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Preparation and physicochemical characterization of phase inverted water/oil microemulsion containing cyclosporin A

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Abstract

To improve the solubility of cyclosporin A, microemulsion systems containing cyclosporin A was developed. The system was optimized by evaluating the solubility of cyclosporin A and the phase-inversion efficiency after the preparation of microemulsions with varying compositions of ethyl oleate, cyclosporin A and surfactant-cosurfactant mixtures (Polypropyleneglycol–20 methyl glucose ether (Glucam®P-20, GP-20), Poloxamer 123 (Pluronic®43, PL43)). Microemulsion systems in this study could markedly improve the solubility of cyclosporin A and the most stable and homogeneous system was obtained with microemulsion containing 15% (w/w) of cyclosporin A prepared with 60% (w/w) of surfactant-cosurfactant mixture (PL43:GP-20 = 1:1). © 1997 Elsevier Science B.V.

Keywords: Cyclosporin A; Phase inverted w/o microemulsion; Phase study; Solubility; Dispersability; Particle size

Cyclosporin A, as an immuno-suppresant, has been widely used for the inhibition of graft rejection in organ transplant recipients. However, its absolute bioavailability and pharmacokinetics after oral administration are markedly variable due to the poor absorption (Kovarik et al., 1994) which is somewhat related to the very high lipophilicity and poor solubility in aqueous fluids.

In recent years, many attempts have been made to develop microemulsion systems for solubilizing poorly water-soluble drugs and thus enhancing its absorption in vivo. In this study, phase-inverted water/oil microemulsion systems containing cyclosporin A were developed to improve the solubility of cycloporin A. The system was optimized by investigating its physicochemical characteristics after the preparation of microemulsions with varying compositions such as oil, surfactant-cosurfacant mixture and cyclosporin A.

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Polypropyleneglycol-20-methyl glucose ether (GLUCAM® P-20, GP-20) was blended with Poloxamer 123 (Pluronic®L43, PL43) in fixed weight ratios (PL43:GP-20 = 1:1, 1:4). Aliquots of each surfactant-cosurfactant mixtures were then mixed with ethyl oleate (EO) and water. The mixtures were kept at room temperature to get equilibrium. The equilibrated samples were marked as being optically clear microemulsions (single phase, o/w or w/o microemulsion) or crude emulsions or gels. The physical states were represented on a pseudo-ternary phase diagram with one axis representing water, the other one oil (EO) and the third one surfactant-cosurfactant mixture (S_{mix}) representing a mixture of surfactant (PL43) and cosurfactant (GP-20).

The solubility of cyclosporin A in each component of microemulsion system (EO, PL43, GP-20) and in the surfactant-cosurfactant mixtures of GP-20 and PL43 with different ratios was assayed using HPLC. HPLC separation of cyclosporin A was achieved with a C_{18} column at 75°C using a temperature control oven. The mobile phase consisted of a mixture of 70% acetonitrile and 30% distilled water, and the flow rate was 1.2 ml/min. The eluent was monitored at 210 nm using an ultraviolet (UV) detector.

Based on the phase diagrams and solubility data, several compositions around single phase region were selected as shown in Table 1. Their physical stability was evaluated by periodic visual observation over 3 months for the presence of macroscopic phase separation.

Each microemulsion containing cyclosporin A equivalent to 1.77 mg of cyclosporin A per ml was diluted with water to make the water-continuous emulsion by phase inversion of viscous w/o microemulsion. Their dispersability was determined by measuring the mean droplet size of phase inverted w/o microemulsions by particle size analyzer.

The isotropic regions (S_μ) in the pseudo-ternary phase diagrams (Fig. 1) were obtained with GP-20 as a cosurfactant at two different surfactant-cosurfactant ratios (PL43:GP-20 = 1:1, 1:4). The phase-inverted microemulsions in the present study were formed spontaneously in the isotropic region at ambient temperature when their components were brought into contact. Comparison between the isotropic regions in Fig. 1A,B revealed that the isotropic region decreased with the increase of cosurfactant content. It indicates that as the relative concentration of cosurfactant increases the maximum amount of water solubilized in the system decreases. Similar results were obtained from microemulsions using Brij 96 as surfactant and glycerin, ethylene glycol, propylene glycol as cosurfactant (Kale and Allen, 1989).

The maximum solubility (% w/w) of cyclosporin A in each component was 49.76 ± 1.04 in GP-20, 30.69 ± 1.16 in PL43 and 32.26 ± 0.87 in EO. The solubility of cyclosporin A in the mixtures of GP-20 and PL43 with different ratios are presented in Table 2. The solubility of cyclosporin A increased markedly by the addition of GP-20 and was almost proportional to GP-20 content ($r = 0.961$). This result suggests that the higher solubility of drug in the oil and surfactant-cosurfactant mixture the more the drug-carrying capacity of microemulsion systems.

The physical stability of w/o microemulsions listed in Table 1 was not changed for at least 3 months. It exhibits that these microemulsions were stable during storage period.

Table 1
Compositions of w/o microemulsions with varying amounts of cyclosporin A and surfactant-cosurfactant mixtures (S_{mix}) with two different ratios

Cyclosporin A (g)	EO (g)	S^b_{mix} (g)	Water (g)
4.47 (5.00[a])	20.00	60.00	5.00
9.44 (10.00)	20.00	60.00	5.00
15.00 (15.00)	20.00	60.00	5.00
21.25 (20.00)	20.00	60.00	5.00
28.32 (25.00)	20.00	60.00	5.00
15.0	20.00	40.0 (50[c])	5.00
15.0	20.00	48.9 (55)	5.00
15.0	20.00	60.0 (60)	5.00
15.0	20.00	74.3 (65)	5.00
15.0	20.00	93.4 (70)	5.00

[a] The percentages of cyclosporin A are written in the parenthesis.
[b] Two different surfactant-cosurfactant mixtures (PL43:GP-20 = 1:1, 1:4) were used.
[c] The percentages of surfactant-cosurfactant mixtures are written in the parenthesis.

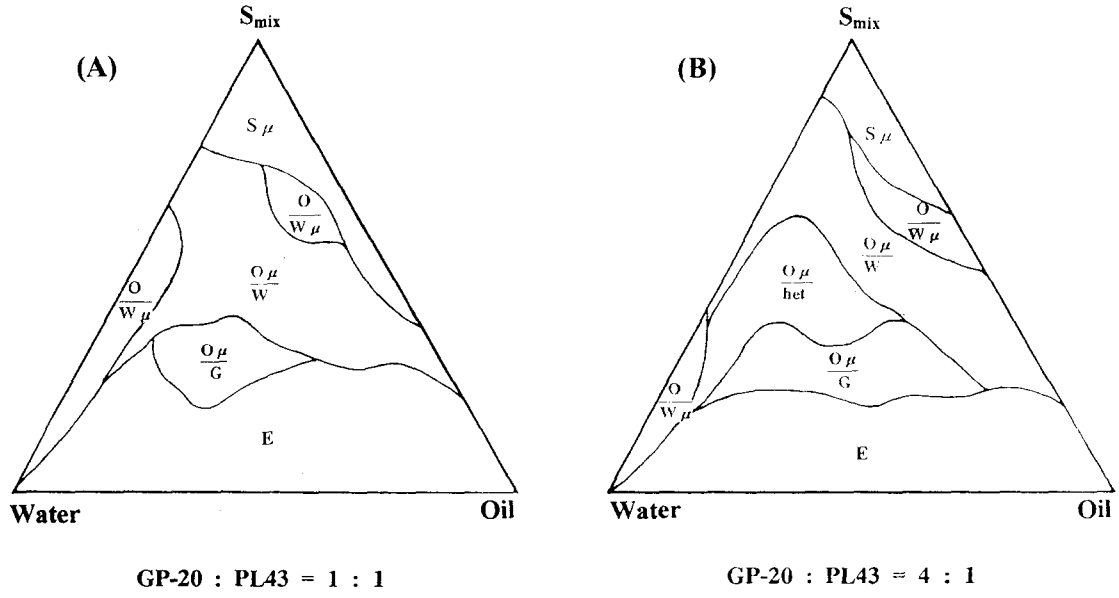

Fig. 1. Pseudo-ternary phase diagrams composed of oil(EO), water and surfactant-cosurfactant mixture (S_{mix}) (PL43:GP-20 = 1:1, 1:4). Key: S_{μ}, single phase microemulsion, O_{μ}, w/o microemulsion; W_{μ}, o/w microemulsion; G, gel; E, crude emulsion; het, heterogeneous phase.

The viscous w/o microemulsions containing 3.75–6.25% (w/w) of water in Table 1 were spontaneously inverted to water-continuous emulsion at ambient temperature after dilution with water. There was also no appreciable effect of the mixing procedure and dilution ratio on the particle size of phase inverted microemulsions under current experimental conditions.

It is known that the particle size distribution is one of the most important characteristics of emulsion determining its stability (Charman et al., 1992) and also in vivo fate of the emulsion (Tarr and Yalkowsky, 1989). Phase inverted emulsion which has droplet size larger than 1 μm suggests that heterogeneous coarse o/w emulsions or multiple (w/o/w) emulsions are formed (Constantinides and Yiv, 1995). In our system, however, the mean particle diameter of phase inverted microemulsions was found between 100–400 nm. It means that the phase inversion of w/o microemulsions in this study was done successfully.

Fig. 2 shows that the droplet size of microemulsions was greatly affected by the composition of

Table 2
Solubility of cyclosporin A in the mixtures of GP-20 and PL43 with different ratios

GP-20:PL43	Solubility*(% w/w)
0:100	30.69 ± 1.16
20:80	38.86 ± 0.93
50:50	40.95 ± 0.80
80:20	44.24 ± 0.91
100:0	49.76 ± 1.04

* Each value represents the mean ± S.D.

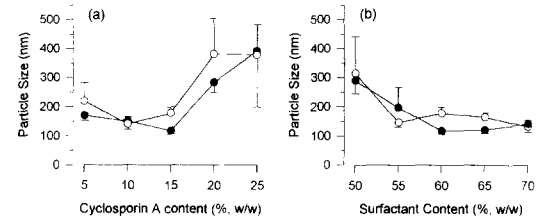

Fig. 2. Particle size of phase-inverted microemulsions prepared with two different surfactant-cosurfactant mixtures (●, PL43:GP-20 = 1:1; ○, PL43:GP-20 = 1:4) (a) containing different content of cyclosporin A (5–25%) (b) containing different content of two surfactant-cosurfactant mixtures (50–70%).

microemulsions. Microemulsions prepared with lower ratio of cosurfactant (PL43:GP-20 = 1:1) was relatively smaller than those prepared with higher ratio of cosurfactant (PL43:GP-20 = 1:4). It indicates that the former microemulsions are more stable than later ones. It is thought that the addition of surfactant causes the interfacial film to condense and to be stable, while the addition of cosurfactant would cause the film to expand (Kale and Allen, 1989). Fig. 2(a) also shows that at fixed S_{mix} and oil content, the droplet size was the smallest in the range of 10–15% (w/w) of cyclosporin A. It means that the proper amount of cyclosporin A should be used to keep the microemulsions stable. The increase of microemulsion size with higher cyclosporin A content (> 20%) may be related to the report that the incorporation of lipophilic drug into an emulsion causes a certain degree of instability (Charman et al., 1992). Fig. 2(b) also demonstrates that the microemulsion is most stable and homogeneous when the microemulsion was prepared with 60% (w/w) of S_{mix} (PL43:GP-20 = 1:1). In summary, the microemulsion containing 15% (w/w) cyclosporin A prepared with 60% (w/w) of S_{mix} (PL43:GP-20 = 1:1) was the most stable and homogeneous system under current experimental conditions.

From the above results, the phase inverted w/o microemulsion system composed of EO, PL43, GP-20 and water could improve the solubility of cyclosporin A. This system was also relatively stable and might be applicable to formulating the liquid and semisolid dosage form of cyclosporin A for enhancing the bioavailability of this drug.

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