Model Compounds

Compounds: (1) CF, (2) AMP, (3) BA, and (4) FP.



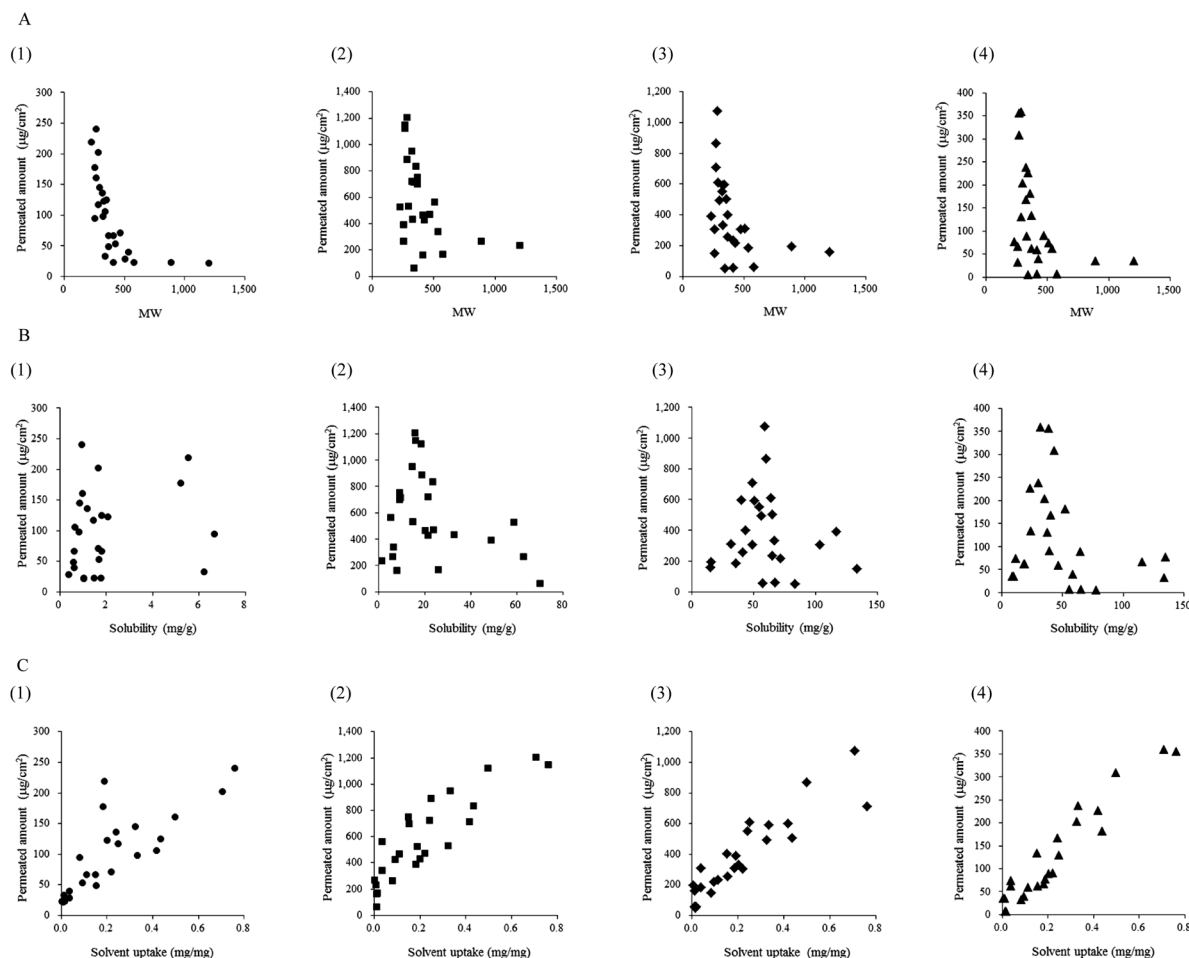


Fig. 3. (A) Relationships between the Molecular Weights (MWs) of the Esters and the Amount of the Model Compounds That Permeated through the Silicone Membrane; (B) Relationships between the Solubility of the Model Compounds in Esters and the Amount of the Model Compounds That Permeated through the Silicone Membrane; (C) Relationships between the Uptake of the Esters and the Amount of the Model Compounds That Permeated through the Silicone Membrane

Compounds: (1) CF, (2) AMP, (3) BA, and (4) FP.

and EHHS, whereas CF had the lowest permeation when it was dissolved in high MW long-chain fatty acids and long-chain alcohol monoesters, triesters, and tetraesters, such as ODR, GTIS, and PETIS.

Figure 3 shows the relationship between the physicochemical properties of esters and the amounts that permeated through the silicone membrane. There was no significant correlation between the amount of model compounds that permeated and the solubility of compounds (Fig. 3B). On the other hand, significant correlations were observed between the MWs of esters and the amounts of model compounds (CF;  $r = -0.876$ , AMP;  $r = -0.471$ , BA;  $r = -0.574$ , FP;  $r = -0.514$ , Fig. 3A). Moreover, there were highly significant correlations between the uptake of the esters through the silicone membrane and the amounts of model compounds that permeated through the silicone membrane (CF;  $r = 0.859$ , AMP;  $r = 0.872$ , BA;  $r = 0.914$ , FP;  $r = 0.931$ , Fig. 3C).

The physical properties of the esters are summarized in Table 4. The physical properties of esters, such as viscosity, wettability, and surface tension also displayed a good relationship with the amounts of model compounds that permeated through the membranes (Table 5). Of note, the wettability of esters on an acrylic plate also showed a significantly good

correlation.

**Strat-M<sup>®</sup> Membrane Permeation by the Model Compounds Form Esters** The amounts of model compounds that permeated through Strat-M<sup>®</sup> are also summarized in Table 3. The amount of CF that permeated through the Strat-M<sup>®</sup> membrane was highest when it dissolved in DIBA, whereas the amounts of AMP and BA were highest in EHIN, and that of FP was highest in IPM. In contrast, the lowest amounts of CF, AMP, and FP that permeated were observed in PETIS, and BA showed its lowest permeation in ODR.

Figure 4 and Table 6 show the relationship between the physicochemical or physical properties of the esters and the amounts of model chemicals that permeated through the Strat-M<sup>®</sup> membrane. Only the amount of CF that permeated through the Strat-M<sup>®</sup> membrane showed a significantly correlation with the solubility of CF ( $r = 0.596$ ), whereas no such correlation was observed for AMP, BA, or FP (Fig. 4B). Similar to the silicone membrane, significant correlations were found between the amounts that permeated and MWs (CF;  $r = -0.876$ , AMP;  $r = -0.822$ , BA;  $r = -0.802$ , FP;  $r = -0.64$ , Fig. 4A) and the uptake of esters (CF;  $r = 0.825$ , AMP;  $r = 0.83$ , BA;  $r = 0.818$ , FP;  $r = 0.656$ , Fig. 4C). Furthermore, the amounts of CF that permeated showed a significant correlation

Table 4. Physical Properties of the Esters Used in This Study

	Viscosity (mPa·s)	Surface tension (mN/m)	Wettability (Contact angle)		
			Acrylic plate	Silicone	Strat-M <sup>®</sup>
DIPA	3.15	28.3	51.0	48.3	11.3
DIBA	4.38	28.0	51.1	47.4	11.5
DES	4.34	32.2	58.6	54.5	10.9
PGDC	6.11	30.0	51.4	47.7	12.7
EHIN	3.18	25.8	39.0	40.5	16.0
DOC	5.04	28.8	52.7	47.0	12.4
IPM	3.52	28.0	48.4	51.1	17.3
ININ	4.27	25.5	41.2	42.8	13.1
NPGDEH	8.74	28.0	48.6	46.1	13.8
PGIS	39.20	30.6	55.9	60.0	13.2
IPP	4.74	28.8	43.3	45.2	16.2
IPL	4.26	26.8	42.1	47.4	11.9
GTEH	23.20	28.9	52.7	50.3	14.3
IPIS	6.59	27.7	49.7	47.2	13.3
ITDIN	7.84	27.2	44.6	45.9	15.9
NPGDC	12.13	29.5	52.5	51.0	11.9
DEHS	13.43	30.6	57.4	53.9	12.6
CEH	8.75	29.8	45.7	49.4	15.1
EHP	8.19	29.3	52.4	49.0	15.0
EHHS	233.00	31.9	58.9	60.0	15.8
ODM	18.17	30.3	58.5	55.4	13.6
ODR	54.23	31.7	54.8	59.3	14.1
ISIS	31.03	30.5	53.8	58.6	13.0
GTIS	118.33	30.6	55.2	59.2	13.8
PETIS	213.67	30.5	56.4	60.1	17.7

Table 5. Correlation Coefficients between the Amounts of the Model Compounds That Permeated through a Silicone Membrane and the Physical Properties of the Esters

		Viscosity	Surface tension	Wettability	
				Acrylic plate	Silicone
Permeated amount	CF	−0.927***	−0.806***	−0.766***	−0.835***
	AMP	−0.662***	−0.845***	−0.759***	−0.792***
	BA	−0.743***	−0.904***	−0.800***	−0.853***
	FP	−0.689***	−0.906***	−0.831***	−0.856***

CF, caffeine; AMP, aminopyrine; BA, benzoic acid; FP, flurbiprofen. \*\*\* $p < 0.001$ .

with the viscosity and wettability of the esters, and with the viscosity, whereas those of AMP, BA, and FP correlated with the wettability of the acrylic plate and the surface tension of the esters (Table 6).

#### Relationship between the Amounts of Compounds That Permeated through the Silicone and Strat-M<sup>®</sup> Membranes

Figure 5 shows the relationship between the amount of compounds that permeated from esters through the silicone and Strat-M<sup>®</sup> membrane. Good relationships were confirmed in the amounts of compounds that permeated between the membrane (CF;  $r=0.78$ , AMP;  $r=0.83$ , BA;  $r=0.84$ , FP;  $r=0.941$ ). The amounts of CF that permeated through the Strat-M<sup>®</sup> membrane were higher than those through the silicone membrane, whereas higher amounts of BA and FP were observed to permeate through the silicone membrane. On the other hand, almost a 1:1 relation was confirmed only for AMP permeation.

#### Discussion

To accurately estimate the efficacy and safety of dermal

products, we need to understand the effects of vehicles on the skin permeation of topically applied compounds. Although esters are widely used in dermal products as the vehicle, few reports are available on their effects on compound permeation through membranes. To reveal the effects of vehicles on compound permeation, in the present study, 25 different esters were selected as vehicles, and artificial membrane permeation was evaluated using compounds with different polarities.

Firstly, solubility studies of the compounds into the esters were performed because permeation through a membrane is related to the thermodynamic activity of compounds in the vehicle.<sup>1)</sup> Because it is well known that the SP distance from solute to solvent is related to solubility, similarly in the present study, a significant negative relationship was found between the SP distance and solubility. Among the relationship between the solubility of compounds and the physicochemical properties of esters, solubility was the most highly correlated with the hydrogen bond SP value. A significant correlation has been reported previously between the solubility of keto-

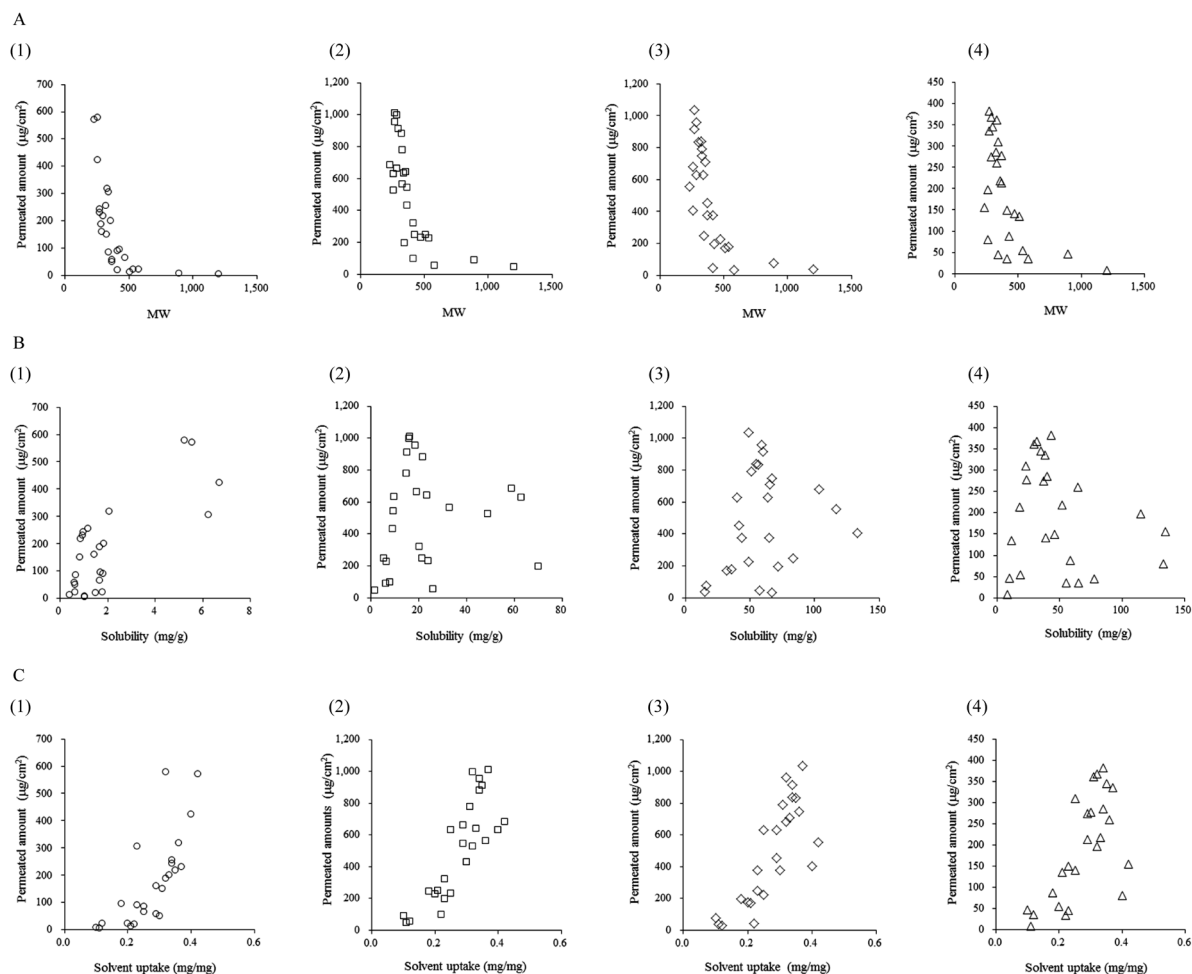


Fig. 4. (A) Relationships between the Molecular Weights (MWs) of Esters and the Amount of the Model Compounds That Permeated through the Strat-M® Membrane; (B) Relationships between the Solubility of the Model Compounds in the Esters and the Amount of the Model Compounds That Permeated through the Strat-M® Membrane; (C) Relationships between the Uptake of the Esters and the Amount of the Model Compounds That Permeated through the Strat-M® Membrane in Esters

Compounds: (1) CF, (2) AMP, (3) BA, and (4) FP.

Table 6. Correlation Coefficients between the Amount of the Model Compounds That Permeated through a Strat-M® Membrane and the Physical Properties of the Esters

		Viscosity	Surface tension	Wettability	
				Acrylic plate	Strat-M®
Permeated amount	CF	−0.778***	−0.398*	−0.412*	−0.484*
	AMP	−0.915***	−0.807***	−0.805***	−0.061
	BA	−0.870***	−0.836***	−0.857***	−0.042
	FP	−0.775***	−0.858***	−0.854***	0.114

CF, caffeine; AMP, aminopyrine; BA, benzoic acid; FP, flurbiprofen. \* $p < 0.05$ , \*\*\* $p < 0.001$ .

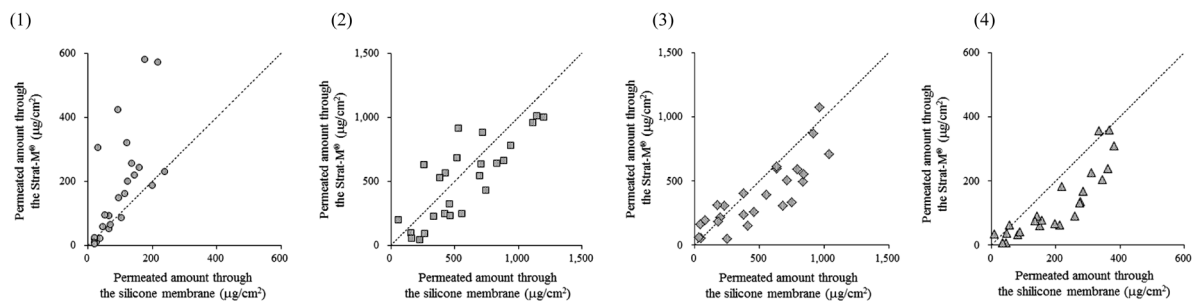


Fig. 5. Relationships between the Amounts of the Model Compounds in Esters That Permeated through the Silicone and Strat-M® Membranes

Dashed lines show the relationships with a 1:1 correlation. Compounds: (1) CF, (2) AMP, (3) BA, and (4) FP.



profen dissolved in fatty acids esters and the hydrogen bond SP value.<sup>16)</sup> The present results suggested that the hydrogen bonds of esters strongly affected the solubility of the model compounds.

Because of the low solubility of CF in the esters, its permeation experiments were conducted with saturated solutions. Therefore, in constant thermodynamic activity conditions, CF permeation through the silicone membrane from the esters should be at equilibrium among the vehicles. On the other hand, the amounts of AMP, BA, and FP that permeated through the silicone membrane were inversely proportional to their solubility, because thermodynamic activity of the compounds decreased with an increase in solubility in the vehicle. However, no correlation was observed between the amount that permeated through the membranes and the solubility of the model compounds in esters, but significant correlations were observed with the uptake of esters into the silicone membrane. Similarly, Twist and Zatz<sup>10,27,29)</sup> reported that the partition coefficient of parabens across a silicone membrane depended on the uptake of the alcohol vehicle into the membrane. This could be a reason why the thermodynamic activity of compounds in esters was not proportionally related to their permeation through the silicone membrane. Furthermore, partitioning of esters into the membrane was related to their viscosity, wettability, and surface tension. The surface tension and wettability on silicone of PGIS ( $\log K_{o/w}$ ; 7.60, MW; 342.6), which has a hydroxyl group, were 30.6 mN/m and 60.0°, respectively, whereas those of NPGDEH ( $\log K_{o/w}$ ; 7.51, MW; 356.6), which does not have a hydroxyl group, were 28.0 mN/m and 46.1°, respectively. The amounts of AMP, BA, and FP that permeated were higher when they were dissolved in NPGDEH (832.7, 505.2, 182.5  $\mu\text{g}/\text{cm}^2$ , respectively) than when they were dissolved in PGIS (62.07, 50.03, 6.493  $\mu\text{g}/\text{cm}^2$ , respectively).

It has previously been reported that isopropyl myristate has a fluidizing effect on the intercellular lipids of the stratum corneum<sup>30)</sup> and that diethyl sebacate can be used to extract lipids,<sup>17)</sup> indicating that both of these esters serve as transdermal absorption enhancers. It has also been found that ketoprofen exhibited increased permeation through hairless rat skin when it was dissolved in low MW esters,<sup>16)</sup> and the permeability of stearyl glycyrrhetinate through Yucatan micropig skin with a finite dose was affected by the surface tension and MW of the vehicle.<sup>19)</sup> Thus, the common properties of these permeation enhancers are their low MWs and surface tension. Similarly, in the present study, it was evident that the amounts of the model compounds that permeated through the membranes were higher when esters with low MWs and low surface tension were used as a vehicle.

On the other hand, although there was a significant positive correlation between the amount of the model compounds that permeated through the silicone and Strat-M® membranes, with higher correlation coefficients being observed for the more lipophilic model compounds, lower correlation coefficients were observed for the Strat-M® membrane between the uptake of the esters and the amounts of the compounds that permeated compared with those with the silicone membrane. Furthermore, the amounts of AMP, BA, and FP that permeated through the Strat-M® membrane were correlated with the viscosity, wettability of the acrylic plate, and surface tension of the esters, whereas the amount of CF was correlated with

the viscosity, wettability of the acrylic plate and Strat-M®, and surface tension of esters. The wettability on an acrylic plate by the esters varied from 39.0 to 58.9°, whereas that of the Strat-M® membrane varied across a much narrower range of 10.9 to 17.7°, which might explain a reason for the absence of a correlation between the wettability on the Strat-M® membrane and the amount of compounds that permeated through it. The solvent uptake into silicone membrane was highly correlated with wettability of the solvent against silicone ( $r=-0.899$ ). On the other hand, that into Strat-M® was highly correlated with the solvent viscosity ( $r=-0.894$ ). Unlike the silicone membrane, the Strat-M® membrane consists of three layers, in which lipid components were found only in the first and second layers.<sup>8)</sup> This might be a reason for the different tendency between the silicone and Strat-M® membranes for the relationship between the physicochemical properties of esters and the amounts of the permeated compounds. In addition, structural differences between them might be related to permeation differences for CF, BA, and FP.

We previously reported that the permeation parameters of chemicals through human skin from aqueous vehicle could be predicted by those through silicone membrane or Strat-M®.<sup>8,9)</sup> In the present study, the effect of esters on artificial membrane permeation were evaluated and the obtained relationships between physical properties and the amount of compounds that permeated were almost similar to previous results that evaluated rat,<sup>15-17)</sup> porcine,<sup>19,20)</sup> and human<sup>18)</sup> skins. Thus, although future research on the correlation between the permeability of artificial membrane and porcine or human skin from esters will be needed, silicone and Strat-M® membranes might be useful in the development of pharmaceutical and cosmeceutical products.

## Conclusion

This study successfully used a high-throughput diffusion cell array to evaluate the permeability of four model compounds (CF, AMP, BA, FP) through silicone and Strat-M® membranes using 25 kinds of esters as vehicles. It also determined the relationship between the amount of each model compound in esters that permeated and the physical properties of the esters. The amount that permeated through the silicone membrane was not correlated with the solubility of the model compound, but was significantly correlated with the wettability, surface tension, and uptake of esters into the membrane. Similarly, the amount of AMP, BA, and FP that permeated through the Strat-M® membrane correlated with the wettability, surface tension, and uptake of esters, whereas the amount of CF that permeated also correlated with its solubility in the esters. There was also a correlation between the amounts of the model compounds passing through the silicone and Strat-M® membranes, with higher correlation coefficients for more lipophilic model compounds. These findings suggested that the type of ester that is used as a vehicle is important for controlling the permeability of topically applied compounds. In addition, evaluation with an alternative membrane such as a silicone or Strat-M® membrane might be useful to reveal vehicle effects on compound permeation from all dermatological products.

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