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Development, Characterization and Skin Interaction of Capsaicin-Loaded Microemulsion-Based Nonionic Surfactant

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The aim of this study was to develop novel microemulsions (MEs) for the transdermal delivery of capsaicin. Microemulsion-based nonionic surfactants consisting of isopropyl myristate as the oil phase, various nonionic surfactants as the surfactant (S), various glycols or alcohol as the co-surfactant (CoS), and reverse osmosis water as the aqueous phase were formulated. Based on the optimal ME obtained from Design Expert®, MEs containing a fixed concentration of oil, water or surfactant were prepared while varying the amounts of the other two fractions. The results indicated that the skin permeation flux of low dose capsaicin (0.15% (w/w)) was significantly higher for the selected ME than the commercial product and capsaicin in ethanol (control) by approximately two- and four-fold, respectively. We successfully demonstrated the feasibility of the transdermal delivery of capsaicin-loaded ME using a low concentration of nonionic surfactant and ethanol. Moreover, the optimization using computer program helped to simplify the development of a pharmaceutical product.

Key words capsaicin; microemulsion-based nonionic surfactant; decyl glucoside; polyethylene glycol (PEG)-7 glyceryl cocoate; cocamide diethanolamine; Design Expert

Microemulsions (MEs) are transparent systems consisting of two immiscible phases that are stabilized by a surfactant or surfactant systems (a mixture of surfactant (S) and co-surfactant (CoS)). MEs are widely used in transdermal drug delivery because they offer several advantages, including simple preparation, a high loading capacity for hydrophilic and lipophilic drugs, thermodynamic stability and a high potential for enhanced skin permeation.¹⁾ Numerous studies have shown that MEs are more effective than other topical formulations, such as solutions, suspensions, gels, creams, hydrogels, micelles, liquid crystalline and liposomes.^{2–9)} Several mechanisms have been proposed to explain how MEs enhance drug penetration into the skin.^{10,11)} In recent years, MEs have continued to be designed and developed as transdermal delivery carriers for various poorly soluble drugs because they can improve the solubilization and bioavailability of lipophilic drugs and provide a large region per concentration ratio for mass transfer. A high ratio of ionic surfactants (which serve as the S phase) to ethanol (which serves as the CoS phase) is generally used to improve the potential for skin delivery by MEs. However, the high S/CoS ratio in the surfactant system used to generate MEs to ensure high skin permeability may also concurrently cause severe skin irritation. Consequently, ME-based nonionic surfactants with low S/CoS ratios need to be developed to avoid safety concerns related to the skin irritation. Software (Design Expert®) is a powerful tool to simplify the complex relationship between the concentration of surfactant systems and ME characteristics (both skin permeability and skin irritation).¹²⁾

Capsaicin (8-methyl *N*-vanillyl-6-nonenamide) is a natural alkaloid (capsaicinoid) and the major active spicy ingredient extracted from chili peppers. Capsaicin is a fat-soluble,

odorless, spicy, off-white solid with a melting point between 62–65°C and a molecular weight of 305.4 kDa. Capsaicin is notable because of its spiciness and ability to cause a burning sensation in mammalian tissues. Because capsaicin is not soluble in water, alcohol and other organic solvents are used to solubilize capsaicin in conventional topical preparations and sprays. Capsaicin is typically applied to treat various diseases, including musculoskeletal inflammation, rheumatism, post-hepatic neuralgia, lumbago and sciatica.¹³⁾ The mechanisms of action of capsaicin have been extensively studied over the past several decades. Capsaicin can release substance P from the afferent nociceptive neurons, and the resulting depletion of substance P desensitizes small afferent sensory neurons.¹⁴⁾ However, orally administered capsaicin undergoes significant first-pass metabolism in rats and mice,¹⁵⁾ and the spiciness of capsaicin limits its clinical applications. Several current studies have reported the topical and transdermal delivery of capsaicin using novel carriers, *e.g.*, niosomes or ME, but these formulations remain limited by their high concentration of capsaicin (0.75% (w/w))⁹⁾ and the use of high levels of ethanol or benzyl alcohol.^{13,16)} Therefore, MEs featuring improved co-surfactant systems may be useful for the transdermal delivery of low-dose capsaicin (0.15% (w/w)) using a low concentration of surfactants.

The aim of the present study was to develop ME systems consisting of novel surfactant systems for the transdermal delivery of capsaicin. Various surfactant systems were screened to be incorporated with capsaicin. The ME systems consisted of isopropyl myristate (IPM) as the oil phase; decyl glucoside (Plantacare® 2000), polyethylene glycol (PEG)-7 glyceryl cocoate (Cetiol® HE) or cocamide diethanolamine (Comperlan® KD) as the surfactant; propylene glycol (PG), ethanol, PEG

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400 or Cetiol® HE as the co-surfactant; and reverse osmosis (RO) water as the aqueous phase. Based on the optimal ME obtained from Design Expert®, the MEs containing a fixed concentration of oil, water or surfactant and various amounts of the other two fractions were prepared. The characteristics of MEs (e.g., droplet size, size distribution and electrical conductivity), capsaicin content and *in vitro* skin permeation were investigated to evaluate the use of these MEs in transdermal drug delivery. The skin permeability of the selected ME (SME), optimal ME (OME), commercial capsaicin product (COM) and capsaicin in ethanol (control) were compared, and their physical and chemical stability were under accelerated aging conditions for 3 months. Furthermore, the interaction of the selected ME and the ME's compositions with the stratum corneum was assessed using Fourier transform infrared (FT-IR) spectroscopy and X-ray diffraction (XRD).

MATERIALS AND METHODS

Materials Synthetic capsaicin (98%) was supplied by Hunan Huacheng Biotech, Inc. (Changsha, China). Isopropyl myristate (IPM) was purchased from Palm-Oleo (Klang) Sdn. Bhd. (KLK Oleo) (Selangor, Malaysia). Decyl glucoside (Plantacare® 2000), PEG-7 glyceryl cocoate (Cetiol® HE) and cocamide diethanolamine (Comperlan® KD) were obtained from BASF (Thai) Co., Ltd. (Bangkok, Thailand). Ethanol was supplied by Commercial Alcohols Inc. (Toronto, Canada). All other chemicals were commercially available and of analytical and high-performance liquid chromatography (HPLC) grade.

Screening of Surfactant Systems for Microemulsions

To select the appropriate pseudo-ternary phase diagrams using a novel surfactant system and consequently develop MEs for transdermal delivery, various surfactants and co-surfactants systems were evaluated. To screen surfactants, a ME consisting of IPM as the oil phase; decyl glucoside (Plantacare® 2000), PEG-7 glyceryl cocoate (Cetiol® HE) or cocamide diethanolamine (Comperlan® KD) as the surfactant; PG as the co-surfactant; and RO water as the water phase was prepared. The S/CoS weight ratio was 1:1. After selecting the appropriate surfactant, the co-surfactants were screened. MEs consisting of IPM as the oil phase; Comperlan® KD as the surfactant; PG, ethanol, PEG 400 or Cetiol® HE as the co-surfactant; and RO water as the water phase were prepared. The S/CoS weight ratio was also 1:1. The appropriate pseudo-ternary phase diagram was selected based on the largest area of the ME.

Construction of Pseudo-ternary Phase Diagrams The pseudo-ternary phase diagrams based on the oil phase, S/CoS and water phase were constructed using the water titration method. The S/CoS weight ratios were 1:1, 2:1, 3:1 and 4:1. For each phase diagram, the oil and S/CoS mixture was prepared at weight ratios of 5:95, 10:90, 20:80, 30:70, 40:60, 50:50, 60:40, 70:30, 80:20 and 90:10 in glass vials at ambient temperature (25°C). The mixture of oil and S/CoS was then continuously titrated with RO water in a stepwise manner under gentle magnetic stirring until the solution became turbid. The behaviors of the phase diagrams during the titration were visually observed. The percentages of the three components (oil, S/CoS and water) were recorded to plot the pseudo-ternary phase diagrams.

Optimization of Optimal ME The model MEs consisted

of three components, the oil phase, the surfactant system and the water phase. The model MEs were prepared according to the ME obtained from the simplex lattice design under the ME areas in the pseudo-ternary phase diagrams.¹²⁾ The concentrations of the oil phase (20–40%), the surfactant system (50–70%) and the water phase (10–30%) were varied to optimize the ME. The capsaicin concentration in all model MEs was fixed at 0.15% (w/w), which was the same concentration as that of a commercial product manufactured in the U.S.A. The optimal ME was defined as the ME exhibiting the maximum skin permeation flux of capsaicin with the minimum concentration of surfactant. The Design Expert® Software, Version 8, approval No. 009503 (Stat-Ease, Inc., MN, U.S.A.) was used to sketch the response surfaces of the skin permeation flux of the optimal ME. Once the predicted response surfaces of the optimal ME were obtained, the optimal ratio of the optimal ME was used to prepare capsaicin-loaded (CAP-loaded) model MEs.

Preparation of CAP-Loaded Microemulsions (MEs)

The MEs were prepared according to the optimal ME obtained from the simplex lattice design. The MEs were consisted of IPM as the oil phase, Comperlan® KD as the surfactant, ethanol as the co-surfactant and RO water as the aqueous phase. Based on the optimal ME, MEs containing a fixed concentration of oil, water or surfactant system and various amounts of the other fraction were prepared (Table 1). All ME components were accurately weighed and mixed well. Capsaicin was accurately weighed and thoroughly mixed and stirred with a magnetic stirrer. CAP-loaded MEs were stored in airtight containers at room temperature (25°C) prior to further investigations.

Characterization of Microemulsions

Droplet Size, Size Distribution and Conductivity Measurement

The average droplet size and size distribution of MEs were determined by dynamic light scattering (Zetasizer Nano ZS; Malvern Instrument, Worcestershire, U.K.). The samples were measured using a helium–neon laser beam at a wavelength of 632.8 nm. The measurement angles were monitored at 12.8, 175 at 25°C. One milliliter of ME was loaded into a disposable zeta cell. The electrical conductivity of the ME formulations was analyzed using a conductivity meter (S230 SevenCompact™, Mettler Toledo, Switzerland). All measurements were performed in triplicates at 25°C.

Capsaicin Content Measurement

Excess capsaicin was added to the ME formulations, and the capsaicin-incorporated ME were continuously shaken at 25±2°C for 48 h. To remove the excess capsaicin, the mixtures were centrifuged at 14000 rpm and 25°C for 30 min, and the supernatants were collected. The CAP-loaded MEs were extracted with methanol (1:1, v/v), filtered through a 0.22 µm nylon filter, and then analyzed by ultra-performance liquid chromatography (UPLC).

In Vitro Skin Permeation Studies

Full-thickness skin from 6- to 8-week-old female mice was used in this study. All skin samples were stored at –10°C and thawed immediately prior to use. The protocols used to generate animal experimental data were approved by the ethics committee for the use of laboratory animals, Faculty of Pharmacy, Silpakorn University (Protocol Number: 001/2014). After thawing, the mouse skin was cut and then immediately

Table 1. ME Compositions and ME Characteristics

Formulation factor	Code	ME compositions (%wt)			Size (nm)		Size distribution		Conductivity ($\mu\text{S}/\text{cm}$)	
		Oil	Surfactant (S/CoS)	Water	Blank ME	CAP loaded ME	Blank ME	CAP loaded ME	Blank ME	CAP loaded ME
Fixed S/CoS	OME ^{a)}	40	50 (37.5/12.5)	10	20.54 $\pm$ 2.38	25.66 $\pm$ 0.49	0.267 $\pm$ 0.052	0.324 $\pm$ 0.098	38.4 $\pm$ 0.4	39.2 $\pm$ 0.2
	ME1	30	50 (37.5/12.5)	20	16.69 $\pm$ 0.89	17.20 $\pm$ 0.64	0.223 $\pm$ 0.006	0.128 $\pm$ 0.036	107.4 $\pm$ 0.6	106.3 $\pm$ 0.2
	ME2	20	50 (37.5/12.5)	30	18.33 $\pm$ 0.08	14.52 $\pm$ 0.76	0.214 $\pm$ 0.010	0.226 $\pm$ 0.024	190.2 $\pm$ 0.3	201.0 $\pm$ 1.0
	ME3	10	50 (37.5/12.5)	40	18.55 $\pm$ 0.20	18.30 $\pm$ 1.35	0.171 $\pm$ 0.008	0.468 $\pm$ 0.092	270.7 $\pm$ 1.5	288.3 $\pm$ 0.6
Fixed oil	ME4	20	45 (33.7/11.3)	35	20.25 $\pm$ 0.01	13.81 $\pm$ 0.45	0.165 $\pm$ 0.006	0.192 $\pm$ 0.025	198.0 $\pm$ 1.8	210.0 $\pm$ 2.0
	ME2	20	50 (37.7/12.5)	30	18.33 $\pm$ 0.08	14.52 $\pm$ 0.76	0.214 $\pm$ 0.014	0.226 $\pm$ 0.024	190.2 $\pm$ 0.3	201.0 $\pm$ 1.0
	ME5	20	55 (41.3/13.7)	25	14.77 $\pm$ 0.25	25.55 $\pm$ 0.85	0.192 $\pm$ 0.014	0.178 $\pm$ 0.062	034.3 $\pm$ 0.2	134.6 $\pm$ 0.7
	ME6	20	65 (48.7/16.3)	15	22.60 $\pm$ 1.88	19.19 $\pm$ 5.32	0.256 $\pm$ 0.024	0.080 $\pm$ 0.010	67.7 $\pm$ 1.2	67.4 $\pm$ 0.2*
	ME7	20	70 (52.5/17.5)	10	19.53 $\pm$ 5.12	22.79 $\pm$ 2.83	0.221 $\pm$ 0.010	0.088 $\pm$ 0.020	40.8 $\pm$ 0.1	41.8 $\pm$ 0.3
Fixed water	ME7	20	70 (52.5/17.5)	10	19.53 $\pm$ 5.12	22.79 $\pm$ 2.83	0.221 $\pm$ 0.010	0.088 $\pm$ 0.020	40.8 $\pm$ 0.1	41.8 $\pm$ 0.3
	ME8	30	60 (45.0/15.0)	10	17.09 $\pm$ 2.63	15.76 $\pm$ 1.53	0.216 $\pm$ 0.012	0.248 $\pm$ 0.123	34.8 $\pm$ 0.2	36.1 $\pm$ 0.1
	OME ^{a)}	40	50 (37.7/12.5)	10	20.54 $\pm$ 2.38	25.66 $\pm$ 0.49	0.267 $\pm$ 0.052	0.324 $\pm$ 0.098	31.0 $\pm$ 0.4	29.2 $\pm$ 0.2
	ME9	50	40 (30.0/10.0)	10	22.52 $\pm$ 0.58	26.20 $\pm$ 1.51	0.356 $\pm$ 0.023	0.449 $\pm$ 0.048	24.3 $\pm$ 0.2	23.1 $\pm$ 0.2
	ME10 ^{b)}	60	30 (22.5/7.5)	10	22.28 $\pm$ 2.41	24.18 $\pm$ 1.71	0.373 $\pm$ 0.011	0.356 $\pm$ 0.095	19.5 $\pm$ 0.0	18.4 $\pm$ 0.1

a) Optimal ME; OPM. b) Selected ME.

mounted on the receptor compartment. The stratum corneum side of the skin sample faced upward into the donor compartment, and the other side was faced downward into the receptor compartment. A Franz diffusion cell with an available diffusion area of 2.3 cm² and a water jacket connected to a 32°C water bath under occlusive conditions was employed. The donor compartment was filled with 2 g of 0.15% (w/w) CAP-loaded ME formulations or tester formulation, *i.e.*, the commercial product (0.15% (w/w) capsaicin topical solution, manufactured in the U.S.A.) or 0.15% (w/w) capsaicin in ethanol. The receptor compartment was filled with 6.0 mL of the mixture of alcohol and phosphate buffer at pH 7.4 (1:1, v/v) under sink conditions. At each time-point (*i.e.*, 30 min, 1, 2, 3, 4, 5, 6, 7 and 8 h), 0.7 mL of receptor medium was withdrawn and replaced with an equal volume of fresh alcoholic phosphate buffer. All samples withdrawn from the receptor medium were stored at 4°C until analysis by UPLC. The cumulative amount of capsaicin that permeated through the skin was plotted as a function of time. The steady-state flux (*J*) was determined by calculating the slope of the linear portion of the plot.

The enhancement ratios of each formulation were derived by calculating the skin permeation flux of either the ME formulation or the commercial product against the control. The enhancement ratios were calculated using the following equation.

$$\text{Enhancement ratio (ER)} = \frac{J_{\text{ME formulation or commercial product}}}{J_{\text{control}}} \quad (1)$$

where $J_{\text{ME formulation or commercial product}}$ is the flux of CAP-loaded MEs or commercial product and J_{control} is the flux of capsaicin in ethanol. The ER was used to determine the skin permeation–enhancement ability of different CAP-loaded ME formulations or commercial products compared with capsaicin in ethanol.

Physical and Chemical Stability Evaluation

The CAP-loaded ME, commercial product of capsaicin and capsaicin in ethanol were stored at 40 $\pm$ 2°C/65 $\pm$ 5%RH for 3 months to investigate the physical and chemical stability under

accelerated aging conditions. Both the physical properties and chemical stability at 0, 1, 2 and 3 months, including the appearance, droplet size, size distribution, electrical conductivity and capsaicin remaining, were evaluated. The clarity and phase separation were assessed by visual inspection. The droplet size and size distribution were determined using the Zetasizer Nano ZS. The capsaicin that remained in the CAP-loaded ME formulations was analyzed by UPLC.

Capsaicin Analysis by UPLC

The UPLC system, which consisted of an ACQUITY UPLC Core system (Waters Corporation, MA, U.S.A.), binary solvent management and 2 switching solvent/DEGAS/ACQUITY TUV Detector and column heater, was used to analyze the capsaicin in all samples in this study. The UPLC column was ACQUITY UPLC BEC C18 analytical column, 2.1 mm $\times$ 100 mm, 1.7 μm (Waters®) (Waters Corporation). The mobile phase consisted of acetonitrile–1% acetic acid (40:60, v/v), which was delivered at a flow rate of 0.3 mL/min. The injection volume was 2.0 μL , and the UV detector was set at 280 nm and 30°C for all measurements.

The Microemulsion–Skin Interaction Studies

The stratum corneum is well recognized as an excellent skin barrier. Therefore, shed snake skin was used as model membrane in this study because of its similarity to human stratum corneum.¹⁷⁾ The skin samples were treated with the selected ME and the ME's compositions (IPM, Comperlan® KD, ethanol and RO water). Following the *in vitro* skin permeation studies, the skin sample was washed with water and blotted dry. The spectrum of the skin sample was recorded in the range of 500–4000 cm^{−1} using a FT-IR spectrophotometer (Nicolet 4700, Thermo Scientific, U.S.A.). An XRD (MiniFlex II, Rigaku Co., Tokyo, Japan) was used to investigate and confirm the possible mechanism underlying the effect of the ME and its composition on the skin permeability of capsaicin. The skin sample was prepared according to a procedure in a previous study.^{18,19)} Briefly, the same skin sample was cut into small pieces, approximately 2 cm $\times$ 2 cm, and attached to an aluminum well sample holder. XRD was used with Cu-K α , scanning from 2 θ =5–45°. The voltage and operating current

were 30 kV and 15 mA, respectively.

Data Analysis All experimental measurements were performed at least in triplicate. The data are expressed as the mean value $\pm$ standard deviation (S.D.). The data were statistically analyzed with a one-way ANOVA. A *p*-value less than 0.05 was considered to indicate significant differences.

RESULTS AND DISCUSSION

Screening of Surfactant Systems for Microemulsion

The surfactants selected for this study were decyl glucoside (Plantacare® 2000), PEG-7 glycerol cocoate (Cetiol® HE) and cocamide diethanolamine (Comperlan® KD). Plantacare® 2000, Cetiol® HE and Comperlan® KD are novel surfactants for both ME and transdermal delivery systems. They have been extensively used as surfactants, cleansing agents, emulsifiers or skin conditioners in cosmetics and various topical personal care products as they rarely cause allergic contact dermatitis.^{20,21)} and they are consequently believed to be mild surfactants based on their denaturing of proteins.^{22,23)} Moreover, Comperlan® KD can be applied to the ocular and nasal mucosa. Thus, it likely does not irritate the skin.²⁴⁾ The ME region is indicated by the shaded area of the pseudo-ternary phase diagram, and the crude emulsion area is represented by the non-shaded area, as shown in Fig. 1. A weight ratio of the surfactant system (S/CoS mixture) of 1:1 maximized the ME area for the S/CoS mixture consisting of Comperlan® KD:PG (Fig. 1C). The ME region decreased when the surfactant in the system was Cetiol® HE or Plantacare® 2000. These results revealed that MEs containing Comperlan® KD as the surfactant resulted in larger ME regions than MEs containing Cetiol® HE or Plantacare® 2000. Therefore, Comperlan® KD was selected as the surfactant in MEs in this study.

To screen co-surfactants, ME systems consisting of IPM as the oil phase, Comperlan® KD as the surfactant, RO water as the water phase, and various types of co-surfactants were prepared and investigated. The selected co-surfactants were PG, ethanol, PEG 400 and PEG-7 glycerol cocoate (Cetiol® HE), as shown in Fig. 2. A weight ratio of the surfactant system of 1:1 maximized the ME area for the S/CoS mixture containing Comperlan® KD:ethanol (Fig. 2B). The ME region decreased when the co-surfactant of the system was Cetiol® HE, PG or PEG 400. Because short-chain alcohols (ethanol) can decrease the interfacial tension between oil and water and adjust the flexibility of the interfacial membrane, ethanol was incorporated as the co-surfactant for pseudo-ternary phase diagrams.^{13,16)} The MEs containing ethanol as a co-surfactant exhibited larger ME regions than MEs containing Cetiol® HE, PG or PEG 400. Thus, the Comperlan® KD:ethanol surfactant system was selected for further study.

Construction of Pseudo-ternary Phase Diagrams Pseudo-ternary phase diagrams were constructed to determine the concentration range of components in the ME region. In this study, the pseudo-ternary phase diagrams for systems containing IPM as the oil phase, Comperlan® KD as the surfactant, ethanol as the co-surfactant and RO water as the water phase were assessed. The surfactant system (S/CoS mixture) was prepared at weight ratios of 1:1, 2:1, 3:1 and 4:1. RO water was drop-wise added to the mixture of surfactant/oil using the water titration method. The ME regions of each phase diagrams are illustrated in Fig. 3. The S/CoS ratio in the mix-

Fig. 1. Pseudo-ternary Phase Diagram of the ME Consisting of Isopropyl Myristate (IPM) as the Oil Phase, Reverse Osmosis (RO) Water as the Water Phase and Various Surfactant Systems (S/CoS)

(A) Plantacare® 2000:PG (1:1), (B) Cetiol® HE:PG (1:1) and (C) Comperlan® KD:PG (1:1).

ture (1:1, 2:1, 3:1 and 4:1) directly correlated with the area of the ME at S/CoS ratios of 1:1, 2:1 and 3:1. However, the area of the ME at a S/CoS ratio of 3:1 was selected because it featured the lowest ethanol ratio (Fig. 3C). Nano-sized formulations that did not exhibit phase separation (stored at the ambient temperature for 1 month) were prepared by varying the ratios of components. To study the effects of oil, the surfactant system and the water ratios on the characteristics and *in vitro* skin permeation of MEs for the transdermal delivery of capsaicin, the 10 ME formulations shown in Table 1 were prepared and evaluated.

Optimization of Optimal ME The CAP-loaded ME formulation was optimized using the Design Expert® software. The criteria for determining the optimal ME was a ME for-

Fig. 2. Pseudo-ternary Phase Diagram of the ME Consisting of Isopropyl Myristate (IPM) as the Oil Phase, Reverse Osmosis (RO) Water as the Water Phase, Comperlan® KD as the Surfactant and Various Types of Co-surfactants

(A) Propylene glycol (PG). (B) Ethanol. (C) Polyethylene glycol 400 (PEG 400). (D) Cetiol® HE.

mulation with excellent skin permeation flux (maximize efficacy) with minimum surfactant system (minimize irritation). Figure 4 illustrates the response surface plot of the skin permeation flux of the optimal ME, which is located at the apex of the simplex lattice design. The desirability of the estimation was 0.8257 from 1.0000. The optimization by Design Expert® suggested that the optimal ME should consist of 40% oil, 50% surfactant and 10% water. However, 50% of the surfactant system used in optimal ME was still high. Thus the optimal ratio of optimal ME was used as the basic model ME for the development of the novel microemulsion-based nonionic surfactant with low concentration of surfactant system in this study.

Droplet Size, Size Distribution and Conductivity Measurement

Droplet Size and Size Distribution

The sizes of blank-ME (ME formulation before capsaicin loading) and CAP-loaded ME (ME formulation after loading with 0.15% (w/w) capsaicin) droplets were on the order of nanometers, *i.e.*, 14.77–22.60 and 13.81–26.20 nm, respectively (Table 1). The incorporation of 0.15% (w/w) capsaicin into the ME formulation did not significantly affect the droplet size of MEs. A previous study suggested that a small droplet size increases the stability of the formulation by preventing sedimentation, flocculation and coalescence.²⁵⁾ The size distribution describes the homogeneity of the droplet size. The size distribution of blank MEs and CAP-loaded MEs ranged from 0.080–0.468, as shown in Table 1. All size distribution values

were smaller than 0.5, indicating that the droplet size exhibited moderate to high homogeneity.²⁶⁾

Electrical Conductivity

The electrical conductivity of blank ME formulations ranged from 19.52 and 270.7 $\mu\text{S}/\text{cm}$. The incorporation of capsaicin into the ME formulations slightly affected the electrical conductivity of the formulations, as shown in Table 1. The results showed that the ME components significantly affected the electrical conductivity of ME formulations. The electrical conductivity of ME formulations significantly increased as the ratio of water increased (OME-ME3). Reciprocally, when the ratio of water decreased (ME4–ME7), the electrical conductivity significantly decreased. Moreover, for a fixed ratio of water (ME8–ME10), the electrical conductivity of ME did not change. These results indicated that the electrical conductivity was affected by the water ratio in ME.¹⁰⁾ The water-in-oil ME exhibited very low conductivity ($<10 \mu\text{S}/\text{cm}$), whereas the conductivity of oil-in-water ME exceeded $10 \mu\text{S}/\text{cm}$.²⁷⁾

Capsaicin Content

The commercially available capsaicin product was used as a benchmark for the 0.15% (w/w) capsaicin topical solution in our study. All ME formulations contained 1.5 mg/mL capsaicin, and the capsaicin content was equal to that in the commercial product. The solubility of capsaicin in various conditions (*e.g.*, ME, oil, S/CoS, water) is shown in Fig. 5. The solubility of capsaicin in ME was $70\text{--}130 \text{ mg}/\text{mL}$. Although capsaicin could be dissolved in oil ($10.13 \pm 0.27 \text{ mg}/\text{mL}$) and S/CoS ($133.37 \pm 0.55 \text{ mg}/\text{mL}$), it was not soluble in water. Thus,

Fig. 3. Pseudo-ternary Phase Diagram of the ME Consisting of Isopropyl Myristate (IPM) as the Oil Phase, Reverse Osmosis (RO) Water as the Water Phase, and Various Ratios of Comperlan® KD/Ethanol as S/CoS

(A) 1:1, (B) 2:1, (C) 3:1 and (D) 4:1.

Fig. 4. The Response Surface Plot of the Skin Permeation Flux of the Optimal ME

0.15% (w/w) capsaicin could be completely incorporated in our ME formulations. As the oil ratio decreased (OME-ME3), the solubility of capsaicin decreased. For ME4–ME7, the solubility of capsaicin significantly increased with the S/CoS ratio. Conversely, when the S/CoS ratio decreased, the solubility of capsaicin in ME8–ME10 was significantly decreased. The IPM and S/CoS used in our ME have been widely used as permeation enhancers for various drug delivery systems^{3,13,27,28};

therefore, the ratio of oil and S/CoS in the ME may affect the solubility of capsaicin. The surfactant system (Comperlan® KD:ethanol (3:1)) and IPM primarily affected the capsaicin loading in ME formulations by influencing the solubility of capsaicin.

In Vitro Skin Permeation Studies

The *in vitro* skin permeation profiles of the CAP-loaded ME formulations (ME1–ME10), OME, commercial product (COM) and capsaicin in ethanol (control) were determined using mice skin. Permeation was plotted as a function of time (Fig. 6), and the skin permeation flux was determined by calculating the slope of the linear portion of the plot, as shown in Fig. 7A. The results suggested that all ME formulations significantly enhanced capsaicin penetration into mouse skin ($p < 0.05$) compared with the capsaicin in ethanol (control). In OME-ME3, the flux decreased as the oil ratio decreased, whereas in ME8–ME10, the flux tended to increase when the oil ratio was increased. Previous studies reported that IPM (oil phase) is an effective penetration enhancer that not only enhances skin penetration by acting as a fluidizer of intercellular lipids but also affects the lipid-rich phase in the stratum corneum, thereby reducing its barrier function.^{3,13,27,28} The flux of ME4–ME7 decreased as the S/CoS increased. These results indicated that the S/CoS ratio in the ME should be lower than 45% because high ratios of S/CoS in the ME simultaneously resulted in high efficacy and improved transepidermal water loss. Our results corroborate those of a previous

Fig. 5. Effect of the Ratios of Oil, Surfactants, and Water on the Solubility of Capsaicin under Various Conditions (Optimal ME; OME)

study showing that the type of S/CoS in the ME affected the skin permeation of capsaicin, whereas a high ratio of S/CoS did not.^{13,28)} The high S/CoS ratios may not have affected the skin permeation of ME but significantly affected the capsaicin content in ME formulations. Thus, the oil, water and S/CoS ratios significantly affected the skin permeation flux of ME (Fig. 7A). Possible mechanisms of the transdermal delivery of MEs have been discussed.^{10,11)} First, the high capsaicin loading capacity of MEs can provide a higher concentration gradient and consequently increase the driving force across the skin. Second, the ME components may improve penetration. Third, the ME components or penetration enhancers (IPM, ethanol, Comperlan KD[®]) can alter the microstructure of the skin, thereby increasing the permeability of the skin to the drug. Furthermore, the CAP-loaded ME droplet may be directly transferred through the stratum corneum.

The enhancement ratios of all CAP-loaded MEs, the commercial product and the control are shown in Fig. 7B. The results indicated that the enhancement ratios of ME10 significantly exceeded those of the control, commercial product, and optimal ME by 4.2-, 1.9- and 1.4-fold, respectively, at the same concentration of capsaicin (0.15% (w/w)). Therefore, a CAP-loaded ME containing a low dose of capsaicin (0.15% (w/w)) and low concentration of surfactant (such as ME10) may be attractive for clinical applications by circumventing the properties of capsaicin that limit its application, such as the spiciness associated with a high dose of capsaicin (0.75% (w/w)). The enhancement ratios of ME4 and ME10 were greater than those of the commercial product and control; thus, these two ME formulations were selected (selected ME) as appropriate ME formulations (Fig. 7B). In general, the efficacy and safety of the appropriate pharmaceutical formulation should be simultaneously considered in the development of all pharmaceuticals. The ME characteristics (efficacy and safety) are reportedly primarily due to the composition of each formulation.²⁹⁾ Therefore, the appropriate ME in our study were selected based on the concentration of the surfactant system. Although the S/CoS ratio of ME4 and ME10 significantly differed (as shown in Table 1), the enhancement ratios were not. Oil, S/CoS and water were the main ME components affecting the characteristics of the ME formulation. The surfactant was related to both the skin permeability (efficacy) and skin irritation (safety) of the ME formulation. Therefore, the S/CoS concentration in the ME should be minimized.²⁶⁾ The S/CoS ratio of ME10 was lower than that of ME4. Thus, skin irritation due to the surfactant should also be the lowest for ME10. A previous study reported that a low level of Tween 80/Span 20 (3:2) (35%) in the ME was an effective vehicle for the delivery of a capsaicin derivative.¹³⁾ Our study success-

Fig. 6. The *in Vitro* Skin Permeation Profiles of the CAP-Loaded ME Formulations (A) ME Fixed S/CoS, (B) ME Fixed Oil, (C) ME Fixed Water and (D) Comparison of CAP Formulation

Fig. 7. Effect of the Ratios of Oil, Surfactants, and Water on (A) the Skin Permeation Flux of Various CAP-Loaded ME Formulations and (B) the Enhancement Ratios of Various CAP-Loaded ME Formulations

* $p < 0.05$ compared with control (selected ME; S-ME, optimal ME; OME, commercial product; COM).

fully developed a potential capsaicin-loaded ME formulation for transdermal delivery using a Comperlan® KD (as surfactant) concentration of only 15% in the ME. Furthermore, the five-fold lower CAP-load in the ME (0.15% (w/w)) in our study may minimize local skin irritation compared with the CAP-loaded ME (0.75% (w/w)) examined in a previous study.⁹⁾ Moreover, both ME systems promoted the transdermal delivery of capsaicin. Thus, ME10 was selected as an appropriate ME formulation for further studies.

Stability Evaluation

Physical and Chemical Stability

The ME10 formulation was subjected to our stability study because of its efficacy and its components (as the selected ME (SEM)). The SME or ME10, OME, commercial product of capsaicin (COM) and capsaicin in ethanol (control) were kept at $40^{\circ}\text{C} \pm 2^{\circ}\text{C}/65\%\text{RH} \pm 5\%\text{RH}$ for 3 months. Both the physical properties and chemical stability of the formulations were evaluated at the start and after 1, 2 and 3 months. All formulations were physically stable (droplet size and size distribution) under these accelerated conditions. Phase separation, sedimentation or flocculation were not evident by visual observation. The droplet sizes of the ME formulation changed slightly from the start but remained within the nano-size range (*ca.* 20 to 25 nm). The size distribution of the ME formulation changed slightly from 0.35 to 0.25 under accelerated conditions, indicating that the droplet size of ME was mono-dispersed.²⁶⁾ The electrical conductivity of the ME formulation significantly increased after 3 months, but the electrical conductivities of the control and commercial product had also slightly changed. Our ME formulation did not exhibit phase inversion because the electrical conductivity was at least $10 \mu\text{S}/\text{cm}$.²⁷⁾ The remaining capsaicin content also slightly decreased under accelerated aging conditions (Fig. 8). The amount of capsaicin remaining in the selected ME slightly decreased after 2 and 3 months of storage, but *ca.* 90% of the capsaicin remained. The results indicated that the CAP-loaded MEs were stable for at

Fig. 8. The Capsaicin Remaining in the CAP-Loaded ME Formulations under Accelerated Aging Conditions at (■) the Start and after (▨) 1, (▤) 2 and (▥) 3 Months

Selected ME; SME, optimal ME; OME, commercial product; COM.

least 2 months of storage.^{29,30)}

The Microemulsion–Skin Interaction Studies

The interaction of skin with the ME formulations and their components (IPM, COM, EtOH and RO water) was investigated using FT-IR and XRD. The possible mechanism of action of the ME was confirmed by the FT-IR spectra and X-ray diffractograms, as shown in Fig. 9. The signals between 1500 and 1700 cm^{-1} of the FT-IR spectra are due to amides I and II bonds. The amide I signals were split into a doublet (1630 to 1680 cm^{-1}), whereas the amide II signal was broad peak (1500 to 1560 cm^{-1}). The modified shapes of the amides I and II signals were used to understand the interaction and organization of hydrogen bonds at the polar interface. The signals between 2800 and 3000 cm^{-1} due to the methylene stretching (CH_2) patterns were perceived as the most intensive signals. The shifts (downward or upward) in asymmetric stretching (*ca.* 2920 cm^{-1}) and CH_2 symmetric stretching (*ca.* 2850 cm^{-1}) frequencies were attributed to changes in the conformational order or alkyl chain packing and hydrocarbon chain fluidity.³¹⁾ In this study, the FT-IR spectra of skin treated with

Fig. 9. Representative (A) FT-IR Spectra and (B) X-Ray Diffractogram of the Shed Snake Skin Sample after Treatment with (a) Selected ME, (b) Isopropyl Myristate, (c) Comperlan KD®, (d) Ethanol, (e) RO Water

ME components (e.g., IPM, COM, EtOH and RO water) did not markedly differ from those of intact skin treated with RO water. In contrast, the CH₂ symmetric stretching and asymmetric stretching peaks of the skin treated with the selected ME shifted from 2850 to 2853 cm⁻¹ and 2920 to 2923 cm⁻¹, respectively. Furthermore, the amide I bands changed from 1620 to 1680 cm⁻¹ to approximately 1640 to 1660 cm⁻¹, whereas the shape of the amide II signal slightly at 1510 to 1560 cm⁻¹ slightly changed, as shown in Fig. 9A.

Additionally, the X-ray diffractograms at 2θ=10 and 20° demonstrated the hexagonal packing of the alkyl chains of lipids in the skin.³²⁾ The X-ray diffractograms of the skin treated with ME components did not significantly differ from those of the intact skin (as treated with RO water), whereas the skin treated with the selected ME exhibited notable differences. The X-ray diffractogram signals of the skin treated with the selected ME significantly decreased at 2θ=10 and 20°, as shown in Fig. 9B. These results revealed that the selected ME can cross the skin barrier because the microstructure of the stratum corneum was disrupted. Lipid is extracted at ethanol concentrations of at least 50%,³³⁾ but the ethanol concentration in the selected ME was 7.5%. Therefore, the selected ME can improve the skin permeability and is simultaneously safe for the skin.

CONCLUSION

In the present study, a novel ME system consisting of isopropyl myristate as the oil phase, Comperlan® KD as the sur-

factant, ethanol as the co-surfactant and reverse osmosis water as the aqueous phase was successfully developed. The ME containing S/CoS at a weight ratio of 3:1 was selected to be loaded with 0.15% capsaicin. The characterization showed that the ME components significantly affected the characteristics and *in vitro* skin permeation of CAP-loaded MEs. The skin permeation flux of the selected ME formulations was significantly higher than that of the commercial product of capsaicin and control by approximately 2- and 4-fold, respectively. The selected ME formulation showed good physical and chemical stability under storage conditions. Overall, these results suggested that our ME systems may be used for the transdermal delivery of capsaicin and could be developed and promoted as a commercially available product. Nevertheless, the skin irritation and skin toxicity of this selected ME formulation are necessary to be evaluated in further study.

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Conflict of Interest The authors declare no conflict of interest.

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