Effects of Oleic Acid/Propylene Glycol on Rat Abdominal Stratum Corneum: Lipid Extraction and Appearance of Propylene Glycol in the Dermis Measured by Fourier Transform Infrared/Attenuated Total Reflectance (FT-IR/ATR) Spectroscopy

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Fourie transform infrared/attenuated total reflection analysis demonstrated that the absorbance intensity of C=O stretching bands, which reflect the amounts of lipids in the stratum corneum, decreased with an increase in the duration of skin treatment with 0.15 M oleic acid/propylene glycol (PG) system, suggesting that the oleic acid/PG system induced the lipid extraction, which was followed by a reorganization of the stratum corneum structures. The spectral peaks which originated from the PG molecule were detected in dermal tissues after 30 min of treatment of the stratum corneum with the same system. This observation suggested that the reorganization of the lipid domains due to the lipid extraction by the oleic acid/PG system helped the PG molecules enter the dermal tissues. It was also suggested that an effective volume within the stratum corneum for solutes and/or solvents which could penetrate through the inter-, and/or intracellular routes could be altered in conjunction with the structural changes of the lipids.

Keywords oleic acid; propylene glycol; stratum corneum; delipidization; dermis; FT-IR/ATR spectroscopy

Oleic acid has been studied as a skin penetration enhancer for drugs primarily *via* its action primarily on stratum corneum lipid structures.[1-3] As to the behavior of oleic acid in the skin, Francoeur *et al.* suggested[4,5] that fluid oleic acid and ordered stratum corneum lipids can coexist when the oleic acid is taken into the stratum corneum at a normal physiological temperature. In their experiment, the action of oleic acid on the stratum corneum resulting in the permeability enhancement of drugs was examined in the single component system of oleic acid. One might suggest that the skin penetration enhancing effect of vehicles would be different depending upon the system used. For example, if one used a combined system consisting of two or more materials which affected the membrane structures, the permeant flux might be enhanced by the complex action of coexisting penetration enhancers. It was reported that the penetration of both polar[6] and non-polar[7] drugs through the skin was enhanced by oleic acid when used in combination with propylene glycol (PG). Such an enhancement was considered to be the result of two different mechanisms in which PG enhanced intracellular drug mobility by solvating alphakeratin in corneocytes, allowing oleic acid to act on the lipid barrier.[8] As to the perturbation of the stratum corneum, the lipid extraction from the stratum corneum could also be an important factor for permeants to penetrate the epidermal lipid barrier. Based on these considerations, factors affecting permeant flux and/or stratum corneum structures should be elucidated on an individual basis considering all components that make up the permeant-containing vehicle.

We have examined the spectral behavior of the rat stratum corneum following treatment of the skin surface with fatty acids and fatty amines in PG using Fourier transform infrared/attenuated total reflection (FT-IR/ATR) in relation to the permeant flux of hydrophilic and hydrophobic solutes, 5- and 6-carboxyfluorescein (CF) and indomethacin, respectively.[9,10] In this experiment, it was found that some fatty acids and fatty amines in PG, as well as PG alone, could alter the conformation of stratum corneum keratinized proteins. Such conformational changes were possibly due to the incorporation of the fatty acid and the fatty amine into the lipid domains and corneocytes when used in combination with PG. In this paper, we have investigated the time profiles of structural changes in the stratum corneum lipids by measuring CH_2 asymmetric stretching vibrations resulting primarily from acyl chains of the stratum corneum lipids. We also examined the ability of the oleic acid/PG vehicle to remove lipids from the rat stratum corneum with increasing duration of exposure of the skin to oleic acid in PG. The appearance of PG into the dermal tissues was also pursued following treatment of the skin with oleic acid in PG by measurement of PG spectra using FT-IR/ATR.

Materials and Methods

Materials Oleic acid and propylene glycol, both of reagent grade, were purchased from Nakaloai Tesque. All other chemicals were also of a reagent grade.

Skin Preparation for FT-IR/ATR Spectroscopy Measurement As previously reported,[9,10] the abdominal skin was removed from male Wistar rats (8—9 weeks old) under pentobarbital anesthesia, shaved with an electric clipper and then with an electric razor. The freshly excised full thickness skin with subcutaneous fat removed was weighed. The skin surface area available for FT-IR/ATR measurement was 1.05 cm^2. The skin samples were then mounted between the two compartments of the diffusion cells with the dermis side facing the receiver compartment. The formulations used were PG with or without oleic acid in a concentration of 0.15 M. One gram of the vehicle was applied into the donor compartment. The donor compartment was sealed from the atmosphere with Parafilm®. The receiver compartment was filled with 14.2 ml of a phosphate buffered solution (PBS; 140 mM NaCl, 2.68 mM KCl, 8.10 mM Na_2HPO_4 and 1.47 mM KH_2PO_4, pH 7.4). The assembled diffusion cells were then immediately immersed in a water bath at 37 °C and the buffer solution was stirred with a magnetic stirrer. The receiver compartments were maintained at 37 °C. Each of the skin samples was taken out from the cell at appropriate time

intervals after the incubation. The surface of the stratum corneum was gently wiped with Kimwipes® and then left as it was for 10 min at ambient temperature. For FT-IR/ATR measurement, the skin was placed on the element with the epidermal surface attached to the reflection surface, then the spectra of the epidermal surface was measured. The same procedure was also used in the measurement of PG that appeared in dermis, with the exception of the skin being placed on the element with the dermal surface attached to the reflection surface so that the spectra of the dermal surface were obtained.

FT-IR/ATR Spectroscopy Measurement IR spectra of the surface of either the stratum corneum or the dermal tissues were obtained at an ambient temperature with a JEOL JTR-100 FT-IR spectrometer equipped with a liquid nitrogen-cooled, narrow band mercury-cadmium-telluride detector (MCT detector) with a resolution of 0.45 cm^{-1}. The internal reflection element was KRS-5 (52 × 20 × 2 mm trapezoidal cut at 45°). The CH_2 asymmetric stretching band peak which originated from alkyl chains in the lipids was obtained by the built-in programmed curve fitting method of the FT-IR/ATR instrument.

Results and Discussion

Time Profile of Stratum Corneum Perturbation The time profiles of the FT-IR/ATR spectra of the surface of the rat skins treated with 0.15 M oleic acid in PG for (b) 5 min, (c) 30 min, (d) 2 h, (e) 12 h, and (a) untreated sample are illustrated in Fig. 1A. As is already known, the CH_2 asymmetric and symmetric stretching vibrations absorbing near 2920 and 2850 cm^{-1}, respectively,[3] result primarily from the methylene groups of the stratum corneum lipid hydrocarbon chains. As for the CH_2 asymmetric stretching vibrations, we have already reported that the degree of the frequency shift depended on the concentration of oleic acid which was applied to the skin.[10] As seen in the spectra of (a) to (e) of Fig. 1A and Table I, after treatment of the skin surface with 0.15 M of oleic acid in PG for 5 min, CH_2 asymmetric stretching vibrations at 2920 cm^{-1} showed a slight shift (0.9 cm^{-1}) toward higher wave numbers. By increasing the duration of exposure of the skin to 0.15 M oleic acid in PG, additional shifting towards high wavenumbers, along with a broadening of the spectra was observed. After 30 min, 2 h and 12 h of treatment, there was an average increase of 2.4, 3.4 and 3.9 cm^{-1}, respectively, showing that the degree of the shift was similar between the 2 and 12 h treatment samples. A similar shift was not observed with the samples treated with PG alone for (b) 2 h and (c) 12 h in comparison with the untreated skin sample (a) as shown in Fig. 1B. These results indicated that disordering of the stratum corneum structures could be initiated at least by 5 min of treatment with 0.15 M oleic acid in PG. Such disordering effects were increased by increasing the duration of exposure of the skin to the skin penetration enhancer in PG, and a maximum effect was reached after 2 h of treatment, suggesting that the uptake of oleic acid and PG into the stratum corneum approached saturation after at least 2 h of treatment.

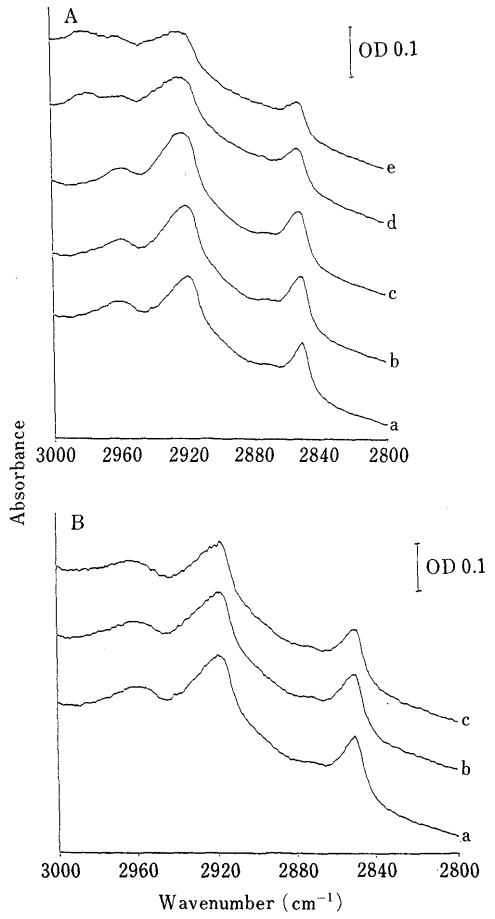

Fig. 1. Representative FT-IR/ART Spectra of Rat Abdominal Stratum Corneum in the CH_2 Asymmetric and Symmetric Stretching Region Resulting Primarily from Stratum Corneum Lipids Following Treatment of the Stratum Corneum with 0.15 M Oleic Acid/PG System and PG Alone

A, the skin was treated with 0.15 M oleic acid in PG for either: a, without treatment; b, 5 min; c, 30 min; d, 2 h; e, 12 h. B, the skin was treated with PG alone either: a, without treatment; b for 2 h; c for 12 h.

Delipidization with Oleic Acid in PG System The peak intensity resulting from CH_2 asymmetric stretching vibrations near 2920 cm^{-1} is known to be affected by stratum corneum proteins.[11] Thus, the peak intensity may not be available for evaluating the amounts of lipids which exist in the stratum corneum. The absorbance at 1741 cm^{-1} in the spectra is assigned to the C=O mode of esterified stratum corneum lipids, and the exogeneously introduced oleic acid produces the characteristic 1710 cm^{-1} absorbance.[3] It is also reported that the absorption band at approximately 1740 cm^{-1}, which originated from the C=O stretching vibrations, is not affected by stratum corneum proteins.[11] Raykar *et al.*[12] took advantage of this observation and evaluated the degree of delipidization of the stratum corneum following treatment of the stratum corneum with a 2:1 chloroform/methanol mixture by measuring changes in absorbance at 1740 cm^{-1}. In our study we also adapted this technique and investigated whether oleic acid extracted lipids from the stratum corneum, when used in combination with PG, by measuring the absorbance intensity at 1740 cm^{-1} as a function of time following

TABLE I. Time Course of Frequency Changes in the CH_2 Asymmetric Stretching Peak Resulting from Rat Abdominal Stratum Corneum Treated with 0.15 M Oleic Acid in PG and/or PG Alone

Treatment time	Frequency (cm^{-1})	
	PG	Oleic acid in PG
0 min	2919.8 ± 0.2 (12)	2919.8 ± 0.2 (12)
5 min	2919.7 ± 0.4 (3)	2920.2 ± 0.1 (3)
30 min	2919.1 ± 0.4 (3)	2922.2 ± 0.1 (3)
2 h	2920.1 ± 0.1 (3)	2923.2 ± 0.3 (3)
12 h	2920.1 ± 0.4 (3)	2923.7 ± 0.5 (3)

The values given are mean ± S.D. Numbers of trials are given in parentheses.

treatment of the skin surface with 0.15 M oleic acid in PG. The results are shown in Fig. 2A. It was apparent that the absorbance intensity at 1740 cm^{-1} decreased with an increase in the skin treatment time with 0.15 M oleic acid in PG. However, no decrease was observed in the samples treated with PG alone for 2 and 12 h, as compared with untreated sample (Fig. 2B). This implied that treatment of the stratum corneum with 0.15 M oleic acid in PG caused the decrease in the amount of the C=O group contributing to the absorbance intensity at 1740 cm^{-1}, suggesting that lipid extraction from the stratum corneum occurred. Another explanation for the decrease in the absorbance intensity was that the spectral characteristic was changed, although no direct evidence was shown at present. However, it was probably reasonable to assume that the structures in the lipid domain were perturbed, either in the case of lipid extraction effects or in the alteration of the absorbance characteristic. Thus, the application of the oleic acid/PG system on the rat skin could reduce the resistance of the stratum corneum by reorganizing the structures of lipid domains. Such conformational alterations would bring about the possible disordering of protein structures in corneocytes.[9] Francoeur *et al.* provided evidence that the lipid phase transitions associated with the intracellular bilayers were markedly affected by treatment with oleic acid.[4] Recently, they further demonstrated[5] that oleic acid lowered the lipid phase transition temperature (T_m) of the stratum corneum lipids in conjunction with increasing the conformational freedom or flexibility of the endogenous lipid alkyl chains above the T_m. Oleic acid did not significantly change the chain disorder of the stratum corneum lipids at the temperature below T_m under those circumstances where oleic acid itself was fully disordered. Of the spectroscopic behavior of stratum corneum lipids, it has been shown that the 0.5 h treatment of human stratum corneum with oleic acid (0.5—10%) in ethanol did not affect the intensity of the 1741 cm^{-1} absorbance, which is characteristic of esterified stratum corneum lipids.[3] This result indicates that oleic acid in ethanol did not extract

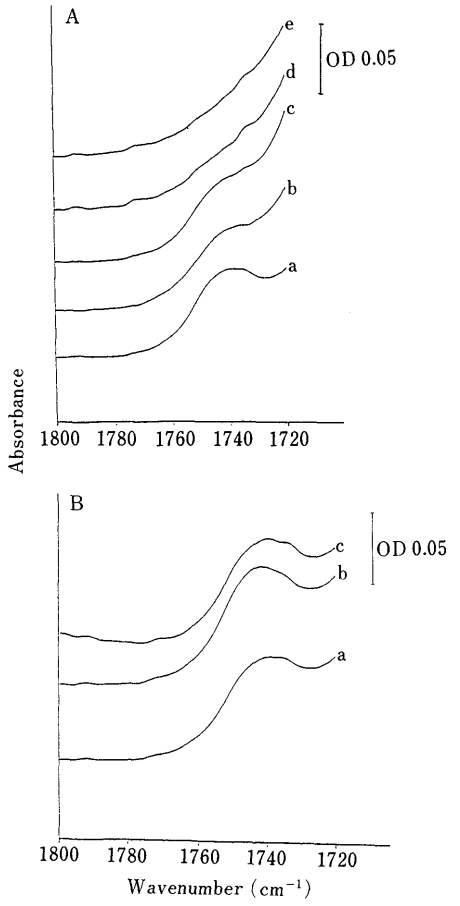

Fig. 2. Representative FT-IR/ATR Spectra of Rat Abdominal Stratum Corneum in the C=O Stretching Region Resulting from Stratum Corneum Lipids Following Treatment of the Stratum Corneum with 0.15 M Oleic Acid in PG and PG Alone

A, the skin was treated with 0.15 M oleic acid in PG either: a, without treatment; b for 5 min; c, 30 min; d, 2 h; e, 12 h. B, the skin was treated with PG Alone either: a, without treatment; b, 2 h; c, 12 h.

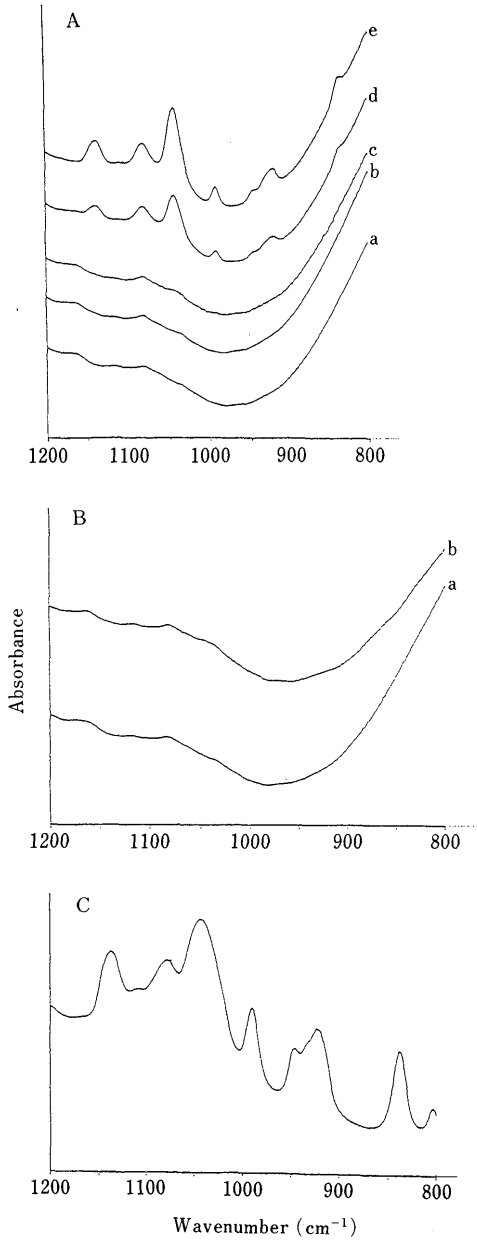

Fig. 3. Representative FT-IR/ATR Spectra of Rat Abdominal Dermal Tissues in the Region from 1200 to 800 cm^{-1} Following Treatment of the Stratum Corneum with 0.15 M Oleic Acid in PG and PG Alone

A, the skin was treated with 0.15 M oleic acid in PG either: a, without treatment; or for b, 5 min; c, 15 min; d, 30 min; e, 2 h. B, the skin was treated with PG alone either: a, without treatment; b, 2 h. C, spectrum for pure PG sample.

any lipids from the stratum corneum under this condition. By contrast, our results indicated that even 5 min treatment of the stratum corneum with 0.15 M oleic acid in PG caused a decrease in absorbance at 1740 cm^{-1}, suggesting that oleic acid in the PG system extracted the stratum corneum lipids. The difference in the action of oleic acid on the lipid extraction, thus, must be due to the difference in the system used in both experiments. It is mentioned that PG molecules may occupy some hydrophilic region between the lipid polar head groups.[8] As to the mechanism of action of oleic acid in the PG system on the stratum corneum lipids, we can speculate that oleic acid in PG may cause breakdown of the intercellular leaflet structure by solubilizing stratum corneum lipids which may then emigrate to the outside of the stratum corneum (donor compartment). Such a structural breakdown could bring about an extension of the effective volume within the stratum corneum for solutes and/or solvents which could penetrate through the inter-, and intracellular routes of dermal and epidermal tissues.

Appearance of PG in Dermal Tissues IR spectra of PG on the dermal side were measured with skin samples which have been treated with 0.15 M oleic acid in PG on the stratum corneum for either 5 min, 15 min, 30 min or 2 h, and with an untreated skin sample. The results are shown in Figs. 3A and 3B. The IR spectrum of the pure PG sample spanning the region from 1200 to 800 cm^{-1} is also shown in Fig. 3C. As indicated, the spectrum obtained following 2 h of treatment of the stratum corneum with 0.15 M oleic acid, for example, was consistent with that of the pure PG sample, and was thus identified as the spectrum resulting from PG molecules. Therefore, the peak with the highest intensity in the absorption band at 1043 cm^{-1} was selected for further study of the appearance of PG in dermal tissues. As seen in Fig. 3A, the peak resulting from PG molecules appeared following treatment with 0.15 M oleic acid in PG for 30 min, and the intensity of the peak increased further with the 2 h of treatment. However, before and following the 5 and 15 min exposure to the 0.15 M oleic acid/PG system, the spectra did not show any peak resulting from PG molecules. PG alone did not cause any change in absorbance at 1043 cm^{-1} following the 2 h of treatment as compared with the untreated sample. These results indicate that PG molecules, when used in combination with oleic acid, penetrated the dermal tissues through the epidermal tissues, and this appearance could take at least 30 min. Penetration of PG molecules through the skin has been reported in several studies which involved the use of two-compartment diffusion cells.[13-15] In these studies, however, they only described the behavior of PG molecules which appeared in the receiver compartment after the diffusion rate became constant, or they only compared the total amount of PG which penetrated the skin between systems with the penetration enhancer, but without any additives (control). Another point to be made here is that it may be possible to calculate the measurement of the steady state level, where the diffusion rate is constant, from the results of the appearance of solutes in the receiver compartment, but the time for reaching the steady state must be obtained by the plot of the amount of solutes penetrated against time. However, our results suggest that one could use FT-IR/ATR spectroscopy to directly measure the time for the steady state level to be attained by pursuing the behavior of the PG which appeared in dermal tissues.

In conclusion, FT-IR/ATR analysis of rat stratum corneum demonstrated that the oleic acid/PG system could perturb the stratum corneum lipid structures by extracting the lipids. The appearance of PG in dermal tissues following treatment of the stratum corneum with 0.15 M oleic acid in PG suggested that the reorganization of lipid domains due to the lipid extraction by the oleic acid/PG system helped PG molecules enter the dermal tissues. It was also suggested that the effective volume within the stratum corneum for solutes and/or solvents which could penetrate through the inter-, and intracellular routes could be altered in conjunction with structural changes of the lipids following treatment with the oleic acid/PG system.

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