DEVELOPMENT OF CAPSAICIN MICROEMULSIONS FOR TRANSDERMAL DRUG DELIVERY

Wisuta Chairat, Praneet Opanasopit[1], Theerasak Rojanarata[1], Tanasait Ngawhirunpat[1, *]

[1]*Pharmaceutical Development of Green Innovations Group (PDGIG), Faculty of Pharmacy, Silpakorn University, Nakhon Pathom 73000, Thailand.*

KEYWORDS: Microemulsions, Capsaicin, Transdermal Drug Delivery

INTRODUCTION

Capsaicin (8-methyl-*N*-Vanillyl-6-nonenamide) is a natural alkaloid (capsaicinoid) extracted from chili peppers, which are plants belonging to the genus *Capsicum*. It is responsible for the hot pungent taste and causes a burning sensation to the mammalian tissue. The capsaicin produces analgesia by depleting substance P in small fiber nociceptor neurons on which Transient Receptor Potential action channel (subfamily V), type 1 (TRPV1) is predominantly located. Capsaicin binds to the vanilloid receptor TRPV1, which acts as a molecular integrator of chemical and physical painful stimuli[1]. Capsaicin is used in topical therapy for a variety of disorders such as rheumatism, lumbago and sciatica. However, capsaicin has strong pungency leading to limitation in clinical use. In addition, it has very poor aqueous solubility resulting in difficulties in the design of pharmaceutical formulation[2].

Microemulsion is defined as a dispersion sytem consisting of oil, surfactant, co-surfactant and aqueous phase, which has a single optically isotropic and thermodynamically stable liquid solution. Microemulsion can be used to deliver drugs to patients via several routes. The topical application of microemulsion has gained increasing interest, however the most difficult aspect of a transdermal delivery system is to overcome the barrier of stratum corneum against foreign substances. Due to its advantages, for example, easy making, good thermodynamically stability, enhanced drug solubility and enhanced skin permeation[3,4], microemulsion has become a potential transdermal drug delivery for both hydrophilic and hydrophobic drugs. The aim of the study is the development of a microemulsion formulation for the transdernal drug delivery of capsaicin using Isopropyl mistate (IPM) as oil phase, Cocamide DEA (Comperlan KD®) as surfactant, ethanol 95% as co-surfactant and RO water as aqeous phase.

MATERIALS AND METHODS

Material Capsaicin powder (synthetic 98%), Isopropyl myristate (IPM), cocamide DEA (Comperlan KD®) were purchased from Sigma-Aldrich (St Lious, USA). All other chemicals used in this study were analytical grade.

Construction of pseudo-ternary phase diagrams Pseudo-ternary phase diagrams were constructed using the water titration method at ambient temperature to obtain the concentration range of the components for microemulsion. The microemulsion system consisted of isopropyl myristate (IPM) as oil phase, cocamide DEA (Comperlan® KD) as surfactant, ethanol 95% as co-surfactant and RO water as water phase. The surfactant/co-surfactant weight ratio was 1:1, 2:1, 3:1 and 4:1. For each phase diagram, the mixtures of IPM and surfactant/co-surfactant were prepared at weight ratios of 5:95, 10:90, 20:80, 30:70, 40:60, 50:50, 60:40, 70:30, 80:20, 90:10, respectively. After the microemulsion regions in the phase diagram were identified, the microemulsion vehicles were selected and prepared at different component ratio in order to study the effect of oil, mixture of surfactant and water ratios to the characteristic of microemulsion.

Preparation of 0.075%w/w capsaicin-loaded microemulsion According to the microemulsion regions in the phase diagrams, the microemulsion formulations were selected as describe in Table 1. Microemulsion formulations were prepared by mixing surfactant mixture, IPM and water by weight ratio using magnetic stirrer at ambient temperature. Capsaicin was accurately weighed and adjusted to weight with the microemulsion formulations, following by stirring with magnetic stirrer at ambient temperature. The finally concentration of capsaicin-loaded microemulsion was 0.075 % (w/w). Drug content was determined by ultra-performance liquid chromatography (UPLC).

Microemulsion characterization The characteristics of microemulsion both before and after loading with 0.075% w/w capsaicin were studied as followed.

pH measurement The pH was determined using pH meter (Metler Toledo, Sevencompact S220). The measurements were performed in triplicate.

Electrical conductivity measurement The conductivity of the microemulsion formulations was determined by using conductivity meter (Metler Toledo, Sevencompact S230) at 25ºC. The measurements were performed in triplicate.

The average particle sizes measurement The particle sizes and polydispersity index were determined by dynamic light scattering (DLS) (Zetasizer Nano ZS, Malvern, UK) using a helium-neon gas laser with beam wavelength 632.8nm Microemlsion were loaded into 1 cm^3 disposable zeta cell. Measurement angles were monitored at 12.8° and 175° and fixed temperature at 25°C.

Determination of capsaicin solubility in microemulsion formulation The excess amount of the capsaicin was added in microemulsion formulations, and samples were continuously shaken for 48h at room temperature. Then the microemulsions were centrifuged (14000rpm, 30mins) to remove the undissolved drug. The supernatants were collected, diluted with methanol, and capsaicin concentration was determined by ultraviolet spectrophotometer at wavelength 280nm.

Table 1 Composition of selected microemulsion formulations.

Formulation No.	ME 1	ME 2	ME 3	ME 4	ME 5	ME 6	ME 7	ME 8	ME 9	ME 10	ME 11	ME 12	ME 13	ME 14	ME 15	ME 16	ME 17
Oil	35	30	25	15	10	10	20	30	40	50	60	70	20	20	20	20	20
S/Co-S (3:1)	50	50	50	50	50	80	70	60	50	40	30	20	45	50	55	60	65
Water	15	20	25	30	40	10	10	10	10	10	10	10	35	30	25	20	15

RESULTS AND DISCUSSION

Construction of pseudo-ternary phase diagram The phase diagrams composed of IPM as oil phase, cocamide DEA (comperlan KD®) as surfactant, ethanol 95% as co-surfactant and RO water as water phase. Surfactant/co-surfactant (S/Co-S) were prepared (w/w) in ratio of 1:1, 2:1, 3:1 and 4:1. RO water was added drop-wise to the surfactant/oil mixtures using the water titration method. Phase diagrams were constructed to visualize the microemulsion forming regions (Figure 1).

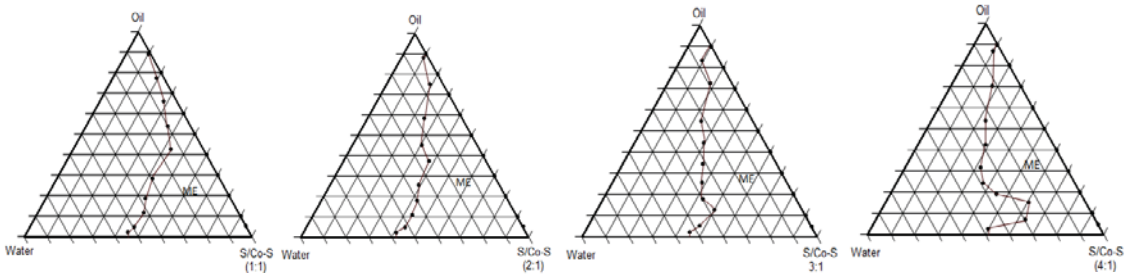

Figure 1 Pseudo-ternary phase diagrams of microemulsion composed of Isopropyl myristate (IPM), Cocamide DEA (Comperlan KD®), Ethanol 95% and RO water.

The previous studies reported that short chain alcohol could decrease interfacial tension between oil and water and adjust the flexibility of interfacial membrane[2,3], so ethanol 95% was incorporated as co-surfactant in this study. With the suitable ratio of the oil, mixture surfactant and water, the mixture was changed to be transparent microemulsion, and the rest of the regions represented the turbid and conventional emulsions based on visual inspection. The microemulsion regions were approximately 35-45% of the phase diagrams. As S/Co-S increased, the area of the microemulsion became enlarged, reaching a maximum at S/Co-S ratio of 3:1. The microemulsion vehicles of S/Co-S ratio 3:1 were selected and prepared at different component ratio. The surfactant mixture at 50%w/w was used in the formulation ME1-ME5, ME9 and ME14; the water was used at 10%w/w in formulation ME6-ME12, and the IPM at 20%w/w was used in formulation ME7, ME13-ME17.

Formulation of 0.075%w/w capsaicin-loaded microemulsion The capsaicin content in capsaicin- loading microemulsion formulations was between 0.0676%w/w and 0.0805%w/w.

Microemulsion characterization:

pH The pH of microemulsion system before loading capsaicin were between 9.84-10.48. The pH of 0.075%w/w capsaicin-loaded microemulsion were 9.82-10.56 (Table 2). The pH of the formulations tended to increase when the ratio of water/surfactant mixture decreased (Figure 2).

Electrical conductivity The electrical conductivity of the microemulsion formulations before loading capsaicin were between 0.11-271 μS/cm^{-1}(Table 2). Water in oil microemulsions represent very low specific conductivity (ca.10^{-9} – 10^{-7} Ω $^{-1}$cm^{-1})[6]. All formulations were o/w microemulsion except the ME12 formulation that was w/o microemulsion. Capsaicin-loaded in the formulation did not sig nificantly affect the conductivity of microemulsion. The conductivity of microemulsion formulations increased as the water content increased. As the water composition is constant and the oil amount increased in the formulations, the conductivity decreased (Figure 3).

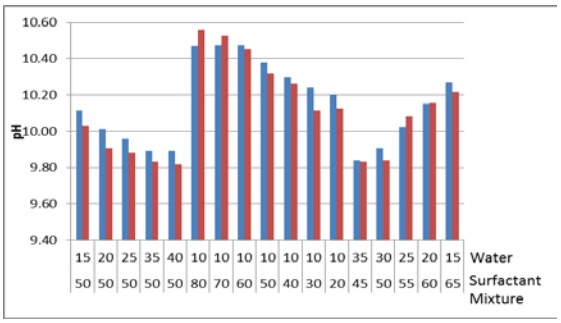

Figure 2 The pH of microemulsion formulations;
( ) Blank ME
( ) 0.075%w/w capsaicin-loaded ME

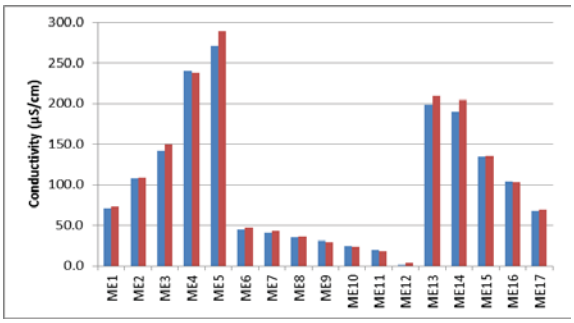

Figure 3 The electrical conductivity of microemulsion formulations;
( ) Blank ME
( ) 0.075%w/w capsaicin-loaded ME

Particle size and polydispersity The droplet sizes of the microemulsion formulations, both before and after loaded 0.075%w/w capsaicin, were in the nano-size range (11.57-257.2 nm). Small droplet sizes provided increased stability against sedimentation, flocculation and coalescence[7]. The 0.075%w/w capsaicin loaded in microemulsion did not significantly influence on the droplet size of microemulsion. The polydispersity value described the homogeneity of the droplet size. The polydispersity values of microemulsion formulations, before and after loading 0.075%w/w capsaicin, were 0.080-0.570. All polydispersity values (except blank ME11) were smaller than 0.5, indicating that the droplet size had high homogeneity[4].

Table 2 Characterization of microemulsion formulations.

Formulation NO.	pH		Conductivity (μs·cm^{-1})		Particle size (nm)		Polydispersity	
	Blank ME	Loaded ME	Blank ME	Loaded ME	Blank ME	Loaded ME	Blank ME	Loaded ME
ME1	10.12±0.02	10.03±0.01	70.9±0.60	72.8±0.79	22.56±1.41	17.46±0.20	0.268±0.061	0.224±0.009
ME2	10.01±0.01	9.91±0.01	107.4±0.56	108.3±0.21	16.69±0.89	15.31±0.10	0.223±0.006	0.171±0.003
ME3	9.96±0.01	9.88±0.02	142.3±0.30	149.7±1.39	16.82±0.03	17.27±0.15	0.197±0.008	0.131±0.007
ME4	9.89±0.01	9.83±0.01	240±0.00	237±3.06	20.17±0.91	18.33±0.17	0.243±0.029	0.115±0.014
ME5	9.89±0.01	9.82±0.01	271±1.53	289±1.53	18.55±0.20	17.49±0.04	0.171±0.008	0.112±0.003
ME6	10.47±0.01	10.56±0.01	44.3±0.20	47.4±0.40	12.72±1.77	11.57±0.29	0.259±0.072	0.195±0.041
ME7	10.48±0.01	10.53±0.01	40.8±0.10	42.9±0.10	19.53±5.12	19.19±5.32	0.221±0.010	0.080±0.010
ME8	10.48±0.01	10.45±0.01	34.8±0.20	36.1±0.10	17.09±2.63	22.79±2.83	0.115±0.030	0.088±0.020
ME9	10.38±0.01	10.32±0.01	31.0±0.30	29.4±0.20	20.54±2.38	17.57±3.96	0.267±0.052	0.090±0.016
ME10	10.30±0.01	10.26±0.01	24.3±0.20	23.1±0.20	22.52±0.58	15.96±0.23	0.356±0.023	0.085±0.027
ME11	10.24±0.01	10.12±0.01	19.52±0.03	18.45±0.05	105.52±12.48	24.18±1.71	0.570±0.148	0.356±0.095
ME12	10.20±0.01	10.13±0.01	0.11±0.03	3.69±0.16	257.2±3.13	51.29±0.43	0.245±0.015	0.445±0.008
ME13	9.84±0.01	9.83±0.01	198.0±1.82	209±3.06	20.25±0.01	23.27±0.39	0.165±0.006	0.105±0.016
ME14	9.91±0.01	9.84±0.00	190.2±0.26	205±0.58	18.33±0.08	17.81±0.18	0.214±0.010	0.113±0.004
ME15	10.02±0.01	10.08±0.01	134.3±0.21	134.8±0.76	14.77±0.25	16.11±1.13	0.192±0.014	0.248±0.042
ME16	10.15±0.01	10.16±0.01	103.2±0.12	102.8±0.20	18.89±2.49	12.27±0.52	0.161±0.007	0.225±0.015
ME17	10.27±0.01	10.22±0.01	67.7±1.19	69.4±0.20	22.60±1.88	17.23±2.07	0.256±0.024	0.164±0.029

Determination of capsaicin solubility in microemulsion formulation Capsaicin solubility of microemulsion formulations were between 94.60-231.83 mg/ml (9.46-23.18 %w/v). The capsaicin solubility increased by increasing the mixture of surfactant and decreasing the water phase (Figure 4).

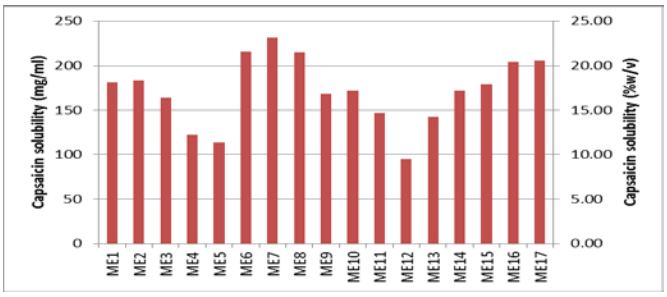

Figure 4 Capsaicin solubility in microemulsion formulation (mg/ml, %w/v).

CONCLUSION

The microemulsion systems for transdermal drug delivery of capsaicin were developed and characterized. The ME systems were composed of isopropy; myristate (IPM) as oil phase, cocamide DEA (Comperlan KD®) as surfactant, ethanol 95% as co-surfactant and RO water as aqueous phase. The mixture surfactant at the ratio of 3:1 provided the largest microemulsion area of the phase diagram. The characterization of microemulsion formulations showed that the oil phase, mixture of surfactant and aqueous phase affected the properties of the microemulsion. As the amount of the ratio of water/mixture surfactant decreased, the pH of microemulsion formulation tended to increase. When the water amount in aqueous phase was increased, the electrical conductivity was increased, however, as the amount of oil phase increased, the conductivity decreased. The droplet sizes of the microemulsion formulations were in nano-size range and the polydispersity value showed the homogeneity of droplet size. Capsaicin loaded in the formulation did not significantly influence the pH, electrical conductivity and droplet size of the microemulsions. The capsaicin solubility in the microemulsion formulations were increased by increasing the surfactant mixture and decreasing the aqueous phase.

ACKNOWLEDGMENTS

This study was supported by Pharmaceutical Development of Green Innovations Group (PDGIG), Faculty of Pharmacy, Silpakorn University, Nakhon Pathom 73000, Thailand.

REFERENCES

1. Hayman, M. and P. C. A. Kam "Capsaicin: A review of its pharmacology and clinical applications." Current Anaesthesia & Critical Care **19**(5-6): 338-343.
2. Huang, Y. B., Y. H. Lin, et al. (2008). "Transdermal delivery of capsaicin derivative-sodium nonivamide acetate using microemulsions as vehicles." International Journal of Pharmaceutics **349**(1–2): 206-211.
3. Z. Pengwei, G. Wenyuan, et.al. "In vitro evaluation of topical microemulsion of capsaicin free of surfactant" Biol. Pharm Bull. **31**(12): 2316-2320.
4. Üstündağ Okur, N., Ş. Apaydın, et al. (2011). "Evaluation of skin permeation and anti-inflammatory and analgesic effects of new naproxen microemulsion formulations." International Journal of Pharmaceutics **416**(1): 136-144.
5. Panomsuk, S. et al. "*In vitro* permeation of drugs through shed snake skin: Species difference." Electronic Proceeding of the 20th FAPA Congress, Bangkok, Thailand, 2004.
6. S.K. Mehta and Gurpreet Kaur (2011). "Microemulsions: Thermodynamic and Dynamic Properties." Thermodynamics, InTech, Available from: http://www.intechopen.com/books/thermodynamics/microemulsions-thermodynamic-and-dynamic-properties
7. Hathout, R. M., T. J. Woodman, et al. (2010). "Microemulsion formulations for the transdermal delivery of testosterone." European Journal of Pharmaceutical Sciences **40**(3): 188-196.