The Effect of Submicron Emulsion Systems on Transdermal Delivery of Kaempferol

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In this study, submicron emulsions have been employed as a carrier for the topical application of kaempferol. The effect of components of submicron emulsions on the physicochemical properties and permeation capability of drug were evaluated. In case of drug-loaded submicron emulsions, the cumulative amount over 12 h (Q12h), lag time and deposition in skin amount ranged from 13.0±3.4 to 236.1±21.2 µg/cm², 1.7 to 5.3 h, and 1.10 to 7.76 µg/cm², respectively, which indicated that the permeation parameters of kaempferol were markedly influenced by the component ratio. Kaempferol dispensed in isopropyl myristate was used as the control. The Q12h lag time and deposition amount in skin were 4.2±1.8 µg/cm², 6.0 h and 2.25±0.60 µg/cm², respectively. The data showed that used appropriate submicron emulsions as vehicle could significantly increase the Q12h and deposition amount in skin and shorten the lag time, demonstrating that submicron emulsions have a potent enhancement effect for kaempferol transdermal delivery.

Key words kaempferol; submicron emulsion; in vitro permeation study; permeation parameter; skin deposition amount

Kaempferol (C15H10O6, molecular weight 286.24, mp 276°C) is one of the phytoestrogens, and it is found in berries and Brassica and Allium species. It has been recognized to have antioxidant, anti-inflammatory, antiallergic, and anticancer properties.1–10) Regarding its anti-inflammatory properties, Wang et al.1) reported that kaempferol dose-dependently inhibited inducible nitric oxide synthase (iNOS) mRNA expression and prostaglandin E2 production, in part through a reducing inflammatory NF-kappaB transcription factor through nuclear factor-kappaB (NF-kappaB) signaling pathway. Park et al.4) reported that kaempferol could inhibit the activation of inflammatory NF-kappaB transcription factor through nuclear factor-inducing kinase (NIK)/IkappaB kinase (IKK) and mitogen-activated protein kinases (MAPKs) in aged rat kidney. Moreover, under daily treatment with 100 µL kaempferol in ethanol 100 µM for 10–30 d, the inflammatory cell infiltrates in the dermis and thickening of the epidermis in the burned area of skin were clearly ameliorated.11) These results demonstrated the efficacy of kaempferol in thermal burn-induced skin injuries. Oral administration of kaempferol results in very low bioavailability (2%) because it exhibits an extensive first-pass metabolism.12) Hence, kaempferol is a good candidate for topical application.

Submicron emulsion systems are fine oil-in-water or water-in-oil dispersions with small droplet sizes in the range of 100–600 nm.13) They offer many advantages over other traditional vehicles including ease of manufacturing, thermodynamic stability, enhanced drug solubilization and increased drug permeation rate.14) Submicron emulsions are typically composed of large amounts of surfactants and oil, which are irritants for skin. However, previous studies15–17) reported that submicron emulsion did not cause barrier perturbation of the skin, and that they can even decrease the skin irritation caused by drug. Moreover, the submicron emulsion system was demonstrated to be a potential vehicle for transdermal and topical delivery of hydrophilic and lipophilic drugs.16–19)

Hence, the submicron emulsion system was used as a vehicle for the poor water solubility molecule, kaempferol, in topical delivery through skin.

It is well known that the composition of formulation will significantly influence the physicochemical properties and permeability of drug. In this study, the pseudo-ternary phase diagrams were constructed to find out the concentration range of components for the existence range of submicron emulsions. Then kaempferol-loaded submicron emulsions with different water/oil/surfactant/cosurfactant ratios were prepared. The physicochemical properties, including viscosity and droplet size, and permeation parameters, including permeation rate, lag time and deposition amount in skin through rat skin, were determined to evaluate the effectiveness of submicron emulsions for topical application of kaempferol.

Experimental

Materials Kaempferol, quercetin, and sorbitan monooctanoate (Span 20) were purchased from Tokyo Chemical Industry (Tokyo, Japan). Polyoxyethylene sorbitan monooleate (Tween 80) was acquired from Showa Corporation (Saitama, Japan). Isopropyl myristate (IPM) and polyethylene glycols 400 (PEG) were purchased from Merck Chemicals (Darmstadt, Germany). All other chemicals and solvents were of analytical reagent grade.

Solubility Determination An excess of drug was placed in contact with 1 mL of selected solvent in sealed glass tubes. The tubes were shaken horizontally at 200 rpm for 24 h. The saturated solution was centrifuged and the supernatant was filtered through a 0.45 µm membrane. The concentration of drug in the filtrate was determined by HPLC after appropriate dilution with the selected solvents.

Construction of Phase Diagrams In order to obtain the concentration range of each component for the existence range of submicron emulsions, the pseudo-ternary phase diagrams were constructed using the water titration method at ambient temperature. Previous studies demonstrated that the emulsified effect was the best, and more rigid structure and minimum

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droplet size of submicron emulsion was obtained when the hydrophilic lipophilic balance (HLB) value of the selected surfactant was equal to that of the required HLB value of oil. The required HLB value of oil phase (IPM) was about 11, hence, the mixture surfactant of Tween 80/Span 20 at the ratio of 2/3 was used as surfactant. Ethanol and PEG 400 were used as cosurfactant. Cosurfactants dissolved in distilled water were used as aqueous phase. The mixtures of oil phase and mixed surfactant at certain weight ratios (1/9, 2/8, 3/7, 4/6, 5/5, 6/4, 7/3, 8/2, 9/1) were diluted with aqueous phase dropwise under moderate agitation until the mixture became clear at a certain point. The amounts of components were recorded in order to complete the pseudo-ternary phase diagrams.

Preparation of Drug-Loaded Submicron Emulsions

Based on the pseudo-ternary diagrams, appropriate concentration ranges of the components were chosen. The compositions of test submicron emulsions are shown in Table 2. The oil and aqueous phase were separately prepared. The oil phase consisted of oil and mixture surfactants of Tween 80 and Span 20, while the aqueous phase consisted of double-distilled water and ethanol. The aqueous phase was added to the oily phase and shaken in a vortex for 2 min at room temperature. Clear submicron emulsions were obtained. Kaempferol of 0.1% was dissolved in the final submicron emulsion formulations by horizontally shaken for 2 h at room temperature. No precipitate was observed after 7 d of storage.

Physicochemical Properties Determination

The viscosity of submicron emulsion was determined by a cone-and-plate viscometer (Brookfield, Model LVDV-II) (Middleboro, Massachusetts, U.S.A.). The drug-loaded submicron emulsions were placed in a cone-and-plate. The sample was sheared at a rate of 120 rpm and maintained at 37°C. Reading was carried out 60 s after measurement was made, when the level had stabilized. All experiments were repeated three times and averaged.

The average droplet sizes of kaempferol-loaded submicron emulsions were determined by photo correlation spectroscopy by laser light scattering (Zetasizer 3000HSA, Malvern Instruments Inc., Malvern, U.K.) using a helium-neon laser with a λ of 633 nm. Samples of 4 mL were loaded into 1 cm² cylindrical cuvettes and placed in a thermostated scattering chamber. Light scattering was monitored at a fixed angle of 90°.

In-Vitro Skin Permeation Study

The animal experimental protocol was reviewed and approved by the Institutional Animal Care and Use Committee of Kaohsiung Medical University in Kaohsiung city, Taiwan. The committee confirmed that the animal experiment followed the guidelines as set forth by the Guide for Laboratory Fact Lines and Care. The extent and rate of skin permeation of kaempferol from submicron emulsion were determined using a modified glass diffusion cell fitted with abdominal skin of excised Sprague Dawley rat (7–9 weeks). The hair of the abdominal region was removed with an electric hair clipper. The abdominal skin was mounted on the receptor compartment with the stratum corneum side facing upwards into the donor compartment and the dermal side facing downwards into the receptor compartment. The donor cell was filled with 1 g of kaempferol-loaded submicron emulsion and occluded by paraffin. The receptor compartment was filled with 20 mL of pH 7.4 phosphate-citrate buffer containing 20% ethanol and 40% PEG 400, and its temperature was maintained at 37±0.5°C by thermostatic water pump during the experiment. The effective diffusion area was 3.46 cm². Approximately 0.5 mL of the receptor medium was withdrawn at determined intervals and replaced immediately with an equal volume of receptor solution to maintain a constant volume. This dilution of the receiver content was taken into account when evaluating the penetration data. The sample withdrawn from the receptor compartment was then analyzed by HPLC. Each data point represents the average of three determinations.

At the end of the in vitro permeation experiment, the deposition amount of drug in skin was also determined by a homogenization method. After washing, the skin was cut into small pieces and placed into a glass tube containing 2 mL ethanol in an ice bath. The sample was homogenized at 17800 rpm for 2 min, and then shaken horizontally for 30 min. The resulting solution was centrifuged for 10 min at 3100×g at 4°C. The supernatant was determined by HPLC.

A HPLC equipped with a Hitachi model L-7100 pump, a Hitachi model L-4000H detector, a Spark Holland basic Marathon autosampler and Merck Lichrocart® C18 column (125×4 mm i.d., particle size 5 μm) was performed. The mobile phase consisted of mixture of 1% acetic acid/acetonitrile/methanol at ratio of 45/15/40. The flow rate was set at 1 mL/min. The UV detection was at 368 nm. Quercetin solution was used as internal standard. The limit of detection was 0.01 μg/mL (signal-to-noise ≥4). The concentration range of the kaempferol was found to have linearity from 0.2 to 50 μg/mL (r²=0.9998). The coefficient variation of accuracy and precision for intraday and interday assay were 8.4 and 10.0%, respectively.

Data Analysis

The cumulative amount of kaempferol penetration through rat skin was plotted as a function of time, and a linear regression analysis was used to calculate the skin permeation rate at steady-state (Jt, μg/cm²/h). Lag time (LT) was defined as the first time of detected drug.

Statistical analyses were performed using an analysis of variance (ANOVA) test. Subgroup comparisons were made using the Newman–Kuels multiple comparisons. A 0.05 level of probability was used as the level of significance.

Results and Discussion

Solubility Determination

The solubilities of kaempferol in selected solvent systems are shown in Table 1. The solubility of kaempferol in ethanol, PEG 400 and water was 13106.67±2606.09, 27696.11±2423.85 and 3.13±0.08 μg/mL, respectively. The solubility of kaempferol in aqueous solution was significantly lower than in other solvent, indicating that kaempferol is a hydrophobic compound.

The solubility was increased with increasing pH value of buffer from pH 6.0 to pH 8.0 (Table 1). The solubility of kaempferol in pH 7.4 phosphate buffer containing 20% ethanol and 40% PEG 400 was 13354.29 μg/mL; hence, the buffer system was used as receptor medium for in vitro permeation studies.

Construction of Pseudo-Ternary Diagrams

The construction of pseudo-ternary diagrams was used in order to obtain appropriate concentration ranges of components in the areas to form submicron emulsions. In this study, submicron emulsion was composed of IPM, mixture surfactant and distilled water containing ethanol or PEG 400 as cosurfactants. As shown in Fig. 1, the area region formed by ethanol was larger than that formed by PEG 400, which indicated that the...
The effect of type of cosurfactant had significant influence on the submicron emulsions formation. Previous studies reported that cosurfactant could reduce the interfacial tension of the surfactant layer in submicron emulsions, resulting in a more flexible and dynamic layer. Cosurfactant with appropriate chain length is good for formulation of microemulsions. In this study, the use of ethanol as cosurfactant resulted in easier formation of submicron emulsions when compared with using PEG 400. The result might be because the short chain of ethanol penetrated further into the interface. Hence, ethanol was used for subsequent experiment. As shown in Fig. 1, it was found that the area region of submicron emulsions became enlarged as the concentration of ethanol increased. In order to evaluate the effect of composition of formulation on the physiochemical properties and permeability of drug, five kinds of submicron emulsion formulations of different component ratios (such as formulation with a higher amount of surfactant or oil, and different ratios of surfactant/cosurfactant) were selected and prepared based on the construction of pseudo-ternary diagrams (Table 2).

**Physicochemical Properties**

The physicochemical parameters of kaempferol-loaded submicron emulsions were measured and these are listed in Table 2. The droplet size of microemulsion was small, with all the formulations having a mean size between 75.3 and 85.4 nm. The viscosity ranged from 12.9 to 24.2×10³ cps. It can be seen that the submicron emulsion containing a higher amount of surfactant showed higher viscosity.

**Permeation Study**

The permeation profiles of five kaempferol-loaded submicron emulsion formulations through rat skin are shown in Fig. 2. A steady increase of kaempferol in the receptor chambers with time was observed, demonstrating that the permeation profiles on submicron emulsions followed zero order release kinetics (r²>0.988). The permeation parameters including cumulative amount over 12 h (Q₁₂ʰ), lag time and deposition amount in skin of kaempferol in submicron emulsions are listed in Table 2. The Q₁₂ʰ ranged from 13.0±3.4 to 236.1±21.2 µg/cm², lag time ranged from 1.7 to 5.3 h, and deposition amount ranged from 1.10 to 7.76 µg/cm². The wide variation indicated that the permeation parameters of kaempferol from submicron emulsions were markedly influenced by the component ratio. Kaempferol of 0.5% dispensed in IPM was used as the control. The Q₁₂ʰ, lag time and deposition amount in skin were 4.2±1.8 µg/cm², 6.0 h and 2.25±0.60 µg/cm² for IPM solution, respectively. The result showed that the permeation and deposition amount of drug could be enhanced and the lag time shortened with the use of submicron emulsion as carrier. These results were in agreement with previous studies which reported that submicron emulsion system is a potential vehicle for transdermal and topical delivery of

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**Table 1. Solubility of Kaempferol in Selected Solvent**

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Solubility (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol</td>
<td>13106.67±26606.09*</td>
</tr>
<tr>
<td>PEG 400</td>
<td>27696.11±2423.85*</td>
</tr>
<tr>
<td>Water</td>
<td>3.13±0.08</td>
</tr>
<tr>
<td>Buffer pH 6.0</td>
<td>1.09±0.01*</td>
</tr>
<tr>
<td>Buffer pH 7.0</td>
<td>2.02±0.02*</td>
</tr>
<tr>
<td>Buffer pH 8.0</td>
<td>5.08±0.02*</td>
</tr>
<tr>
<td>Receptor medium</td>
<td>13354.29±1736.21*</td>
</tr>
</tbody>
</table>

a) Ethanol/PEG400/buffer pH 7.4=1/2/2. *p<0.05 compared with water.

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![Fig. 1. Pseudo-Ternary Diagrams of Submicron Emulsions Composed of Oil (Isopropyl Myristate), Surfactant (Tween80/Span20 at Ratio of 3/2) and Aqueous Phase Containing Different Kinds and Amounts of Cosurfactant (Ethanol, 30–50%)](image-url)

(A) 40% PEG 400, (B) 30%, ethanol, (C) 40% ethanol, (D) 50% ethanol. The dotted area is unstable submicron emulsion.
hydrophilic and hydrophobic drugs.\textsuperscript{15,16,18,19}

In comparison of the effect of composition of submicron emulsions on kaempferol permeation (Table 2 and Fig. 2), it was found that $Q_{12\text{h}}$ and lag time showed no change and the deposition amount was increased when the oil amount was increased from 5\% to 15\% (SM1 and SM2). With surfactant/cosurfactant at the ratio of 1 (SM1 and SM3), the $Q_{12\text{h}}$ and deposition amounts were significantly increased by decreasing the concentration of surfactant/cosurfactant (ME1 and ME3). A previous study reported that the progress of emulsification might be compromised by viscous liquid crystalline gel forming at the surfactant/water interface when submicron emulsion contained higher levels of surfactant, which results in the decrease of drug diffusion through the double layer submicron emulsion.\textsuperscript{20} In addition, the thermodynamic activity of drug in the submicron emulsion is a significant driving force for the release and permeation of the drug into skin.\textsuperscript{29} The thermodynamic driving force for release reflects the relative activities of the drug in different phases,\textsuperscript{30} since the drug can be released from the internal phase to external phase and then from the external phase to the skin; the relative activities may determine the skin permeation capability. The thermodynamic activity of the drug in submicron emulsion was decreased with an increase in the concentration of surfactants.\textsuperscript{31} In comparison of the effect of ratio of surfactant/cosurfactant (1/2, 1/1 and 2/1; SM3–SM5) submicron emulsions on kaempferol permeation, the $Q_{12\text{h}}$ was significantly increased from 38.4 to 236.1 $\mu$g/cm$^2$ and lag time was shortened from 5.3 to 1.7h by decreasing the ratio of surfactant/cosurfactant from 2/1 to 1/2. This result might be attributed to the interfacial tension of the interfacial tension of the surfactant file may decrease when higher concentration of cosurfactant is added, resulting in a more flexible and dynamic layer cosurfactant, which thus increases the partitioning and diffusion of drug into the stratum corneum.\textsuperscript{14,16,26}

However, the $Q_{12\text{h}}$ could be increased and the lag time shortened by using submicron emulsion as vehicle, indicating that submicron emulsion had a potent enhancement effect for transdermal delivery. These results may be due to the following: 1) increased thermodynamic activity of the drug may enhance its partitioning into the skin; 2) the components of submicron emulsion may act as permeation enhancers to decrease the diffusional barrier of the stratum corneum and increase the permeation capability of drug through skin; and 3) the hydration effect of submicron emulsion on the stratum corneum may facilitate the permeation of formulations.\textsuperscript{14,16,18,23,32}

### References


### Table 2. The Composition, Physicochemical Properties and Permeation Parameters of Kaempferol-Loaded Submicron Emulsions

<table>
<thead>
<tr>
<th></th>
<th>SM1</th>
<th>SM2</th>
<th>SM3</th>
<th>SM4</th>
<th>SM5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mixed surfactant</td>
<td>0.300</td>
<td>0.300</td>
<td>0.225</td>
<td>0.150</td>
<td>0.300</td>
</tr>
<tr>
<td>Ethanol</td>
<td>0.300</td>
<td>0.300</td>
<td>0.225</td>
<td>0.300</td>
<td>0.150</td>
</tr>
<tr>
<td>Water</td>
<td>0.350</td>
<td>0.250</td>
<td>0.500</td>
<td>0.500</td>
<td>0.500</td>
</tr>
<tr>
<td>IPM</td>
<td>0.050</td>
<td>0.150</td>
<td>0.050</td>
<td>0.050</td>
<td>0.050</td>
</tr>
<tr>
<td>Viscosity (×10$^3$ cps)</td>
<td>19.7±0.4</td>
<td>18.7±0.1</td>
<td>14.1±1.0</td>
<td>12.9±0.1</td>
<td>24.2±0.5</td>
</tr>
<tr>
<td>Droplet size (nm)</td>
<td>75.3±0.7</td>
<td>87.8±0.4</td>
<td>79.8±2.0</td>
<td>85.4±0.5</td>
<td>77.6±0.6</td>
</tr>
<tr>
<td>$Q_{12\text{h}}$ (µg/cm$^2$)</td>
<td>38.4±10.5</td>
<td>44.5±14.3</td>
<td>74.1±47.9</td>
<td>236.1±21.2</td>
<td>13.0±3.4</td>
</tr>
<tr>
<td>Lag time (h)</td>
<td>3.3±1.2</td>
<td>3.0±0.0</td>
<td>3.0±1.0</td>
<td>1.7±0.6</td>
<td>5.3±1.2</td>
</tr>
<tr>
<td>Residual in skin (µg/cm$^2$)</td>
<td>1.74±1.50</td>
<td>2.59±1.09</td>
<td>2.56±0.57</td>
<td>7.76±2.90</td>
<td>1.10±0.37</td>
</tr>
</tbody>
</table>

$q_{12\text{h}}$: cumulative amount at 12h.