Relationship between formulation factors and physicochemical characteristics of microemulsions by response surface method

Sureewan Duangjit¹, Leilah Maria Mehr²,³, Mont Kumpugdee-Vollrath², Tanasait Ngawhirunpat³*  

Abstract

Introduction: Generally, the development of microemulsions (MEs) is based on trial and error methods. To obtain the appropriate MEs for transdermal drug delivery with simultaneously desirable physicochemical characteristics including high skin permeation, high stability and high safety. Therefore an optimization process based on computer program is an alternative method to apply in the development of MEs. Materials and Method: The model MEs were prepared according to the ME region obtained from the pseudo-ternary phase diagram. Using simplex lattice design as a model experimental design, the MEs were experimentally formulated and investigated. The ME systems were formulated with oleic acid, Cremophor® RH40, ethanol, water and meloxicam and their physicochemical characteristics (e.g., droplet size, charge, conductivity, pH, drug loading capacity) and skin permeation flux were evaluated. The ME’s compositions and the physicochemical characteristics were defined as formulation factors (X_n) and response variables (Y_n), respectively. The
relationship between formulation factor and physicochemical characteristics was investigated using Design Expert® program. **Results:** The response surfaces estimated by Design Expert® program exhibited obvious relationship between formulation factor and physicochemical characteristics. The formulation factor directly affected the physicochemical characteristics of MEs. The complicated relationship between formulation factor and physicochemical characteristics was clarified using the response surface method. **Conclusion:** The response surface method was beneficial for the development of MEs for transdermal drug delivery. Using the response surfaces, the physicochemical characteristics of MEs can be predicted without experimentally formulated and characterization.

**Keywords:** Response surface method, Formulation factor, Physicochemical characteristics, Microemulsion, Meloxicam

---

**Introduction**

Generally, the development of microemulsions (MEs) is based on trial and error methods. A high ratio of surfactants in MEs may relate to high skin permeation; but the skin irritation should be simultaneously considered. Nowadays, the complicated relationships between the formulation factors ($X_n$) of ME compositions and their physicochemical characteristics ($Y_n$) were not fully understood. To obtain the appropriate MEs for transdermal drug delivery with simultaneously desirable physicochemical characteristics including high skin permeation, high stability and high safety. Therefore an optimization process based on computer program is an alternative method to apply in the development of MEs (Duangjit et al. 2014).

Meloxicam (MX) is a non-steroidal anti-inflammatory drug (NSAID), used for rheumatoid arthritis, osteoarthritis and other joint diseases (Seedher and Bhatia 2005). The NSAIDs are very effective drug via oral route, but clinical applications are often limited because of the gastrointestinal side effects (e.g., peptic ulceration and irritation) (Ngawhirunpat et al. 2009). Although, MX appears to have partial cyclooxygenase-2 (COX-2) specificity, in practice it still has incidence of GI side effects at high doses on long term treatment (Duangjit et al. 2010, Duangjit et al. 014). Consequently, the development of MX as a transdermal drug delivery carriers indicate many challenges.

Thus, this study aims to discover the optimal ME for the transdermal delivery of MX that has sufficient skin permeation (maximum efficacy) with minimum skin irritation (minimum surfactant system) by using the computer program (Design-Expert®). The MX-loaded ME composed of oleic acid ($X_1$), water ($X_2$) and Cremophor® RH40/ethanol mixture ($X_3$) were prepared. The physicochemical characteristics, e.g., droplet size ($Y_1$), charge ($Y_2$), conductivity ($Y_3$), pH ($Y_4$), drug loading capacity ($Y_5$) and skin permeation flux ($Y_6$) were experimentally investigated and then the response surface was estimated by Design-Expert®.

**Materials and Method**

**Materials** Meloxicam (MX) and oleic acid was supplied by Sigma-Aldrich Production GmbH (Buchs, Switzerland). Polyethylene glycol (PEG)-hydrogenated castor oil (Cremophor® RH 40) was purchased from NS group (Bangkok, 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 compositions (oil phase, water phase and surfactant system) was constructed using water titration method (Ngawhirunpat et al. 2013). The surfactant system composed of the surfactant (S) and co-surfactant (CoS) mixture at weight ratios of 1:1, 2:1, 3:1 and 4:1 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. Each ratio of the S/CoS mixture and oil phase was continuously titrated with water phase. The manner of each ternary phase system during the titration was optically observed. The ternary phase system was calculated and plotted on triangular co-ordinates to construct the pseudo-ternary phase diagrams.

Design of experimental

The simplex lattice design was used as a model experimental design (Duangjit et al. 2014). Three compositions of the ME, including the oil phase ($X_1$), water phase ($X_2$) and surfactant system ($X_3$), were selected as formulation factors. The upper and lower limits of the levels of each composition were defined as Eqs. (1)–(4). The total concentration of the three compositions was adjusted to 100%.

\[
15 \leq X_1 \leq 40 \text{ (%)}
\]  
\[
5 \leq X_2 \leq 30 \text{ (%)}
\]  
\[
55 \leq X_3 \leq 80 \text{ (%)}
\]  
\[
X_1 + X_2 + X_3=100 \text{ (%)}
\]

Preparation of microemulsion

The MEs were formulated according to the formulation obtained from the simplex lattice design under the pseudo-ternary phase diagrams (Figure 2B). Eight model ME of MX-loaded ME composed of oleic acid as the oil phase, water as the water phase, Cremophor® RH 40 as the S absolute and ethanol as the CoS were prepared (Table 1). All components were accurately weighed and mixed thoroughly, and the resulting ME were stored in airtight containers at room temperature, prior to further evaluation.

Table 1 Experimental design and model MEs

<table>
<thead>
<tr>
<th>Form.</th>
<th>$X_1$ Oil (%)</th>
<th>$X_2$ Water (%)</th>
<th>$X_3$ S/CoS (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>15.0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>0.5</td>
<td>27.5</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>40.0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>0.5</td>
<td>27.5</td>
<td>0.5</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>15.0</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td>0</td>
<td>15.0</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>0.33</td>
<td>23.5</td>
<td>0.33</td>
</tr>
<tr>
<td>8</td>
<td>0.33</td>
<td>23.5</td>
<td>0.33</td>
</tr>
</tbody>
</table>

Droplet size, charge, conductivity and pH

The droplet size and charge of the MEs were measured by Zetasizer (Zetasizer Nano series, Malvern Instrument, U.K.). Thousand microliters of the MEs were loaded in disposable zeta cells. All samples were performed at 25°C, at least three independent samples were analyzed. The conductivity of the MEs was measured using conductivity meter (S230 SevenCompact™, Mettler Toledo, Switzerland). The pH was measured using pH meter (S220 SevenCompact™, Mettler Toledo, Switzerland). The samples were analyzed in triplicate at room temperature (25°C).

Drug loading capacity determination

The MX loading capacity in the MEs was determined by HPLC. The mixture of ME and methanol (1:1) was centrifuged at 10,000 rpm at 25°C for 10 min. The supernatant was filtered with the 0.45 μm nylon syringe filter and analyzed by HPLC. The MX loading capacity the MEs was calculated according to the calibration curve and/or the following equation:

\[
\text{loading capacity (\%)} = \left(\frac{D_t}{M_t}\right) \times 100
\]

where $D_t$ is the initial weight of MX in the MEs and $M_t$ is the total weight of MX.

Skin permeation study

The shed snake skin of *Naja kaouthia* as a kind gift from the Queen Saovabha Memorial Institute, Thai Red Cross Society, Bangkok,
Thailand, was used as a skin model in this study (Ngawhirunpat, Panomsuk et al. 2006). The skin model was cut and then immediately placed on a Franz diffusion cell. The diffusion cell with an available diffusion area of 2.01 cm$^2$ was employed. The donor chamber was filled with 1 g of model ME, 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 chamber. The concentration of MX in the aliquot was analyzed using HPLC.

**HPLC analysis** The HPLC 1100 system (Agilent 1100 Series HPLC System, Agilent Technologies, U.S.A.) was utilized to analyze the all samples. The analytical column was Eclipse XDB-C18 column (4.6×150 mm), and the mobile phase composed of 0.1% phosphoric acid and methanol (25:75). The flow rate was set at 1 mL/min, and the UV detector used was 254 nm. The calibration curve for MX was in the range of 1–50 μg/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%.

**Computer program** The simplex lattice experimental design was studied based on a three-composition system: oil phase ($X_1$), water phase ($X_2$) and surfactant system ($X_3$). The physicochemical characteristics of MEs, e.g., droplet size ($Y_1$), charge ($Y_2$), conductivity ($Y_3$), pH ($Y_4$), drug loading capacity ($Y_5$) and skin permeation flux ($Y_6$) were taken as the response variables. The response surfaces of all model MEs were estimated and sketched by Design-Expert® program, Version 8, Approved No. 009503 (Stat-Ease, Inc., MN, U.S.A.).

![Figure 1](image_url) **Figure 1** Pseudo-ternary phase diagram of the ME composed of oil, water and S/CoS: (A) 1:1, (B) 2:1, (C) 3:3 and (D) 4:1.
Results

As shown in Figure 1, the crude emulsion regions area is displayed by the area outside, and the ME region is indicated by the shaded area of the pseudo-ternary phase diagram. The largest ME area was observed in the S/CoS mixture of Cremophor® RH40: ethanol at weight ratio of 1:1. The ME region decreased when the ratio of S/CoS was 2:1, 3:1 and 4:1, respectively. Therefore, the ME system composed of Cremophor® RH40: ethanol (1:1) was selected as the acceptable pseudo-ternary phase diagram for further study (Figure 2A).

The response surfaces for each physicohemical characteristics were predicted by Design Expert®, based on the experiment. The appropriate upper and lower concentration of oleic acid (as oil phase), water (as water phase) and the mixture of Cremophor® RH40: ethanol (S/CoS) (as surfactant system) were varied according to the ME region obtained from the pseudo-ternary phase diagram using the simplex lattice design, as shown in Figure 2B.

To study the effects of ratios of oil, the water and the surfactant system on the physicochemical characteristics and skin permeation flux of ME for the transdermal delivery of MX, 8 formulations of ME were prepared and evaluated (Table 1). The percentage of oleic acid, S/CoS and water were selected as formulation factors. The physicochemical characteristics of ME e.g., droplet size, charge, conductivity, pH, drug 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 formulation factors and the response variables (Duangjit et al. 2014).

The effect of the oil phase, the water phase and the S/CoS system on the physicochemical characteristics of MX-loaded ME is shown in Figure 3. The response surface indicated that three compositions of ME (oil, water, S/CoS) directly affected the physicochemical characteristics of MEs.

The response surface suggested that an increase in S/CoS resulted in a significant decrease in droplet size and a significant increase in drug loading capacity. An increase of water resulted in a significant increase in conductivity and skin permeation flux. The composition of ME may affect the charge and pH of the ME, but we cannot be significantly observed in this study. This might be because the compositions of ME used in the MEs have the same intrinsic physicochemical properties, such as the charge of water and ethanol is zero, or weakly negative or positive (less than ±1) in the experimental condition, while oil phase (oleic acid) and surfactant system (Cremophor® RH40) are non-ionic compounds. Thus, 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); therefore, the different ratios of the ME compositions may not significantly affect its net pH.
Figure 3 The response surface of the physicochemical characteristics of MEs: (A) droplet size, (B) charge, (C) conductivity, (D) pH, (E) drug loading capacity and (F) skin permeation flux

The response surface of droplet size was useful for preparing the various droplet sizes of ME. To formulate the ME with large droplet size, the percentage of S/CoS in the ME should be low; in contrast, a low level of water resulted in ME with small droplet size. The high level of water resulted in the high conductivity of ME. MEs with low conductivity (< 10 µS/cm) was classified as oil-in-water (O/W) ME, whereas ME with conductivity higher than 10 µS/cm was recognized as water-in-oil (W/O) ME (Baroli et al. 2000). The response surface of conductivity was useful for indentifying the types of ME: O/W ME and W/O ME, this type of response surface could be used for predicting the types of all MEs in this diagram without the experimental preparation. The drug loading capacity of the ME was high, by increasing the ratio of S/CoS from medium to high. The S/CoS act as solubilizing agents in ME, thus increasing in ratio of S/CoS in the ME resulted in an increase in the amount of drug incorporated in the ME (Pappinen and Urtti 2006). The response surface of skin permeation flux indicated that a high level of water, medium level of S/CoS and low level of oil ratio resulted in a significant increase in the flux of the ME formulations. This is because skin permeability appears to depend on various factors, e.g., specific formulation designs or drug concentration in the formulation (drug content) (Baroli et al. 2000). Therefore, an increase in penetration enhancers in ME may not always increase in the skin permeation flux (Lee et al. 2003; Mei et al. 2003; Peltola et al. 2003). The large amount of water in ME is not only to result in high skin permeation efficacy but also to improve the safety of the ME formulation, simultaneously (Williams 2003). The response surface of skin permeation flux was useful for the development of
ME for transdermal delivery of MX. To design the ME with high potential as high skin permeation efficacy, the ratio of oil in the ME should be in the range of 15-30 (%), the ratio of water should be in the range of 15-30 (%) and the ratio of surfactant should be in the range of 66-70 (%). Although the surfactant used in ME (Cremophor® RH40) was safe as its non-ionic property, however the further study should be evaluated for the skin irritation study of all ME components (e.g., oleic acid, Cremophor® RH40 and ethanol).

**Conclusion**

The response surfaces suggested an obvious relationship between the formulation factors and the physicochemical characteristics. Considering the response surface of the skin permeation flux, we were successful in showing the feasibility of ME for the transdermal delivery of MX. Using the response surfaces, the physicochemical characteristics of MEs can be predicted without experimentally formulated and characterization.

**Acknowledgements**

The authors gratefully acknowledge the Thailand Research Funds through the Basic Research Grant (Grant No. 5680016), the Faculty of Pharmacy, Silpakorn University, Nakhon Pathom, Thailand, and the Faculty of Pharmaceutical Sciences, Ubon Ratchathani University, Ubon Ratchathani, Thailand for facilities and financial support.

**References**


