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Physicochemical characterization and evaluation of a microemulsion system for oral delivery of cyclosporin A

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Abstract

The purpose of this study was to improve the solubility and enhance the bioavailability of poorly water-soluble cyclosporin A loaded in o/w microemulsion systems. Microemulsions with varying weight ratios of surfactant to cosurfactant were prepared using caprylic/capric triglyceride (Captex 355®) as an oil, polyoxyethylated castor oil (Cremophor EL®) as a surfactant, Transcutol® as a cosurfactant and saline. The area of o/w microemulsion region in pseudo-ternary phase diagram was increased with increasing ratio of Cremophor EL® to Trancutol®. The solubility of cyclosporin A in microemulsion systems reached the maximum with 2:1 mixture of Cremophor EL® and Trancutol®. The dispersion rate of oil–surfactant–cosurfactant mixture with varying ratios of Cremophor EL® to Trancutol® in aqueous media assuming the condition of gastric fluid decreased with the increase of Cremophor EL® to Trancutol® weight ratio. The droplet size of microemulsion without cyclosporin A was decreased with the increase of Cremophor EL® content. The droplet size increased on increasing the incorporation of cyclosporin A. The droplet size of cyclosporin A loaded microemulsion was minimized with microemulsions prepared with 2:1 mixture of surfactant to cosurfactant (Cremophor EL®:Transcutol®:Captex 355®, 10:5:4). The maximal blood concentration (C_{max}) of cyclosporin A and the area under the drug concentration-time curve (AUC) after oral administration of this cyclosporin A loaded microemulsion was 3.5 and 3.3 fold increased compared with Sandimmun®. No significant difference of C_{max} and AUC was observed between this microemulsion system and Sandimmun Neoral®. The absolute bioavailability of cyclosporin A loaded in this microemulsion system was increased about 3.3 and 1.25 fold compared with Sandimmun® and Sandimmun Neoral®. The enhanced bioavailability of cyclosporin A loaded in this microemulsion system might be due to the reduced droplet size of microemulsion systems. © 1998 Elsevier Science Ireland Ltd.

Keywords: Cyclosporin A; Microemulsion; Size; Solubility; Bioavailability

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1. Introduction

With the rapid progress in biotechnology, peptide drugs are becoming as important as therapeutic agents. A wide variety of peptides have been used as drugs including hormones, synthetic peptides, enzyme substrates and inhibitors (Lee, 1990). Although they are highly potent and specific in their physiological functions, most of them are difficult to administer orally because of the unique physicochemical properties of peptides. These properties include molecular size, poor solubility, short plasma half life, requirement for specialized mechanisms for membrane transport and susceptibility to enzymatic breakdown (Lee et al., 1991).

Many different approaches have been used to improve the oral absorption and enhance the bioavailability of peptide drugs. In recent years, enhanced bioavailability after oral administration has been reported by using microemulsion systems (Ritschel, 1991; Sarciaux et al., 1995). Microemulsions are thermodynamically stable, isotropically clear dispersions of two immiscible liquids such as oil and water, stabilized by an interfacial film of surfactant molecules (Eccleston, 1992). The advantages of microemulsions as drug delivery systems is the improvement of drug solubilization and protection against enzymatic hydrolysis, as well as the potential for enhanced absorption due to surfactant-induced permeability changes.

For selecting a suitable microemulsion system for peptide drug delivery, it is important to know about the physicochemical properties of the microemulsion system such as the drug solubility, the area of the microemulsion region on the phase diagram and the resulting size of microemulsion. The most popular peptide drug which has been studied for the optimization of microemulsion systems is cyclosporin A, which is an immunosuppressant for organ transplant patients. It is known that the absolute bioavailability of cyclosporin A is low due to the poor absorption which is related to the relatively high molecular weight, very high lipophilicity (log $p = 2.92$) (Taylor et al., 1993) and poor solubility in aqueous fluids (Ismailos et al., 1991). Several microemulsion systems loaded with cyclosporin A have been reported (Kahan, 1989; Thomson and Neild, 1991). However, there has been no report on the influence of surfactant to cosurfactant ratio on the physicochemical characteristics of microemulsion systems obtained by using polyoxyethylated castor oil (Cremophor EL®) as a surfactant, Transcutol® as a cosurfactant and caprylic/capric triglyceride (Captex 355®) as an oil.

In this study, microemulsions were prepared with varying weight ratio of compositions such as oil, surfactant–cosurfactant mixture and cyclosporin A, in addition the effects of composition on the physicochemical characteristics of each microemulsion systems were investigated for the optimization of microemulsion system. Particularly, the effect of weight ratio of surfactant to cosurfactant on the area of o/w microemulsion regions was studied, as well as the stabilization of microemulsions (particle size) and the solubilization of cyclosporin A. After optimization of the microemulsion system, the bioavailability of cyclosporin A loaded in the microemulsion systems was also compared with commercial cyclosporin A preparations (Sandimmun®, Sandimmun Neoral®) in rats.

2. Materials and methods

2.1. Materials

Cyclosporin A was supplied by Re-Yon (Seoul, Korea). Cremophor EL® and Transcutol® were obtained from Gattefosse (Saint-Priest Cedex, France). Captex 355® was supplied by Stepan company (Maywood, NJ). All other chemicals were of reagent grade and used without further purification.

2.2. Preparation of pseudo-ternary phase diagram

Surfactant was mixed with cosurfactant in fixed weight ratios (0.5:1, 1:1, 2:1 and 4:1). Aliquots of each surfactant–cosurfactant mixture (S_{mix}) were then mixed with oil and finally with aqueous phase (saline or 0.1 N HCl). Mixtures were gently shaken or mixed by vortexing and kept at ambi-

Table 1
Compositions (%, w/w) of the oil–surfactant–cosurfactant systems for preparing cyclosporin A microemulsions

Formula / Ingredients	I	II	III	IV	V
Captex 355® (oil)	4	4	4	4	4
Cremorphor EL® (surfactant)	5	7.5	10	11.25	12
Transcutol® (cosurfactant)	10	7.5	5	3.75	3

ent temperature (25°C) to get to equilibrium. The equilibrated samples were assessed visually and determined as being clear and transparent microemulsions, or crude emulsions or gels.

The physical states were represented on a pseudo-ternary phase diagram with one axis representing water, one representing oil and the third representing the S_{mix}. The influence of weight ratio of surfactant to cosurfactant on the area of o/w microemulsion region was investigated on the pseudo-ternary phase diagram.

2.3. Preparation of microemulsion containing cyclosporin A

Once the microemulsion region was identified, cyclosporin A varying from 15 to 40 mg was taken and put into the 0.2 ml of oil–S_{mix} mixture with varying ratios of surfactant to cosurfactant in fixed ratios of total S_{mix} and oil content as described in Table 1. The oil–S_{mix} mixture was then added to saline in a ratio of 19:81 (w/w), and o/w microemulsion containing cyclosporin A was produced by vortexing the resultant mixture at ambient temperature. Microemulsions were stored at 4°C and room temperature. Their physical stability was measured by periodic inspection over 3 months for the presence of macroscopic phase separation as shown by cloudiness or the formation of two distinct layers.

2.4. Solubility of cyclosporin A

The solubility of cyclosporin A was determined in each component of microemulsion systems such as surfactant, cosurfactant and oil, and also in the oil–surfactant mixture, oil–cosurfactant mixture and in the oil–S_{mix} mixture, as well as in the dispersed solutions of each mixture after addition of saline as an aqueous phase on each mixture, respectively. An excess amount of cyclosporin A was introduced to 2 ml of each dissolution medium and the mixture was stirred for 48 h at 25°C. Triplicated samples were centrifuged at $3000 \times g$ for 5 min (Starstedt MH2, Germany) to remove the excess amount of drug undissolved. Then, aliquots of supernatant were taken and the content of cyclosporin A was quantified by HPLC after dilution with methanol of analytical grade.

2.5. Partitioning of cyclosporin A between lipophilic and aqueous phase (C_o:C_w)

A total of 15 mg of cyclosporin A was added to 0.2 ml of oil–S_{mix} mixture with varying ratios of surfactant to cosurfactant as described in Table 1. Then 0.05 ml of this mixture was taken and 0.5 ml of saline was added to produce the cyclosporin A loaded microemulsion system. It was placed in Microcon™ (50 kD, Amicon, Beverly, MA) and centrifuged at $3000 \times g$ for 5 min at 25°C. The filtered aliquot was diluted with methanol of analytical grade and the content of cyclosporin A in aqueous phase was assayed by using HPLC. The concentration of cyclosporin A in the lipophilic phase was calculated by subtracting the content of cyclosporin A in aqueous phase from the initial loaded content of cyclosporin A.

2.6. Determination of cyclosporin A

The amount of cyclosporin A in various systems was quantified using a HPLC system consisting of solvent delivery system, C18 column (NUCLEOSIL®, Macherey-Nagel, Dúren; 10 μm particle diameter), column oven (Hitachi, model 655A-52, Japan), multiwavelength detector and

integrator. The mobile phase consisted of a mixture of distilled water and acetonitrile (40:60) filtered through 0.45 μm membrane filter and delivered at the flow rate of 1.7 ml/min (Hitachi, model L-6000 pump, Japan) at 75°C. Effluents were monitored at 210 nm using a ultraviolet (UV) detector (Hitachi, model L-4200, Japan).

2.7. Dispersability and particle size determination

A volume of 0.2 ml of oil–S_{mix} mixture, prepared as described in Table 1, was placed at bottom of a UV cell and 0.8 ml of 0.1 N HCl solution (pH 1.2, assuming the pH of gastric fluid) was gently added on the mixture as the water phase. the oil–S_{mix} mixture was gradually dispersed into the aqueous phase and the microemulsion formed spontaneously. The dispersability was observed by monitoring the turbidity change at 450 nm at 37°C.

A laser particle analyzer (model LPA-3000, 3100, Photal Otzuka Electronics, Japan) was used to measure the mean droplet size of various microemulsion systems. Systems without surfactant or cosurfactant were prepared and then the droplet size of each was measured to investigate the effect of surfactant and cosurfactant on the resultant droplet size, respectively. Then, with addition of both surfactant and cosurfactant with varying ratios, the droplet size of microemulsions with/without cyclosporin A was also measured in water and in 0.1 N HCl at 25 ± 1°C. The effect of the volume of the aqueous phase for producing the microemulsions was also investigated.

2.8. Animal studies

Male Sprague-Dawley rats weighing 300 ± 25 g were used. After anesthesia with diethylether during surgery, the femoral vein and artery was cannulated with 23 gauge-polyethylene cannula. All of the incisions were covered with wet cotton and the cannula was flushed with 0.1 ml of 3% EDTA normal saline solution to prevent the blood clotting. After recovering from anesthesia, Sandimmun®, Sandimmun Neoral® and pre-microemulsion concentrate (oil–surfactant–cosurfactant mixture in a ratio of 10:5:4, 20 mg of cyclosporin A/0.2 ml of oil–S_{mix} mixture) equivalent to 7 mg/kg of cyclosporin A was administered orally to rats using oral sonde, respectively. Each preparation was dispersed into 1 ml of normal saline by simply vortexing for 40 s immediately prior to dosing. On the other hand, the microemulsion preparation equivalent to 1 mg/kg of cyclosporin A was given intravenously to rats via the femoral vein.

Blood samples (200 μl) were withdrawn at designated time intervals. The concentration of cyclosporin A in whole blood was measured by radioimmunoassay (RIA) method using CYCLO-Trac SP-Whole Blood RIA kit (INCSTAR corporation-stillwater, MN), based on a double-antibody competitive-binding assay.

2.9. Pharmacokinetic data analysis

The non-compartmental pharmacokinetic parameters, including area under the drug concentration-time curve (AUC), were calculated using the trapezoidal rule (Gibaldi and Perrier, 1982). The maximal plasma concentration of drug (C_{max}) and the time to reach maximum plasma concentration (T_{max}) were also obtained from blood data. The data between different formulations were compared for statistical significance by the one-way analysis of variance (ANOVA). The statistical significance of means among different formulations was then compared by multiple range method of least significant difference. All results were expressed as mean $\pm$ S.D.

3. Results and discussions

For preparing microemulsion systems for oral delivery of cyclosporin A, Cremophor EL® was selected as a surfactant because non-ionic surfactants are known to be less affected by pH and ionic strength changes (Constantinides and Yiv, 1995). It was also considered that Cremophor EL® can enhance the intestinal permeability of drugs (Manoj et al., 1996). Transcutol®, purified diethylene glycol monoethyl ether by distillation, was used as a cosurfactant which is known to enhance the permeability of drugs. Captex 355®,

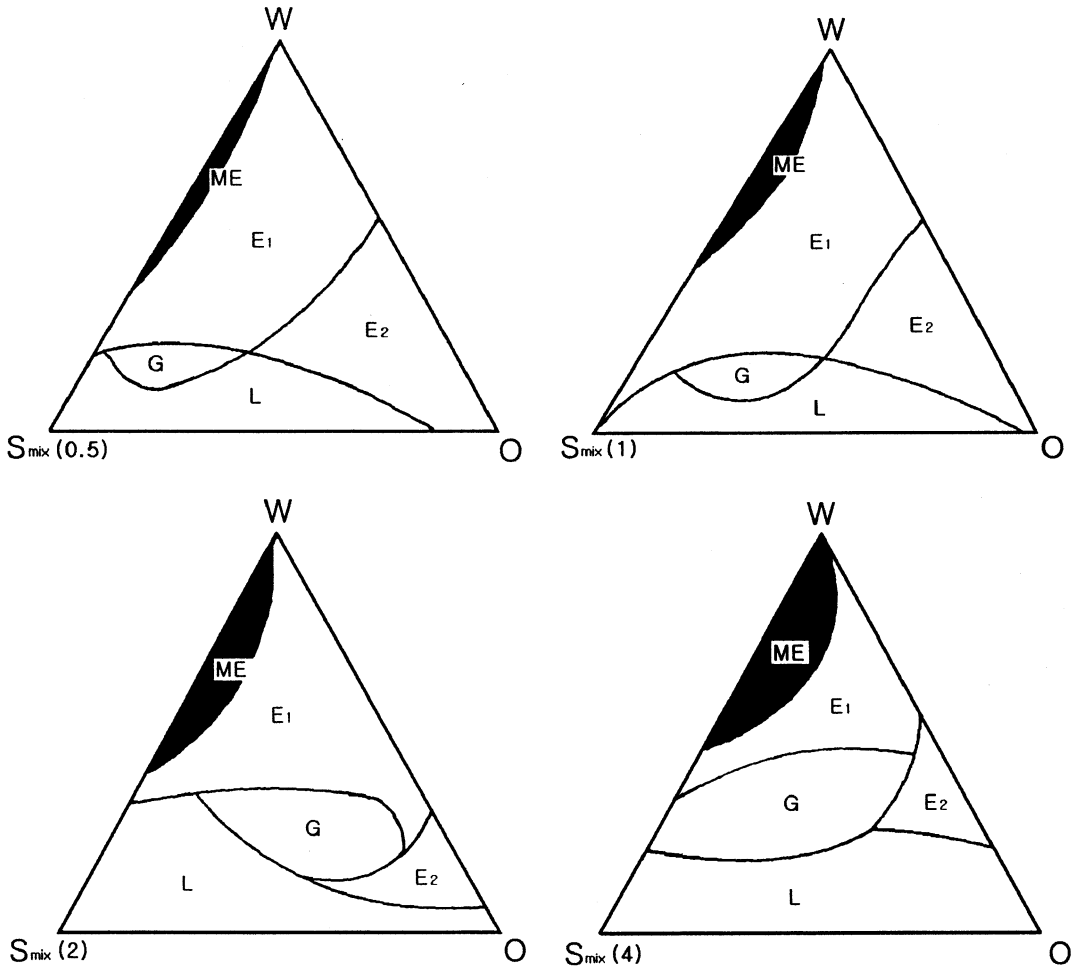

Fig. 1. Pseudo-ternary phase diagrams composed of Captex 355®, Cremophor EL®–Transcutol® mixture (S_{mix}) (Cremophor EL®:Transcutol® = 0.5:1, 1:1, 2:1 and 4:1) and water. G, gel; L, isotropic region; ME, single phase o/w microemulsion; E_1, crude emulsion; E_2, w/o emulsion region.

medium-chain glycerides derived from coconut oil, was selected as an oil because it is a food grade product and also known to improve the intestinal absorption of co-formulated drugs (Swenson, 1992).

3.1. Phase studies

Phase studies were done to investigate the effect of surfactant to cosurfactant ratio on the extent of stable o/w microemulsion region. The microemulsions in the present study formed spontaneously at ambient temperature when their components were brought into contact. It is advantageous for developing oral dosage forms of peptide drugs because easy formulation at ambient temperature is particularly advantageous for thermolabile drugs such as peptides (Constantinides et al., 1994).

The areas of microemulsion and isotropic regions increased with increasing ratio of surfactant to cosurfactant (Fig. 1). It indicates that the maximum proportions of oil incorporated in microemulsions increased significantly with increasing the ratio of surfactant to cosurfactant. A similar result was obtained from mineral oil/water solu-

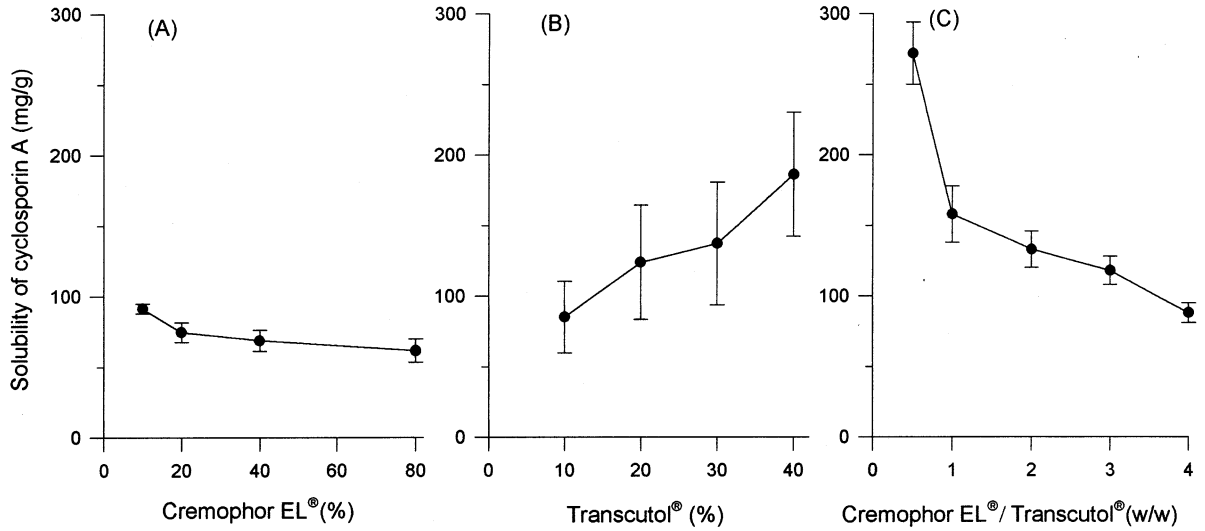

Fig. 2. (A) Effect of the content of Cremophor EL® on the solubility of cyclosporin A in a mixture of Cremophor EL® and Captex 355®; (B) effect of the content of Transcutol® on the solubility of cyclosporin A in a mixture of Transcutol® and Captex 355®; (C) effect of the weight ratio of Cremophor EL® to Transcutol® on the solubility of cyclosporin A in a mixture of Cremophor EL®–Transcutol®(S_{mix}) and Captex 355® (S_{mix}:Captex 355® = 15:4).

tion using Brij 96 as surfactant and glycerin, ethylene glycol and propylene glycol as cosurfactants (Kale and Allen, 1989). From a formulation viewpoint, the increased oil content in microemulsions may provide a greater opportunity for the solubilization of poorly water-soluble drugs. A phase study revealed that the addition of Cremophor EL®–Transcutol® mixture in a ratio greater than 2:1 can produce clear and transparent microemulsions in the subsequent study.

3.2. Solubility of cyclosporin A

The solubility of cyclosporin A in each component was 98.72 ± 7.75 mg/g in Captex 355®, 56.51 ± 4.90 mg/g in Cremophor EL® and very soluble in Transcutol®. The solubility of cyclosporin A in the oil–surfactant mixture slightly decreased with increasing the content of Cremophor EL® (Fig. 2A). In contrast, the solubility of cyclosporin A in the oil–cosurfactant mixture increased linearly with increasing the content of Transcutol® (Fig. 2B). Fig. 2B shows that the solubility of cyclosporin A in the oil–S_{mix} mixture with varying surfactant–cosurfactant ratio decreased with increasing the surfactant content. It indicates that the solubilization of cyclosporin A was greatly affected by the cosurfactant.

After adding the aqueous phase, the solubility of cyclosporin A in oil–surfactant–water system increased linearly with increasing Cremophor EL® (Fig. 3A). The solubility of cyclosporin A in oil–cosurfactant–water system also increased dramatically with more than 0.5% of Transcutol® (Fig. 3B). The solubility of cyclosporin A in a system containing all components for producing microemulsions (Fig. 3C) increased markedly compared with those of systems without surfactant (Fig. 3B) or cosurfactant (Fig. 3A) and it reached a maximum (21.95 ± 1.48 mg/ml) when the ratio of surfactant to cosurfactant of S_{mix} was 2:1 (Fig. 3C). The maximized solubilization is thought to be achieved by the formation of transparent microemulsion with small droplets. At a ratio greater than 2:1, although the mixtures formed microemulsions, the lower Transcutol® content in the microemulsion systems decreased the solubilizing capacity of microemulsion. In contrast, at a ratio less than 2:1, the clear microemulsion cannot be produced due to the insufficient amount of surfactant. The slight increase in solubility of cyclosporin A at the ratio of 0.5:1 is

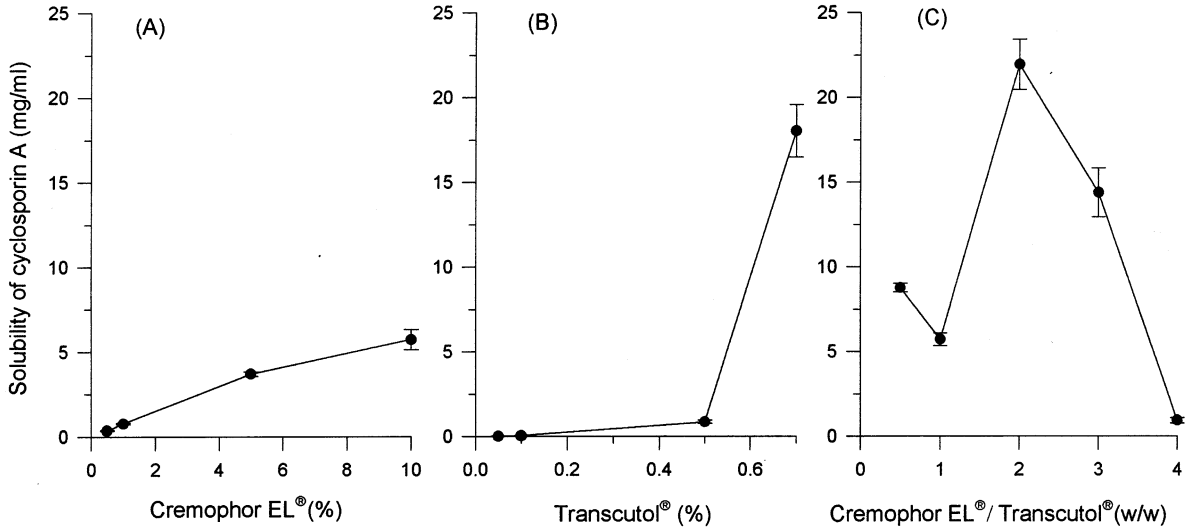

Fig. 3. (A) Effect of the content of Cremophor EL® on the solubility of cyclosporin A in a mixture of Cremophor EL®, Captex 355® and saline (Cremophor EL®–Captex 355®:saline = 19:81); (B) effect of the content of Transcutol® on the solubility of cyclosporin A in a mixture of Transcutol®, Captex 355® and saline (Transcutol®–Captex 355®:saline = 19:81); (C) effect of the weight ratio of Cremophor EL® to Transcutol® on the solubility of cyclosporin A in microemulsion systems obtained by adding saline to a mixture of Cremophor EL®–Transcutol® (S_{mix}) and Captex 355® (S_{mix}:Captex 355® = 15:4).

thought to be due to the fact that although the clear microemulsion cannot be formed, an excess amount of cosurfactant exists in the water phase and increased the solubility of cyclosporin A. This was confirmed by determining the partitioning of cyclosporin A between lipophilic and aqueous phases in the subsequent study (Fig. 4).

3.3. Partitioning of cyclosporin A between lipophilic and aqueous phase (C_o:C_w)

Partitioning using the aqueous and oil phases of corresponding microemulsion are known to be correlated to the observed oral bioavailability and/or in vitro permeability (Constantinides and Yiv, 1995).

The concentration ratio of cyclosporin A in the lipophilic phase to that in the aqueous phase (C_o:C_w) was greatly affected by the ratio of surfactant to cosurfactant of S_{mix} (Fig. 4). C_o:C_w was maximized when the microemulsion system was prepared with S_{mix} at 1:1 ratio of surfactant to cosurfactant. When the ratio was at above or below 1:1, the C_o:C_w ratio was reduced because the excess amount of surfactant or cosurfactant existed in the aqueous phase and contributed to increasing the concentration of cyclosporin A in this phase. However, if considering the absolute solubility of cyclosporin A in the lipophilic phase, the maximum concentration of cyclosporin A

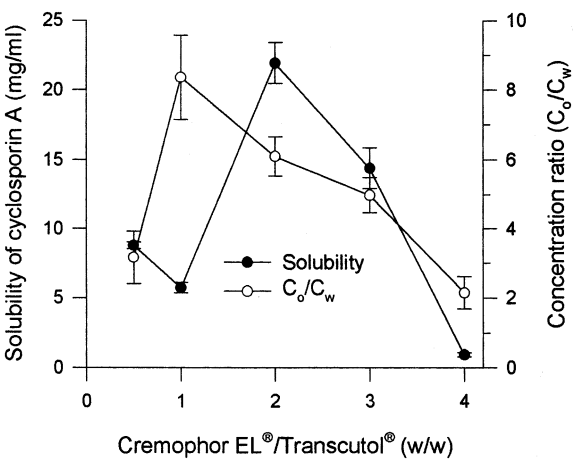

Fig. 4. Effect of the weight ratio of Cremophor EL® to Transcutol® on the concentration ratio of cyclosporin A in lipophilic phase and that in aqueous phase of microemulsion systems (C_o:C_w).

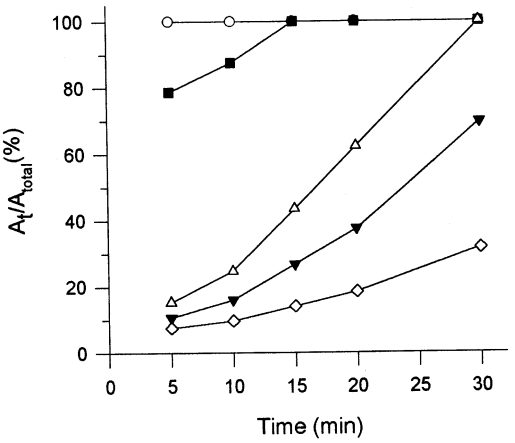

Fig. 5. Dispersion rate of mixture of Cremophor®–Transcutol® (S_{mix}) (Cremophor EL®:Transcutol® = 0.5:1 (○), 1:1 (■), 2:1 (△), 3:1 (▼) and 4:1 (◇)) and Captex 355® (S_{mix}:Captex 355® = 15:4) into aqueous media assuming the pH condition of gastric fluid.

could be achieved with microemulsions containing the 2:1 mixture of surfactant to cosurfactant.

3.4. Dispersion rate

To use the oil–S_{mix} mixture as a pre-microemulsion concentrate, it must be readily dispersed in the stomach to form a fine microemulsion (Shah et al., 1994). Thus, the dispersability of oil–S_{mix} mixtures prepared with varying weight ratios of surfactant to cosurfactant was compared in aqueous media, assuming the pH condition of gastric fluid. The dispersion occurred more slowly with increasing surfactant to cosurfactant ratio (Fig. 5). Too slow a dispersion rate of pre-microemulsion concentrate prepared with $S_{mix} > 3{:}1$ ratio of surfactant to cosurfactant might retard the absorption of drugs in the gastrointestinal tract, therefore the ratio of surfactant to cosurfactant must not exceed 2:1 when the oil–S_{mix} mixture is used as a pre-microemulsion concentrate.

3.5. Particle size

It is known that the particle size distribution is one of the most important characteristics of emulsion for the evaluation of its stability (Charman et al., 1992) and also in vivo fate of emulsion (Tarr and Yalkowsky, 1989).

At first, the effect of each component of microemulsion systems on the resultant droplet size was investigated. The surfactant content below 20% of the mixture did not affect the droplet size significantly (Fig. 6A). However, with increasing the cosurfactant content in the oil–cosurfactant–water system, the droplet size decreased linearly (Fig. 6B). The droplet size of microemulsions prepared with S_{mix} was markedly reduced compared with those prepared with surfactant or cosurfactant alone. It demonstrates that the small and stable microemulsion was formed by the addition of both. The droplet size of microemulsion decreased with increasing the surfactant to cosurfactant ratio and became constant at above 2:1 ratio of surfactant to cosurfactant (Fig. 6C). This result is in accordance with the report that the addition of surfactant to the microemulsion systems causes the interfacial film to condense and to be stable, while the addition of cosurfactant causes the film to expand (Kale and Allen, 1989). The smallest droplet size of microemulsion (22 nm) was obtained from a S_{mix} at more than 2:1 ratio of surfactant to cosurfactant, where it can produce clear and transparent microemulsions. However, as mentioned in the proceeding sections, S_{mix} at 3:1 or 4:1 ratio of surfactant to cosurfactant produced microemulsions with a reduced drug-solubility capacity and slow dispersion rate, which can be disadvantageous for use as an oral delivery system of cyclosporin A.

When cyclosporin A was added at concentrations from 15 to 40 mg/ml, the droplet size increased with increasing incorporation of cyclosporin A as shown in Fig. 7A. The size increased greatly when an excess amount of cyclosporin A was added up to its solubility limit in each system. This demonstrates that the proper amount of cyclosporin A should be used to keep the microemulsion stable. The increase of microemulsion size with higher cyclosporin A content may be compared with the incorporation of lipophilic drugs into emulsions which causes a certain degree of instability (Charman et al., 1992).

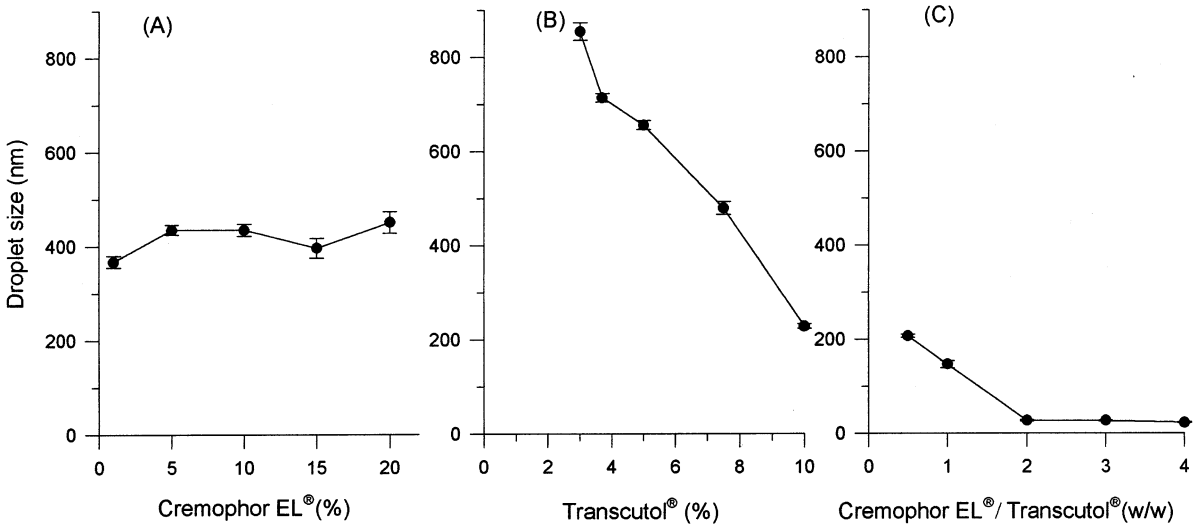

Fig. 6. (A) Effect of the content of Cremophor EL® in a mixture of Cremophor EL®, Captex 355®, and saline (Cremophor EL®–Captex 355®:saline = 19:81) on the resultant mean droplet size; (B) effect of the content of Transcutol® in a mixture of Captex 355®, Transcutol® and saline (Transcutol®–Captex 355®:saline = 19:81) on the resultant mean droplet size; (C) effect of the weight ratio of Cremophor EL® and Transcutol® in empty microemulsion obtained from a mixture of Cremophor®–Transcutol® (S_{mix}), Captex 355® and saline (S_{mix}:Captex 355®:saline = 15:4:81) on the resultant mean droplet size.

The droplet size of cyclosporin A loaded microemulsions was minimized with microemulsions prepared with 2:1 mixtures of surfactant and cosurfactant. The S_{mix} at a ratio of 3:1 and 4:1 of surfactant to cosurfactant formed bilayers instead of microemulsions at cyclosporin A concentration over 15 and 20 mg/ml, respectively.

Fig. 7B shows that the volume of aqueous phase added to the oil–S_{mix} mixture did not affect the droplet size of the microemulsion. Furthermore, the droplet size of microemulsions produced by adding 0.1 N HCl was almost identical with that produced by adding saline as an aqueous phase. It demonstrates that the oil–S_{mix} mixture as a pre-microemulsion concentrate containing cyclosporin A can be administered as a liquid dosage form after dilution with a water phase such as saline or it can also be administered as a capsule-filled solid dosage form, which forms microemulsions in the gastrointestinal tract after administration.

Taken together, from the investigations on the physicochemical characteristics of microemulsions with varying compositions, the microemulsion with the smallest droplet size and proper cyclosporin A solubility and fast dispersion rate was obtained with 10:5:4 mixture of Captex 355®:Cremophor EL®:Transcutol® loaded with 20 mg of cyclosporin A per 0.2 ml of oil–S_{mix} mixture. This system was stable (single and optically clear phase) for at least 3 months.

3.6. Pharmacokinetic analysis

Fig. 8A shows the blood concentration-time profiles of cyclosporin A after oral administration of various formulations to rats. The blood concentration-time profiles of cyclosporin A after intravenous administration of cyclosporin A microemulsion was also represented in Fig. 8B. The non-compartmental pharmacokinetic parameters in Table 2 were calculated based on the observed blood data. The C_{max}, T_{max}, and AUC of Sandimmun®, Sandimmun Neoral® and microemulsion in this study were 1.285, 2.859, 3.275 (μg/ml) and 2.333, 3.000, 3.667 (h) and 12.531, 33.171, 41.332 (μg/h per ml), respectively. The C_{max} of cyclosporin A loaded in the microemulsion system in the present study was markedly increased compared with Sandimmun®

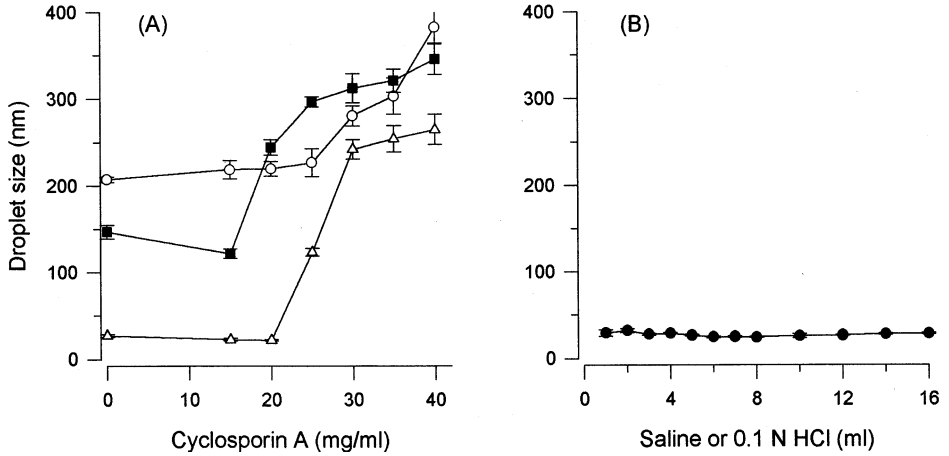

Fig. 7. (A) Effect of the weight ratio of Cremophor EL® to Transcutol® (Cremophor EL®:Transcutol® = 0.5:1 (○), 1:1 (■), 2:1 (△)) on the droplet size of microemulsion systems (S_{mix}:Captex 355®:saline = 15:4:81) loaded with cyclosporin A from 15 to 40 mg/ml; (B) effect of the volume of aqueous phase for producing microemulsions from a mixture of Cremophor®–Transcutol®, Captex 355® as a pre-microemulsion concentrate.

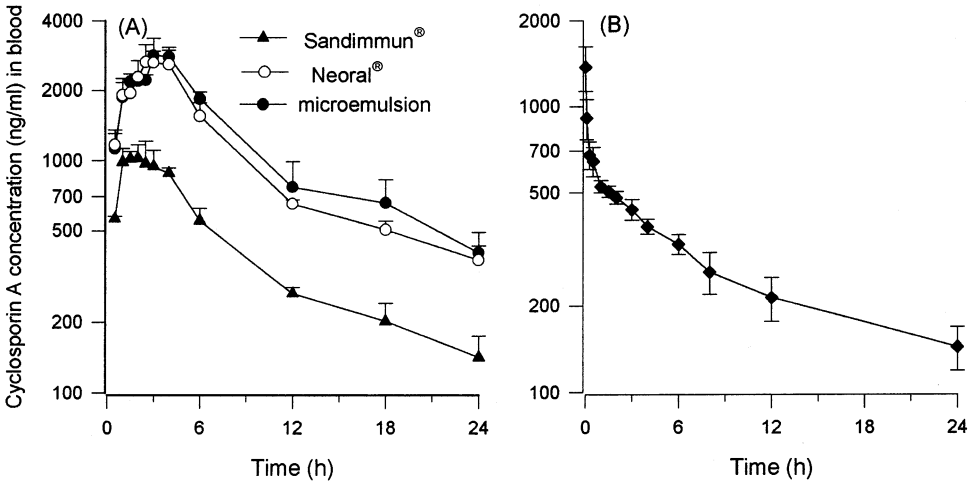

Fig. 8. (A) The blood concentration-time profile of cyclosporin A after oral administration of Sandimmun®, Sandimmun Neoral® and microemulsion system in the present study (Cremophor EL®:Transcutol®:Captex 355® = 10:5:4, loaded with 20 mg/ml of cyclosporin A) equivalent to 7 mg/kg as cyclosporin A to rats. Bars represent the standard deviation; (B) the blood concentration-time profile of cyclosporin A after intravenous administration of microemulsion system equivalent to 1 mg/kg as cyclosporin A to rats in the present study.

and was not significantly different compared with Sandimmun Neoral®. The AUC of cyclosporin A after the oral administration of microemulsion in this study was significantly increased ($p < 0.05$, 3.30 fold), when compared with Sandimmun®. However, no significant difference was found between the AUC of microemulsion and Sandimmun Neoral® ($p > 0.05$, 1.25 fold). There was no significant change in T_{max} ($p > 0.05$) among the three products.

The absolute bioavailability (F) of microemulsion optimized in this study increased about 3.30 and 1.25 fold compared with Sandimmun® and Sandimmun Neoral®. The bioavailability of cy-

Table 2
Analysis of noncompartmental pharmacokinetic parameters after oral administration of cyclosporin A products to rats

Parameters	Intravenous	Oral		
		Sandimmun®	Sandimmun Neoral®	Microemulsion
C_{max} (μg/ml)	—	1.285 ± 0.088	2.589 ± 0.322	3.275 ± 0.367[a]
T_{max} (h)	—	2.333 ± 0.441	3.000 ± 0.354	3.667 ± 0.333
AUC (μg/h per ml)	11.390 ± 0.193	12.531 ± 0.088	33.171 ± 5.534[a]	41.3221 ± 4.532[a]
Absolute bioavailability (F)[b]	—	0.157	0.416	0.518

[a] $p < 0.05$ by the student t-test when compared with Sandimmun®.
[b] $F = [(AUC_{oral})/(dose_{oral})] \div [(AUC_{i.v.})/(dose_{i.v.})]$.

closporin A incorporated in the optimized microemulsion did not show a significant difference when compared with Sandimmun Neoral® (0.416 vs 0.518) and 3.30 fold increased compared with Sandimmun® (0.157 vs 0.518). It is thought that this result supports the report that for lipophilic drugs and peptides, where absorption is dissolution rate limited, a strong correlation exists between the particle size of emulsions and bioavailability (Kararli et al., 1992). In this study the particle size of Sandimmun®, Sandimmun Neoral® and microemulsion was 864, 39 and 22 nm, respectively.

4. Conclusion

The surfactant to cosurfactant ratio of S_{mix} greatly affected the physicochemical characteristics of the resultant microemulsion systems obtained using Cremophor EL® as a surfactant, Transcutol® as a cosurfactant and Captex 355® as an oil. The stable microemulsion with its high solubility of cyclosporin A, small droplet size and fast dispersion rate was obtained from a mixture composed of 10:5:4 ratio of Cremophor EL®:Transcutol®:Captex 355®.

The enhancement of bioavailability of cyclosporin A by using o/w microemulsion optimized in this study is thought to be due to the combination of factors including the drug solubilization effect and the increase of drug permeability through the intestinal membrane. In other words, the bioavailability of drugs loaded in microemulsions was dependent on the physicochemical properties of drug and o/w microemulsion.

This system might be applicable to formulate liquid and solid dosage forms of cyclosporin A for enhancing its bioavailability after oral administration. This formulation approach can also help to improve the oral bioavailability of other poorly soluble peptide drugs as is the case for cyclosporin A.

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