Role of Simplex Lattice Statistical Design in the Formulation and Optimization of Microemulsions for Transdermal Delivery

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Microemulsions (ME) have gained attention as an alternative pharmaceutical formulation for transdermal delivery systems. However, the complicated relationships between various ME compositions (causal factors) and their characteristics (response variable) have not been fully comprehended. To overcome this problem, the design and development of ME for transdermal delivery was performed in our study using Design Expert[®] Software. The model formulations of ME were prepared according to the ME region obtained from pseudo-ternary phase diagrams using the simplex lattice design as an optimization technique. In this study, ketoprofen-loaded ME composed of oleic acid, Cremophor[®] RH40, ethanol and water were prepared, and their characteristics (*e.g.*, size, charge, conductivity, pH, viscosity, drug content, loading capacity and skin permeation flux) were evaluated. The ME having an appropriate skin permeation flux was used as the basis for optimization. The skin permeation flux of the experimental ME was very close to the flux predicted by Design Expert[®] Software and was significantly greater than that for the commercial product. Possible mechanisms for the enhancement of the skin permeation of the ME were also investigated using Fourier transform infrared (FT-IR) spectroscopy and X-ray diffraction (XRD). This finding provided an understanding of the relationship between the causal factors and response variables, as shown in the response surfaces. Moreover, these results indicated that the simple lattice design was beneficial for the pharmaceutical development of ME for transdermal delivery.

Key words simplex lattice design; microemulsion; optimization; transdermal drug delivery; Fourier transform infrared (FT-IR) spectroscopy; X-ray diffraction (XRD)

Colloidal drug delivery carriers such as liposomes, niosomes, micelles, microemulsions, nanospheres, liquid crystal and particulate dispersions consisting of small particles of 10 to 400 nm diameters have exhibited great promise as drug delivery systems. Colloidal drug delivery carriers are one of the most important entities in pharmaceutical formulations designed primarily for targeted drug delivery systems. Using colloidal drug carriers for targeted drug delivery could minimize systemic side effects and also improve the therapeutic efficacy of various drugs.¹⁾

Self-emulsifying systems known as microemulsions (ME) are optically isotropic transparent colloidal systems consisting of two immiscible liquid systems stabilized by surface tension reducing agents (surfactants).²⁾ ME consist of an oil phase, water phase and surfactant or a mixture of surfactants, usually in combination with a co-surfactant, that spontaneously emulsify when mixed in optimal ratios. ME have long been utilized as drug delivery systems in various routes of delivery including topical,³⁾ oral,⁴⁾ ocular,⁵⁾ nasal⁶⁾ and parenteral⁷⁾ routes. Moreover, ME have gained considerable interest as an alternative pharmaceutical formulation for transdermal delivery because of several advantages.²⁾ First, the incorporation of surfactant and co-surfactant in ME may introduce a high solubilizing capacity to incorporate a large amount of drugs in the formulation. Second, the surfactant and co-surfactant in ME may act as penetration enhancers, resulting in the disruption of major skin barrier functions of the stratum corneum. Third,

the skin permeability of drug-loaded ME may be increased by modifying the affinity of the drug to the internal phase in the ME, to enhance dissolving or partitioning into the stratum corneum. Moreover, ME are not only highly efficient for transdermal delivery but also highly stable, being thermodynamically stable,⁸⁾ compared to other carrier systems.

Previously, the development of ME has been based on trial and error techniques to obtain an acceptable ME formulation with multiple desirable characteristics (*e.g.*, high efficacy, high stability and high safety). The complicated relationships between various ME compositions (causal factors; X_n) and their characteristics (response variable; Y_n) were not fully comprehended. A high concentration of a mixture of surfactants may relate to high skin permeability efficacy; however, skin irritation should be concurrently considered. Therefore, an optimization technique based on a computer rationale was a powerful method for the development of ME with optimal characteristics. Moreover, the complicated relationship between the causal factors and ME characteristics could be easily understood by using the computer program.

This study investigates the development of ME for the transdermal delivery of ketoprofen (KP). In the development of ME for transdermal delivery, it is important to design an optimal ME formulation that has sufficient skin permeation (maximum efficacy) with minimum surfactant/co-surfactant system levels (minimum skin irritation). The challenge in achieving this objective is to discover the optimal ME formulation for the transdermal delivery of KP by using the computer program. The model ME formulations for the transdermal delivery of KP were obtained by using a simplex lattice statistical design based on the ME regions obtained from

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Fig. 1. The Phase Diagrams

(A) Pseudo-ternary phase diagram of microemulsions (ME). (B) Simplex lattice design for the three factors under the ME region. Eight formulations were prepared according to the results obtained using the simplex lattice design with duplicate centroids.

pseudo-ternary phase diagrams. The KP-loaded ME composed of oleic acid, Cremophor® RH40, ethanol and water were prepared. Their characteristics, *e.g.*, size, charge, conductivity, pH, viscosity, drug content, loading capacity and skin permeation flux were investigated. The ME were optimized using Design-Expert® Software and then prepared and compared with the commercially available product. Finally, possible mechanisms for the enhancement of skin permeation of the ME was evaluated and confirmed by Fourier transform infrared (FT-IR) spectroscopy and X-ray diffraction (XRD).

MATERIALS AND METHODS

Materials Oleic acid, KP and indomethacin was supplied by Sigma-Aldrich Production GmbH, (Buchs, Switzerland). Polyethylene glycol (PEG)-hydrogenated castor oil (Cremophor® RH 40) was obtained from NS group (Bangkok, Thailand). Absolute ethanol (99.9%) was purchased from Qrec Chemical Co., Ltd. (Chonburi, Thailand). All other chemicals and solvents were of analytical reagent grade.

Construction of the Pseudo-Ternary Phase Diagram The pseudo-ternary phase diagram of three components (oil phase, water phase and emulsifier phase) were constructed using aqueous titration method.⁹ The emulsifier phase composed of the surfactant (S) and co-surfactant (CoS) mixture at weight ratios of 1:1, 1:2, 1:3 and 1:4 were dissolved in oil phase, in the ratio of 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2 and 9:1 in glass vials at room temperature. Each ratio of the S/CoS mixture and oil phase was titrated continuously drop by drop with distilled water, using a burette and gentle magnetic stirring until the solution became turbid. The behavior of each ternary phase system during the titration was visually observed. The percentage of each component in the ternary phase system was calculated, and the phase diagrams were plotted on triangular co-ordinates to construct the pseudo-ternary phase diagrams.¹⁰

Experimental Design The simplex lattice design was used for the optimization of pharmaceutical products in accordance with previous studies.^{11,12} Three components in the ME, including the oil phase (X_1), emulsifier phase (X_2) and water phase (X_3), were selected as causal factors. Based on the ME region obtained from the pseudo-ternary phase diagram shown in Fig. 1A, the upper and lower limits of the levels of

each component were set as follows:

$$15 \leq X_1 \leq 40 (\%) \quad (1)$$

$$55 \leq X_2 \leq 80 (\%) \quad (2)$$

$$5 \leq X_3 \leq 30 (\%) \quad (3)$$

$$X_1 + X_2 + X_3 = 100 (\%) \quad (4)$$

Therefore, the feasible experimental region in the simplex lattice design was the largest triangular shape under the ME region. The components in the ME system were studied by varying their concentration simultaneously, and the total concentration of the three components was adjusted to 100%, as defined in Eqs. (1)–(4).

Microemulsion Preparation The ME were prepared according to the formulation obtained from the simplex lattice design under the ME regions in the pseudo-ternary phase diagrams. As shown in Fig. 1B, 8 model formulations (with duplicate of the centroid) of KP-loaded ME were prepared, composed of oleic acid as the oil phase, Cremophor® RH 40 as the surfactant, absolute ethanol as the co-surfactant and distilled water as the water phase (Table 1). Previous studies indicated that oleic acid and Cremophor® RH 40 improve the transdermal delivery of various drugs.^{9,13} All components were accurately weighed and mixed thoroughly, and the resulting ME were stored in airtight containers at room temperature, prior to further evaluation.

Determination of Droplet Size, Charge, Conductivity and pH The droplet size and charge of the ME were measured by photon correlation spectroscopy (Zetasizer Nano series, Malvern Instrument, U.K.). The samples were analyzed using a He–Ne laser at 633 nm. Thousand microliters of the ME were filled in disposable zeta cells. All analyses were performed at 25°C, at least three independent samples were analyzed, and the droplet size and charge were measured in triplicate. The electrical conductivity of the ME was determined using conductivity meter (S230 SevenCompact™, Mettler Toledo, Switzerland), with at least three independent samples. The pH was measured using pH meter (S220 SevenCompact™, Mettler Toledo, Switzerland) at room temperature (25°C). The samples were analyzed in triplicate.

Table 1. Experimental Design and Model Formulations of Ketoprofen-Loaded Microemulsions

Formulation	X_1	Oleic acid (%)	X_2	S/CoS (%)	X_3	Water (%)
1	0	15.0	1	80.0	0	5.0
2	0.5	27.5	0.5	67.5	0	5.0
3	1	40.0	0	55.0	0	5.0
4	0.5	27.5	0	55.0	0.5	17.5
5	0	15.0	0	55.0	1	30.0
6	0	15.0	0.5	76.5	0	17.5
7	0.33	23.5	0.33	63.5	0.33	13.5
8	0.33	23.5	0.33	63.5	0.33	13.5

Determination of Viscosity The viscosity of the ME was measured using a Brookfield Rheometer (Brookfield Engineering Laboratories, U.S.A.) fitted with a CP-40 spindle. The Brookfield Rheocalc operating software was used to control the rheometer. A minimum sample volume of 2 mL of ME was transferred into the viscometer chamber and the temperature was controlled at 25°C by circulating water bath. The time required for the fluid to flow between two specific points was measured and calculated in triplicate.

Determination of Drug Content and Loading Capacity The drug content and loading capacity of KP in the ME formulations were determined by HPLC. The KP-loaded ME were extracted with methanol at a 1:1 v/v ratio. The mixture of ME/methanol was centrifuged at 10000rpm at 25°C for 10min. The supernatant was filtered with the 0.45 μ m nylon syringe filter and analyzed by HPLC. The drug content and loading capacity of KP in the ME was calculated according to the calibration curve and/or the following equation:

$$\text{loading capacity (\%)} = \left(\frac{D_t}{M_t} \right) \times 100 \quad (5)$$

where D_t is the total weight of KP in the ME formulation and M_t is the total weight of ME formulation.

In Vitro Skin Permeation Study The shed snake skin of *Naja kaouthia* was used as a model membrane for the *in vitro* skin permeation study.¹⁴⁾ The shed snake skin was a kind gift from the Queen Saovabha Memorial Institute, Thai Red Cross Society, Bangkok, Thailand. It was stored at -10°C prior to use. After thawing, the shed snake skin was cut and then immediately placed on the diffusion cell. A Franz diffusion cell with an available diffusion area of 2.01 cm² was employed. The donor chamber was filled with the model ME (1 g), and the receiver chamber was filled with 6.5 mL of phosphate buffer saline (pH 7.4, 32°C) under sink condition. At appropriate times, an aliquot of the receiver solution was withdrawn, and the same volume of fresh buffer solution was replaced in the receiver side. The concentration of KP in the aliquot was analyzed using HPLC.

The skin sample after the skin permeation study was characterized using a FT-IR spectroscopy (Nicolet 4700 spectrophotometer; Thermo Fisher Scientific, Waltham, MA, U.S.A.) and an X-ray diffractometer (MiniFlex II, Rigaku Co., Tokyo, Japan) for investigating the possible mechanism underlying the effect of the ME on the skin permeability of KP. Following the skin permeation study, the skin sample was prepared according to the procedure in the previous study.^{15,16)} Briefly, the skin sample was washed with distilled water, blotted dry and kept in desiccators. The spectrum of each skin sample was recorded in the range of 500–4000 cm⁻¹ using a FT-IR

spectrophotometer. Then, the same skin sample was cut into a small sheet, approximately 2 cm × 2 cm, and attached to an aluminum well sample holder. XRD was used with CuK α , scanning from 2 θ =5–45°. The voltage and current operated were 30 kV and 15 mA, respectively.

Determination of Ketoprofen Concentration The HPLC 1100 system (Agilent 1100 Series HPLC System, Agilent Technologies, U.S.A.) was used to analyze the samples. The analytical column was Eclipse XDB-C18 column (particle size=5 μ m; column dimension=4.6 × 150 mm), and the mobile phase consisted of methanol and 0.1% phosphoric acid (75:25), set at a flow rate of 1 mL/min, and the UV detector was set at 254 nm for all determinations. The calibration curve for KP was in the range of 0.2–50 μ g/mL with a correlation coefficient of 0.999, using indomethacin as the internal standard. The intra-day and inter-day percent relative standard deviations (%RSD) were less than 0.2%.

Simultaneous Optimization Simplex lattice statistical design was used to estimate the relationship between the causal factors and response variables and statistically determine the optimal ME formulation. The experimental design was studied based on a three-component system: oil phase (X_1), emulsifier phase (X_2) and water phase (X_3). The ME's characteristics, e.g., droplet size (Y_1), charge (Y_2), conductivity (Y_3), pH (Y_4), viscosity (Y_5), drug content (Y_6), loading capacity (Y_7) and skin permeation flux (Y_8) were taken as the response variables. The response surfaces of all model formulations were estimated and sketched by Design-Expert® Software, Version 8, Approved No. 009503 (Stat-Ease, Inc., MN, U.S.A.). The best fitting mathematics (e.g., linear, quadratic, cubic and special cubic) was suggested based on the model summary statistics: the standard deviation (S.D.), the multiple correlation coefficient (R^2), the adjusted multiple correlation coefficient (adjusted R^2), the predicted multiple correlation coefficient (predicted R^2) and the predicted residual sum of square (PRESS), verified by Design-Expert® Software. The simultaneous optimal ME solution was predicted to have a droplet size as small as possible (nano-sized), non-charge (or a bit charge), conductivity (in range), pH (close to 5.5), viscosity (in range), drug content (maximum), loading capacity (maximum), skin permeation flux (maximum) and surfactant/co-surfactant system level (minimum). The predicted values and experimental values were evaluated to validate the reliability of the response surface.

RESULTS AND DISCUSSION

The ternary phase diagrams were constructed by varying the ratio of the S/CoS mixture as 1:1, 1:2, 1:3 and 1:4.

Fig. 2. The Response Surfaces of the ME's Characteristics Estimated by Design-Expert® Software, as a Function of the Percentage of Oleic Acid, S/CoS and Water Included

(A) Droplet size, (B) charge, (C) conductivity, (D) pH, (E) viscosity, (F) drug content, (G) loading capacity and (H) skin permeation flux.

Fig. 3. The Linear Correlation Plot between the Predicted and Actual Values for Various Responses

(A) Droplet size, (B) charge, (C) conductivity, (D) pH, (E) viscosity, (F) drug content, (G) loading capacity and (H) skin permeation flux.

Based on the visual observation, the 1:1 S/CoS mixture was selected as the largest ME region. The shaded area of the ternary phase diagram indicates the ME region, while the non-shaded area shows the turbid region, as shown in Fig. 1A.

Prediction of Response Surface Using Design Expert®

The response surfaces for each response variable were predicted by Design Expert®, based on the experimental data. The appropriate concentration of oleic acid (oil phase), Cremophor® RH40: ethanol (S/CoS) (emulsifier phase) and water (water phase) were varied according to the ME region obtained from the pseudo-ternary phase diagram using the sim-

plex lattice design, as shown in Fig. 1B. Eight model formulations of ME were prepared. The concentration of oleic acid, S/CoS and water were selected as causal factors. The characteristics of ME *e.g.*, droplet size, charge, conductivity, pH, viscosity, drug content, loading capacity and skin permeation flux were selected as the response variables. The response surface estimated by Design Expert® shows an obvious relationship between the causal factors and the response variables.¹⁷⁾

The effect of three ME components (oil, S/CoS, water) on the characteristics of KP-loaded ME is shown in Fig. 2. The response surface indicated that a decrease in S/CoS resulted

Fig. 4. The Corresponding Residual Plot between Run Number and Internally Studentized Residuals for Various Responses
(A) Droplet size, (B) charge, (C) conductivity, (D) pH, (E) viscosity, (F) drug content, (G) loading capacity and (H) skin permeation flux.

in a significant increase in droplet size, whereas an increase in S/CoS resulted in a significant increase in drug content and loading capacity. Moreover, an increase of water resulted in a significant increase in conductivity, viscosity and skin permeation flux. Although the ME compositions might affect the charge and pH of the ME formulation, this could not be significantly observed in this study. This might be because the ME components used in the ME formulation have the same intrinsic physicochemical properties. The charge of water and ethanol is zero, or weakly negative (less than -1) or weakly positive (less than $+1$) in the experimental condition, while oleic acid and Cremophor® RH40 are non-ionic substances. Therefore, the difference in the ratio of each component might not significantly affect its total net charge. Moreover, the individual pH of each ME component used was in the same range (pH 5–8); thus, varying the ratios of the ME components might not significantly affect its net pH.

The response surface of droplet size could be useful for preparing the ME with various droplet sizes. To prepare the ME with small droplet size, the percentage of water in the ME should be low; in contrast, a low level of S/CoS resulted in ME with large droplet size. The response surface of conductivity was useful for classifying the types of ME: water-in-oil (W/O) and oil-in-water (O/W). The high level of oleic acid might result in the low conductivity of ME. ME formulations with low conductivity ($<10 \mu\text{S}/\text{cm}$) was classified as W/O ME, whereas ME with conductivity higher than $10 \mu\text{S}/\text{cm}$ was recognized as O/W ME.¹⁸ This response surface could be used for predicting the types of all ME formulations in this region without the preparation of experimental ME. Moreover, the response surface of viscosity suggested that a high level of water significantly increases the viscosity of ME. The contact time of pharmaceutical formulations is directly affected by their viscosity, which could increase the therapeutic effect of the drug.^{19–21} The drug content and loading capacity of the ME formulation could increase by increasing the level of S/CoS from medium to high. Because Cremophor® RH40 and ethanol act as solubilizing agents, increasing S/CoS in the ME resulted in an increase in the amount of drug incorporated in the ME. The solubility of the drug in the ME depended on the solubilization capacity of the total ME system and on the ME structure. The structure of ME containing high surfactant levels (20–80%) enables to incorporate large amounts of both lipophilic and hydrophilic drugs in the ME formulation.⁸ The response surface of skin permeation flux indicated that a low level of oil, medium level of S/CoS and medium to high level of water ratio resulted in a significant increase in the flux of the ME formulations. Various studies suggested that an increase in some penetration enhancers in ME increased the skin permeability,^{22,23} but not always.^{18,24} This is because skin permeability appears to depend on other factors, e.g., specific formulation designs or drug concentration in the formulation (drug content).¹⁸ The partition coefficient of a drug between the oil and water phase of ME at the high level of water region may appropriate for drug permeability. The solubility of KP in oleic acid and S/CoS was higher than in water. The high level of water in the ME may not prevent KP diffusion to the skin surface,^{25,26} but KP in other ME regions at high levels of oleic acid or S/CoS might prefer to localize in the ME, instead of permeating into the skin.²⁷ Furthermore, water is natural and safe and thus most widely used as a pen-

etration enhancer to improve transdermal drug delivery, by increasing the water content of the tissue.²⁸ Generally, increasing skin hydration tends to increase the transdermal delivery of both hydrophilic and lipophilic drugs. High water levels in ME is not only to result in high efficacy but also to improve the safety of the ME formulation, simultaneously.

The linear correlation plot between the predicted and actual values for various response variables are shown in Fig. 3. The linear correlation coefficient is a statistical parameter used to define the linear relationship between the predicted and actual value, indicating the reliability and stability of the response surface. The linear correlation coefficient results for droplet size, viscosity, drug content, loading capacity and skin permeation flux were relatively high (0.8–0.9), whereas some of them (charge and pH) were quite low (0.6–0.7). The reliability of these results was confirmed by the corresponding residual plot between run number and internally studentized residuals for various response variables, as shown in Fig. 4. Based on the completely randomized run, the vertical spread of the internally studentized residuals was not out of the line from bottom-to-top, indicating that all points fall within the limits (at 95% confidence level). Our results indicated that the Design Expert® successfully estimated the response surface that exhibited the relationship between the ME composition and the ME characteristics.

Optimization of ME The ME formulation of KP was optimized based on the experimental data using Design Expert® Software. The response variables criteria for estimating the optimal ME were set to determine the ME formulation with good efficacy for transdermal drug delivery. The constraint of optimization was formulated to maximize skin permeation flux (maximize efficacy) and to minimize the concentration of S/CoS (minimize irritation) with equal importance and equal constraints in the triangular experimental region defined in Eqs. (1)–(4).

$X_1=19.22(\%)$, $X_2=61.70(\%)$ and $X_3=19.08(\%)$ were estimated as the optimal ME formulation. Figure 5 shows the three dimensional (3D) response surface plot of the desirability of the optimal ME, which is located near the centroid of the simplex lattice design. The desirability of the prediction was 0.8184. To evaluate the accuracy of the optimal ME formulation predicted by Design Expert®, the ME formulation composed of the optimal composition ratio was prepared and

Fig. 5. The Three Dimensional (3D) Response Surface Plot of Desirability at the Prediction

Table 2. The Response Variables of the Optimal ME

Response	Predicted optimal ME	Experimental optimal ME	Bias (%)
Y_1 (nm)	23.23	34.12 ± 3.39	-31.92
Y_2 (mV)	-0.37	-0.34 ± 0.05	8.82
Y_3 ($\mu\text{S}/\text{cm}$)	22.56	22.10 ± 1.49	2.08
Y_4	5.51	5.52 ± 0.01	-0.18
Y_5 (mPa·s)	38.68	39.51 ± 0.24	-2.10
Y_6 ($\mu\text{g}/\text{mL}$)	2462.81	2479.50 ± 58.05	-0.67
Y_7 (%)	27.28	27.14 ± 0.74	0.52
Y_8 ($\mu\text{g}/\text{cm}^2/\text{h}$)	1.70	1.68 ± 0.10	1.19

*Bias was calculated as $\{(\text{predicted value} - \text{experimental value}) / \text{experimental value}\} \times 100\%$.

investigated experimentally. The ideal characteristics estimated by Design Expert® and the experimental characteristics measured for the optimal ME are shown in Table 2. The results indicated that the characteristics of the optimal ME demonstrated by the experiment were very close to the predicted values estimated by the computer program. The reliability of this study was displayed as the percentage bias.²⁹ Moreover, this result suggested that at the minimum S/CoS level, the skin permeation flux of optimal ME was sufficiently high for the transdermal delivery of KP. A previous study reported that the skin permeation flux of meloxicam-loaded menthosomes³⁰ and ondansetron hydrogels³¹ predicted by computer program matched with the experiment flux measured by *in vitro* skin permeation studies.

Comparative Study of Optimal ME and Commercially Available Product Figure 6 shows the cumulative skin permeation per area and the skin permeation flux of 2.5% KP gel (commercial product) and 2.5% KP-loaded ME (optimal ME). The cumulative skin permeation per area of both formulations increased linearly after 3 h. The skin permeation flux of KP through the skin sample was determined as a slope of the linear portion of the plot. The result indicated that the skin permeation flux for the optimal ME was significantly higher than that for the gel ($p < 0.05$). The ME system may promote skin permeability by various mechanisms. According to the skin permeation profile, some ME droplets may interact with the stratum corneum; this localization effect may result in increased permeation of the drug across the skin due to the high local concentration at the skin surface or the site of action. The other possible mechanism is that the fusion of the ME droplet to the skin results in a potential penetration-enhancing effect. However, the drug in the continuous phase might also partition into the skin, and the drug will consequently be released from the droplet to the continuous phase, compensating for the permeated drug as the free drug mechanism.^{8,32,33} However, the mechanism of our ME in delivering the drug through the skin is not fully understood, and should be confirmed by further study.

The effect of gel and ME formulations on the stratum corneum of the skin sample was investigated by FT-IR and XRD. Moreover, the possible mechanisms of action of the ME were confirmed by FT-IR spectra and XRD pattern, as shown in Fig. 7. In the FT-IR spectra region between 2800 to 3000 cm^{-1} the methylene stretching (CH_2) bands were remarked as the most intensive spectra. The downward shifts in CH_2 symmetric stretching (*ca.* 2850 cm^{-1}) and asymmetric stretching (*ca.* 2920 cm^{-1}) frequencies reflected the changes in hydrocarbon chain fluidity and conformational order or alkyl chain

Fig. 6. The Skin Permeation Profile and Skin Permeation Flux of the Optimal ME and the Commercially Available Product

Each value represents the mean $\pm$ S.D. ($n=3$). * indicates statistical significance ($p < 0.05$). The white circle represents gel formulation and the fill circle represents optimal ME.

packing.³⁴ While the FT-IR spectra region between 1500 to 1700 cm^{-1} originate from amide I (mostly from C=O stretch (80%)) and amide II (mostly mixed N-H in plan bend (60%) and C-N stretch (40%)). The amide I band was split into doublet (1620 to 1690 cm^{-1}), but the amide II band was broad peak (1510 to 1560 cm^{-1}). The alteration in the shape of amide I and amide II was used as a marker for understanding the interaction and organization of hydrogen bonds in the polar interface.³⁵ In this study, the skin treated with gel formulations showed no difference in the FT-IR spectra, when compared to that from intact skin. In contrast, the CH_2 symmetric stretching and asymmetric stretching peaks of the skin treated with the ME formulations were shifted from 2850 cm^{-1} and 2920 to 2849 cm^{-1} and 2918 cm^{-1} , respectively. Additionally, the shape of the amide I and amide II bands was altered from 1620 to 1690 cm^{-1} to near regions 1640 to 1660 cm^{-1} and from 1510 to 1560 cm^{-1} to 1520 to 1550 cm^{-1} , respectively, as shown in Fig. 7A. Furthermore, the X-ray diffractograms at $2\theta=10$ and 20° indicated the hexagonal packing of alkyl chains of lipid in the skin.³⁶ The XRD pattern of the skin treated with gel formulations and ME formulations was not significantly different from the intact skin as shown in Fig. 7B. However, when the X-ray diffractograms of all skin patterns were overlaid (as shown in the enlarge picture), the X-ray diffractograms showed that the intensity of the XRD pattern of the skin treated with the ME formulations was slightly decreasing and increasing at $2\theta=10$ and 20° , respectively. These results suggested that ME formulations could deliver the drug through the skin, while the microstructure of the skin was not considerably disrupted. A previous study found that the effect of ethanol on the lipid

Fig. 7. Representative (A) FT-IR Spectra and (B) X-Ray Diffraction Patterns of (a) Intact Shed Snake Skin and (b) Shed Snake Skin Sample after Treatment with Gel Formulation (Commercial Product) and (c) ME Formulation

extraction in hairless mouse skin was obvious at 50% of ethanol or higher.³⁷⁾ In the present study, the ethanol used was approximately 30%; therefore, the extraction effect was not pronounced. These results indicated that the effect of ethanol in the ME was not the extraction of lipid (thus this was safe for skin) but the disruption of the organization of lipid structure for improving skin permeability; thus, the disruption of the organization of lipid structure may be affected by the other components of the ME.

CONCLUSION

Based on a simplex lattice statistical design, an optimal ME formulation for the transdermal delivery of KP was developed. The formulation was composed of oleic acid as the oil phase, Cremophor® RH40 as the surfactant, ethanol as the co-surfactant and water as the aqueous phase. Using Design Expert® Software, the response surfaces indicated a clear relationship between the causal factors and the response variables. The optimal ME was defined as the formulation with an appropriate skin permeation flux and minimum S/CoS. This

optimal formulation was estimated using a computer program, as well as determined experimentally. The experimental value of the skin permeation flux of the optimal ME was very close to the flux predicted by the computer program and was significantly greater than the commercial product. Considering the skin permeability of the optimal ME, we were successful in showing the feasibility of ME for the transdermal delivery of KP. Furthermore, the results indicated that the simple lattice design was beneficial as a powerful technique for the pharmaceutical development of ME for transdermal delivery.

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