Enhanced microemulsion formation in lipid-based drug delivery systems by combining mono-esters of medium-chain fatty acids with di- or tri-esters.

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ABSTRACT

To develop strategies for selecting appropriate lipids from mono-, di- and tri-esters of medium-chain fatty acids for the development of lipid-based drug delivery systems, ternary phase diagrams of propylene glycol (PG) monocaprylate (Capryol® 90; HLB~7), PG dicaprylocaprate (Labrafac™ PG; HLB~2) and glycerol tricaprylocaprate (Labrafac™ Lipophile WL1349; HLB~2) were determined in combination with a common surfactant, PEG-35 castor oil (Cremophor® EL, HLB~13), and water. Particle size and viscosity in different regions of the phase diagrams were measured, solubility of a model drug, danazol, in different lipid-surfactant mixtures was determined, and dispersion testing by diluting selected preconcentrates with 250 ml 0.01 NHCl was performed. Further, phase diagrams were constructed using binary mixtures of lipids (monoester with diester, or monoester with triester) in place of single lipids. The phase diagrams of PG dicaprylocaprate and glycerol tricaprylocaprate were similar, while it was distinctly different for PG monocaprylate. The microemulsion regions in phase diagrams were rather limited for individual lipids, and additionally, the di- and tri-esters showed pronounced gel regions in the phase diagrams, which could influence drug release from preconcentrates. The mixing of PG monocaprylate (monoester) with PG dicaprylocaprate (diester) or glycerol tricaprylocaprate (triester) had dramatic effects on the performance of lipids as evidenced by the greatly reduced gel phases, much larger microemulsion regions, faster dispersion of the preconcentrates in an aqueous medium, and smaller particle size of the microemulsions subsequently formed.

KEY WORDS: Lipid-based drug delivery, SEDDS, medium chain lipid, propylene glycol ester, triglyceride, phase diagram, drug solubility, dispersion test

INTRODUCTION

Lipid-based drug delivery systems have been studied extensively with the aim to increase the bioavailability of poorly water-soluble drugs (1-9). In addition to providing enhanced bio-availability, the lipid-based oral delivery system is known to reduce the effects of food as it presents the drug to the gastrointestinal system in a solubilized state (10-12). It also helps in lowering first-pass metabolism of certain drugs, although to a very limited extent, by channeling them to the lymphatic drug transport system (8).

Lipid-based formulations are usually developed as preconcentrates consisting of lipid, surfactant and, if necessary, co-surfactant and/or

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co-solvent. They form emulsions or micro-emulsions upon dilution with aqueous media in the gastrointestinal tract. Depending on the size of the lipid globules formed in the aqueous media, the preconcentrates are usually called self-emulsifying drug delivery system (SEDDS) or self-microemulsifying drug delivery system (SMEDDS). There has long been a controversy over what constitutes a microemulsion and whether it is indeed an emulsion or a micelle (13). It is now generally recognized as a thermodynamically stable micellar or swollen micellar system of lipid, surfactant and water (14). In a pharmaceutical application, it is a thermodynamically stable clear or translucent dispersion of lipids with particles usually less than 200 nm, formed spontaneously upon the addition of water to SMEDDS containing lipid/surfactant or lipid/surfactant/co-surfactant mixtures (15, 16). Therefore, the SMEDDS is also referred to as microemulsion preconcentrate (9). The term 'micro' in SMEDDS refers to 'small' particles and the actual size of particles formed is indeed in the lower nanometer range (usually <250 nm). In contrast, SEDDS produce opaque and milky emulsions upon dilution with an aqueous media with particle size of lipid globules >250 nm that may continue to grow or separate because the dispersion is thermodynamically unstable.

To develop a rational basis for differentiating various lipid-based formulations, Pouton (17, 18) introduced a lipid formulation classification system (LFCS) that categorizes various formulations in four classes, namely, Types I, II, III and IV. The classification is based on the compositions of the formulations, as well as, the potential effects of dilution with aqueous media on the dispersion of the formulations, digestion of lipids and possible precipitation of the drugs. Among them, Type III formulations form microemulsions of lipid-surfactant mixtures with particle sizes in the range of 50 to 250 nm. According to the LFCS, it is preferable that the lipid-based systems for bioavailability enhancement of poorly water-soluble drugs are developed as Type III formulations since the drugs may be absorbed from the micro-emulsions without the digestion of lipids and/or surfactants present. Thus the drugs remain in a solubilized state until their absorption (17, 18). Pouton (18) further divided Type III formulations into IIIA and IIIB where lipid contents are 40-80 and <20 % w/w, respectively. Type IIIA may have advantages over Type IIIB as more lipid is available to solubilize the drug in the preconcentrate, as well as, in the microemulsion formed after the dilution with aqueous media.

Many lipids are available for the development of lipid-based systems (19-21). They include vegetable oils, glycerides and partial glycerides of medium chain and unsaturated long chain fatty acids, and polyalcohol (e.g., propylene glycol) esters of medium chain fatty acids. The number of lipid-based drug products for poorly water-soluble drugs have remained limited despite the availability of many different lipids, and there are numerous poorly water-soluble drugs in discovery that could benefit from being formulated with lipids. Mullertz *et al.* (21) reported that, as of 2010, there were only five Type III lipid-based drug formulation on the market (ciprofloxacin, cyclosporine A, lopinavir, ritonavir and tipranavir). The authors suggested that there is a lack of adequate knowledge and understanding of the various issues involved in the development of such delivery systems. For example, the physico-chemical properties of lipids must be understood clearly to use them in the development of Type III formulations. The lipids differ depending on their hydrophilic-lipophilic properties. Certain lipids, especially triglycerides, are completely lipophilic with HLB values of zero or close to zero because of the absence of any hydrophilic moiety. On the other hand, among the lipids containing hydrophilic moieties, there can be a difference in the degree of hydrophilicity depending on their chemical structure (22, 23). For example, mono- and di-glycerides of long and medium chain fatty acids have hydrophilic properties due to the presence of free -OH groups and their hydrophilic-lipophilic balance (HLB) depends on whether

one or two -OH groups are free and the rest are esterified. Complicating the situation, most of the commercially available lipids are not available as pure species. Rather, they are mixtures of lipids with differing hydrophilic-lipophilic properties and differing fatty acid chain lengths. No systematic study has been reported in the literature on how the hydrophilic-lipophilic properties of lipids influence the development of Type III lipid-based formulations. The impact of combining two lipids with different degrees of hydrophilic-lipophilic properties in a formulation is also not fully understood.

In order to develop a rational approach for selecting lipids for their suitability in the development of LFCS Type III formulations, comprehensive laboratory studies were carried out. Of particular interest was their ability to form microemulsions and emulsions in the presence of a surfactant and water (16, 24). In a previous study (24), the effects of fatty acid chain lengths of medium chain lipids (C_8 versus C_{12}) were compared based on their performance in lipid-based drug delivery systems. Propylene glycol (PG) monoesters and PG diesters with C_8 to C_{12} fatty acids were used.

The focus of the present study was to determine the effect of the degree of esterification of lipids based on their performance in lipid-based drug delivery systems using PG esters of lower chain length (C_8 or mixtures of C_8 and C_{10}) only. The effects of the degree of esterification of glycerides on lipid-based drug delivery systems has been reported previously (16). It was, therefore, of interest to determine whether PG esters would perform in a similar manner. PG monocaprylate (C_8-fatty acid) and PG dicaprylocaprate (mixture of C_8 and C_{10}-fatty acids) were, respectively, used as mono- and di-esters, and, since PG cannot form a triester, glycerol tricaprylocaprate (mixture C_8 and C_{10}-fatty acids) was used as the tri-ester for comparison. Ternary phase diagrams of lipid-surfactant-water mixtures were determined for individual lipids and combinations of lipids using a common surfactant, PEG 35 castor oil. The various phases in different phase diagrams were characterized by particle size analysis and by measuring the viscosity. The solubility of a poorly water-soluble model drug, danazol, was studied in different lipids and lipid-surfactant mixtures. Additionally, drug solutions in different lipid-surfactant mixtures were evaluated for their ability to emulsify or disperse in aqueous media. An ancillary aspect of this present study was to compare PG esters from two different suppliers. As mentioned earlier, most of the commercially available lipids are mixtures of more than one species of esters, and therefore, there could be differences between materials obtained from two different manufacturers. Since the PG esters used here were obtained from a different manufacturer than those used in a previous study (24), it was of interest to determine whether the materials from the two suppliers would exhibit similar phase diagrams.

MATERIALS AND METHODS

Materials

PG monocaprylate (Capryol® 90), PG dicaprylocaprate (Labrafac™ PG) and glycerol tricaprylocaprate (Labrafac™ Lipophile WL1349) were donated by Gattefosse Corp, Paramus, New Jersey, USA. PEG-35 castor oil (Cremophor® EL) was donated by BASF, Tarrytown, New York, USA. The pharmaceutical grade danazol was received as a gift from a pharmaceutical company in the USA. The lipids are not commercially available in pure forms, they are usually mixtures of more than one lipid with different chain lengths and varying degree of esterification. The PG monocaprylate contained >90% monoester of propylene glycol with caprylic acid, while PG dicaprylocaprate was a mixture of PG dicaprylate (50 to 80%) and PG dicaprate (20 to 40%). Glycerol tricaprylocaprate was a mixture of the triglycerides of caprylic acid (50 to 80%) and capric acid (20 to 50%). Structures of the major components of the three lipids used are shown

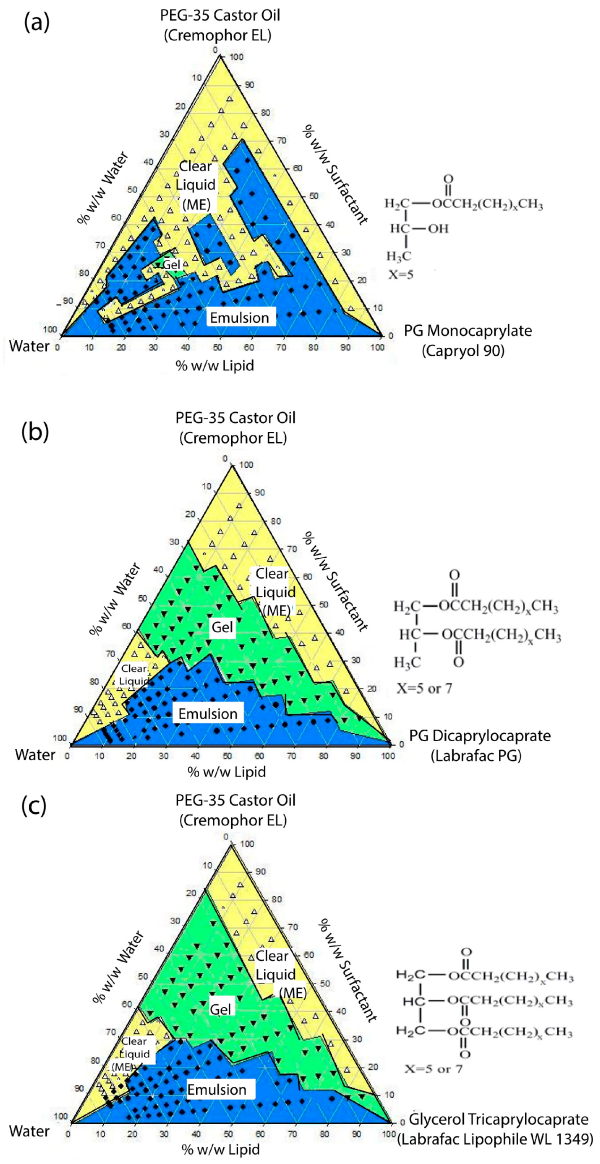

Figure 1 Phase diagrams of (a) PG monocaprylate, (b) PG dicaprylocaprate, and (c) glycerol tricaprylocaprate in combination with Cremophor® EL (PEG-35 castor oil) and water. Key: ● Emulsion; ▼ Gel; △ Clear liquid. The chemical structure of the primary component of the lipid used is given next to each phase diagram.

in Figure 1 on the right-hand sides of their respective phase diagrams.

To prevent possible variance due to batch-to-batch variability of the lipids or the surfactant, the same batches of materials were used throughout this study.

Methods

Construction of phase diagrams

Individual lipid-surfactant-water phase diagrams were constructed for PG monocaprylate, PG dicaprylocaprate and glycerol tricaprylocaprate, as well as, for combinations of PG monocaprylate with PG dicaprylocaprate or glycerol tricaprylocaprate at 1:3 and 1:1 ratios using a method described previously (16). Briefly, the lipid (or the combination of lipids) was mixed with the surfactant in 100 ml volumetric flasks at ratios of 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9 w/w to prepare a total of 4 grams of each mixture. A fixed-speed vortex mixer (Henry Tromner LLC, Thorofare, NJ, USA) was used to mix the lipid and the surfactant to a uniform consistency. Distilled water was added to the lipid-surfactant mixtures in 5% w/w increments. The concentration of water represented the percentage in the total mixture, i.e., the total amount of lipid, surfactant and water. Therefore, as the combined weight of lipid and surfactant was kept constant at 4 grams, the weight of water added increased with the increased concentration of water in the mixture. For example, 0.21 grams of water was necessary for the initial 5% water (increase from 0 to 5% w/w) so that the added water was 5% w/w of the total weight of 4.21 grams, while the addition 0.889 g of water was necessary to raise the concentration of water from 50 to 55% w/w thereby decreasing the concentration of lipid plus surfactant to 45% w/w (the amount of added water increased from 4 grams to 4.889 grams, making the total weight 8.889 grams). After each addition of water, the mixture was shaken using a wrist action shaker (Burrell Scientific, Pittsburgh, Pennsylvania, USA) by immersing the flasks in a water bath maintained at 25°C. Phase changes caused by each increment of water was observed visually. Based on the results of the preliminary experiments, the clear liquid or turbid liquid mixtures were shaken for 15 minutes and mixtures with gel-like consistency were shaken

for at least 40 minutes to attain equilibration during the construction of the phase diagrams.

Particle size analysis

The lipid-surfactant-water mixtures of the water-rich regions in the phase diagrams (70% w/w or more water) were analyzed for particle size of the lipid globules formed. Mixtures for particle size analysis were prepared independently from those prepared earlier for the purpose of constructing the phase diagrams. However, the method of preparation for the mixtures for both the construction of phase diagrams and the analysis of particle size was the same. Approximately 2-3 ml of the prepared mixture was transferred to a disposable cuvette (Beckman Coulter, Brea, California, USA) and the particle size was then determined using a Delsa Nano C (Beckman Coulter) particle analyzer. After particle size analysis, the sample was returned into the flask and additional water was added to each mixture to increase water content consecutively to 80%, 90% and 99% w/w. The particle size was determined after each dilution. The last dilution (99% w/w) was made in a 500 ml beaker as the total volume of liquid was 400 ml.

Determination of apparent viscosity

The apparent viscosity was determined for the gels formed from the dilution of the three lipid-surfactant ratios, 30:70, 50:50 and 70:30 w/w, to water contents of 20%, 30%, 40%, 50% and 60% w/w (the apparent viscosity was not measured for any of the mixtures that did not form a gel). For each measurement of viscosity, a fresh sample of gel was prepared according to the same procedure as that used for the construction of the phase diagrams. Approximately 0.5 ml of the gel was transferred to the cup of the viscometer and the viscosity was measured using a Brookfield RVDV III Ultra CP viscometer (Brookfield, Middleboro, Massachusetts, USA) with a CPE-52 cone at 150 RPM.

Determination of drug solubility

The solubility of danazol in the surfactant (Cremophor® EL), individual lipids and lipid-lipid and lipid-surfactant mixtures was determined by shaking an excess of danazol with each solvent (approximately 10 ml each) in 25 ml volumetric flasks for 24 hours at 25°C in a water bath using a wrist action shaker (Burrell Scientific) at its highest speed. Since the shaker provided high agitation, the preliminary experiments showed that the equilibrium of the solution was reached in about 8 hours. The concentration did not change as the shaking was continued for 24 hours. The equilibrated suspensions were filtered through 0.45-μm pore size polypropylene filters. The first 2-3 ml of filtrate was discarded each time to prevent any drug loss due to the possible adsorption onto the filter. Appropriate quantities of filtered aliquots were diluted with methanol, and the UV absorbance was measured at 286 nm using a UV-visible spectrophotometer (Beckman DU 650i, Beckman Coulter, Brea, California, USA). Similarly diluted surfactant or lipid-surfactant mixtures (without drug) in methanol served as controls for the UV analysis.

Dispersion test

Dispersion tests were carried out using 250 ml of 0.01N HCl (pH ~ 2) as the dispersion medium in a USP dissolution apparatus II (Distek Inc., North Brunswick, New Jersey, USA) at 50 RPM and 37°C. Dispersion testing was performed for the preconcentrates with 15 mg of danazol dissolved per gram of 1:1 mixture of surfactant and lipid (individual lipids or mixtures of them). One gram of each of the preconcentrates and the controls (containing the same amount of lipid-surfactant mixture but no dissolved drug) was transferred to the bottom of each dissolution vessel using a Pasteur pipette (exact weight determined by weighing the pipette before and after the transfer of the material). A visual observation was made of the the dispersion/emulsification

time, when the preconcentrate had mixed uniformly in the medium. A 3-ml aliquot was collected at 5, 10, 15, 30, 60 and 120 minutes and analyzed for particle size and concentration of danazol. The danazol concentration was determined using the UV spectrophotometric procedure mentioned previously. Aliquots were filtered through 0.45 μm filters and diluted with methanol before measuring the UV absorbance. Selected samples were also analyzed without filtration through 0.45 μm filter to determine the effect of filtration on concentration of the drug. After each collection of aliquot, 3 ml of fresh 0.01N HCL was added to the dissolution vessel to maintain the volume constant at 250 ml.

RESULTS AND DISCUSSION

Phase diagrams for the individual lipids

Figure 1 shows comparative phase diagrams of the three lipids used. Each phase diagram was constructed from the results obtained by mixing the lipid with the surfactant, Cremophor® EL, at different ratios, as indicated on the right side of each diagram, and then adding water. Distilled water was used as the aqueous phase although it is recognized that the lipid-surfactant mixtures are diluted with gastro-intestinal fluids upon oral administration as preconcentrates. Since lipids and the surfactant used are non-ionic, preliminary studies showed that a change in pH or the addition of electrolytes at low concentrations did not have a significant impact on the phase diagrams produced. The phase diagram of the PG monocaprylate in Figure 1a shows that when water was added to its mixture with surfactant, either a clear liquid or a milky emulsion was formed. The particle size analysis for different mixtures with water contents from 70 to 90 % w/w demonstrated that the difference between the clear liquid and emulsion regions was the particle size of lipid globules present. The particle size within the clear region was <200 nm, while the particle size within the emulsion region was greater (Table 1). Because the clear and emulsion regions were irregular as shown in Figure 1a, the phase diagram was constructed twice to ensure reproducibility. Both phase diagrams were found to be identical. Indeed, partial phase diagrams with 70 to 99% w/w water content were constructed an additional two times during the particle size determination. In all cases the results were similar. The phase diagram in Figure 1a is also essentially similar to one reported earlier where PG monocaprylate from a different manufacturer was used (24). Although irregular in appearance, the phase diagram was thus found to be highly reproducible. It is apparent from Figure 1a that the water-in-oil (w/o) microemulsions or emulsions were likely first formed when the water was added to the lipid-surfactant mixtures, which then turned into oil-in-water (o/w) microemulsions or emulsions after dilution to a high water content. However, the region of microemulsion at high water content was rather limited, only the 10:90-mixture of PG monocaprylate and Cremophor® EL became clear liquid after the dilution to 99% w/w water, i.e, 1 to 100 dilution of pre-concentrate with water.

The phase diagrams of Figures 1b and 1c represent ternary phase diagrams for PG dicaprylocaprate and glycerol tricaprylocaprate, respectively, in presence of Cremophor® EL and water. The two phase diagrams are essentially similar. At low water concentrations, the lipid-surfactant mixtures were clear liquids that were apparently w/o microemulsions. As the concentration of water increased, the clear liquids turned into gels, which, upon further addition of water, produced either an o/w microemulsion (clear or translucent liquid) or an o/w emulsion (turbid liquid) regions. In Figure 1b, the formation of gel occurred with the addition of ~30% w/w of water at 10:90, 20:80, and 30:70 lipid-surfactant ratios, ~20% w/w of water at 40:60 and 50:50 lipid-surfactant ratios and 10% w/w water at 10:90 and 70:30.

Table 1 Average particle diameters (nm) of emulsions and microemulsions formed upon dilution of mixtures of the individual lipids and the surfactant with water in the range of 70 to 99% w/w. Each value depicts the average of two determinations.

LIPID USED AND % WATER	LIPID/SURFACTANT RATIO (w/w) 90:10	70:30	50:50	30:70	10:90
PG monocaprylate					
70	2632 (1014,4250)	706 (860,553)	2276 (2476,2077)	2562 (2315,2809)	49 (51,47)
80	1159 (706,1612)	411 (487,336)	768 (1012,525)	1300 (1359,1242)	13 (14,13)
90	763 (438,1088)	272 (319,226)	85 (128,43)	370 (368,372)	12 (12,12)
99	1354 (389,2319)	198 (228,168)	60 (56,64)	151 (151,152)	14 (14,14)
PG dicaprylocaprate					
70	610 (506,714)	572 (622,523)	451 (418,484)	50 (37,63)	45 (40,50)
80	494 (354.634)	365 (448,282)	172 (192,153)	46 (40.53)	22 (28,16)
90	733 (494,972)	289 (266,313)	124 (117,131)	36 (30,43)	16 (16,17)
99	2192 (1092,3292)	220 (232,208)	125 (87,65)	37 (49,26)	16 (17.16)
Glycerol tricaprylocaprate					
70	1233 (564,1902)	1002 (465,1539)	374 (429,319)	40 (35,45)	35 (32,38)
80	860 (558,1162)	521 (565,478)	170 (199,141)	35 (22,48)	23 (18,28)
90	1099 (878,1320)	454 (377,532)	103 (117,89)	60 (32.89)	22 (17,28)
99	4497 (3105,5889)	320 (311,329)	82 (75,89)	65 (31,99)	17 (17,18)

In Figure 1c, the clear w/o microemulsion region appears to be slightly smaller. Between lipid-to-surfactant ratios of 10:90 and 70:30, the gel formed was clear, while at lipid-to-surfactant ratios of 80:20 and 90:10, it was turbid.

Particle size analysis confirmed that the clear liquid at high water content represents an o/w microemulsion with particle sizes <200 nm, and the o/w emulsions formed had particle sizes >200 nm (Table 1). It may be observed from individual values of particle sizes given in Table 1 in parentheses that the reproducibility of the data was high when microemulsions with particle sizes <200 nm were formed. In the case of emulsions with larger particle sizes, especially for the 90:10 lipid-to-surfactant ratio, a larger variability in particle size, even as much as 4 to 5-fold, was observed. This was expected because the microemulsions represent thermodynamically equilibrium systems (14), while the emulsions are nonequilibrium systems where the particle sizes of the lipid globules may differ depending on experimental conditions and time. Although the phase diagrams were constructed by visual observation and the particle size analysis was carried out by preparing fresh samples of appropriate lipid-surfactant-water ratios, there was a good agreement between the visual observations and the experimental data as the clear or translucent regions had particle sizes of ~200 nm and less.

PG monocaprylate, PG dicaprylocaprate and glycerol tricaprylocaprate have certain similarities in that all of them are esters of propane polyols with medium-chain fatty acids of C_8 and C_{10} chain length. However, their dissimilarities are also distinct. PG monocaprylate is a monoester with one free –OH group, and therefore, it is a relatively more hydrophilic lipid, with a HLB value of 7, as compared to the other two lipids used. Although PG dicap-

rylocaprate and glycerol tricaprylocaprate are, respectively, di- and tri-esters of fatty acids, they do not have any free –OH group and thus both of them are either practically lipophilic or only very slightly hydrophilic (HLB~2). The difference in the phase diagram of the PG monocaprylate, as compared to the other two and, the similarity in the phase diagrams of the PG dicrylocaprate and glycerol tricaprylocaprate, suggest that the hydrophilic-lipoophilic properties of the lipids (as indicated by the HLB values), and not the degree of esterification, was the determining factor for the nature of the phase diagrams produced by the three lipids used here.

Phase diagrams with mixtures of lipids

Pseudoternary phase diagrams were constructed using mixtures of two lipids instead of individual lipids (the term pseudoternary is used because the lipid phase consists of mixtures of two lipids instead of only one). There were two primary reasons for investigating the effects of lipid mixtures on the appearance of phase diagrams. First, the medium chain lipids, such as mono- and di-fatty acid esters of propylene glycol, are not commercially available in pure forms, rather they are available as mixtures with one component being the predominant one. It was, therefore, of interest to determine how a change in the mixing ratio of the lipids would influence the phase diagrams. Second, as shown in Figure 1, the dilution of the lipid-surfactant mixtures with water produced comparatively small regions of o/w microemulsions with the monoester (Figure 1a), and for the diester (Figure 1b) and the triglyceride (Figure 1c), the o/w microemulsions were formed only when the percentage of lipid in the lipid-surfactant mixtures was low (less than ~40%). A previous study showed that mixing a monoglyceride with di- or triglyceride increased the size of the microemulsion regions (16). Therefore, it was also of interest to determine whether mixing the two PG esters or the PG monoester with the triglyceride would provide larger o/w microemulsion regions during the development of the formulation. This would then lead to LFCS IIIA formulations with higher lipid contents.

Pseudoternary phase diagrams of mixed lipids were constructed combining PG monocaprylate with PG dicaprylocaprate or glycerol tricaprylocaprate (Figure 2). Preliminary studies indicated that combining PG dicaprylocaprate with glycerol tricaprylocaprate did not have any major influence on the nature of the phase diagrams as compared to those with individual lipids. This was expected, as both had similar polarities. Therefore, mixtures of a diester with a triester were not used. Figures 2a and 2b represent pseudoternary phase diagrams of, respectively, 1:3 and 1:1 mixtures of PG monocaprylate (monoester) with PG dicaprylocaprate (diester), while Figures 2c and 2d show the effect of mixing PG monocaprylate (monoester) with glycerol tricaprylocaprate (triester) at, respectively, 1:3 and 1:1 ratios. Effects of combining the PG monocaprylate with PG dicaprylocaprate or glycerol tricaprylocaprate at 1:3 ratios were essentially similar. In contrast to the phase diagrams obtained for the individual di- and tri-ester lipids in Figures 1b and 1c, the combination of one part of monoglyceride with three parts of di- or tri-esters in Figures 2a and 2c, respectively, increased the w/o microemulsion regions (up to 20-25% water content), decreased both the gel phase and the emulsion region, and expanded the o/w microemulsion regions to higher lipid contents. A more dramatic effect was observed when the PG monoester was mixed with the PG diester or the triglyceride at 1:1 ratios (Figures 2b and 2d, respectively), where the clear liquid microemulsion regions greatly increased, the emulsion regions were further decreased, and the gel phases were practically eliminated.

The results of particle size analysis with 70% w/w and higher concentrations of selected combinations of mixed lipids with the surfactant are given in Table 2, showing that the o/w microemulsions were obtained at ratios

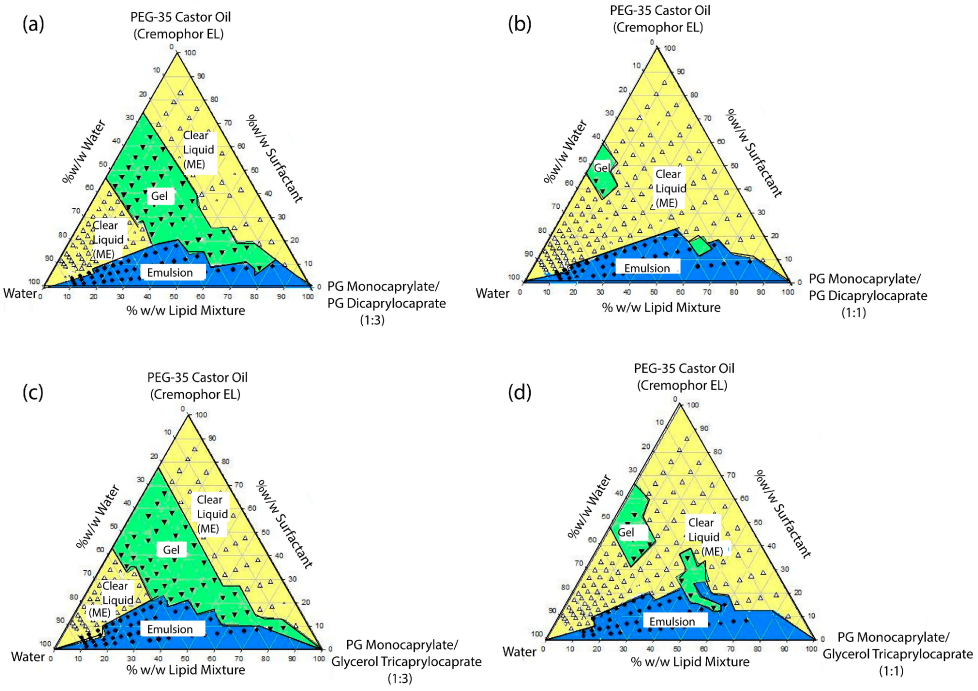

Figure 2 Phase diagrams of lipid mixtures in combination with Cremophor® EL (PEG-35 castor oil) and water. Lipid mixtures used: (a) PG monocaprylate + PG dicaprylocaprate, 1:3 w/w; (b) PG monocaprylate + PG dicaprylocaprate, 1:1 w/w; (c) PG monocaprylate + glycerol tricaprylocaprate, 1:3 w/w; and (d) PG monocaprylate + glycerol tricaprylocaprate, 1:1 w/w. Key: ● Emulsion; ▼ Gel; △ Clear liquid.

of between 70:30 and 10:90 of mixed lipids to surfactant. Thus, LFCS IIIA formulations with relatively high lipid loads (up to 70% w/w) may be obtained by combining the PG monoester with the PG diester or triglyceride. This is important as it has been reported that a lipid-surfactant mixture with relatively low lipid content (i.e., high surfactant content) may lead to the precipitation of the drug upon dilution with water (25, 26).

Therefore, increasing lipid content in a formulation by mixing two lipids instead of using individual ones could provide better drug formulations.

Apparent viscosity of the gels formed

The gels observed in the phase diagrams of PG dicaprylocaprate and glycerol tricaprylocaprate were characterized for apparent viscosity. The results are presented in Figure 3. The apparent viscosities of the gels formed in the construction of the phase diagrams with the two lipids were found to be similar. The apparent viscosities obtained ranged from about 100 to 200 mPa.s and relatively low if compared with the viscosities of water and glycerol at identical temperatures are approximately 1 and 1000 mPa.s., respectively. For gels formed at different lipid-surfactant ratios, the apparent viscosities increased for different lipid-surfactant ratios up to certain points and then decreased until the gels disappeared. In comparison, similar apparent viscosities of the gel phases were observed when the mixtures of the PG monocaprylate to the PG dicaprylocaprate or glycerol tricaprylocaprate at 1:3 ratios were used. As mentioned previously, the viscous gel phase practically vanished when the monocaprylate was mixed with either the dicaprylocaprate or the tricaprylocaprate at 1:1 ratios.

Table 2 Average particle diameters (nm) of emulsions and microemulsions formed upon dilution of combinations of mixed lipids and the surfactant with water in the range of 70 to 99% w/w. Each value depicts the average of two determinations, except where indicated, and the individual values are given in parentheses.

LIPID MIXTURES AND % WATER	MIXED LIPID/SURFACTANT RATIO (W/W)				
	90:10	70:30	50:50	30:70	10:90
PG monocaprylate + PG dicaprylocaprate (1:3 w/w)					
70	452 (336,706,406,360)[a]	395 (377,414)	218 (196,240)	38 (25,51)	22 (20,24)
80	333 (322,345)	214 (214,215)	68 (57,79)	33 (33,34)	13 (14,13)
90	304 (222,386)	142 (137,148)	41 (39,43)	18 (19,18)	13 (13,14)
99	676 (255,1133,957,357)	116 (112,121)	39 (38,40)	21 (22,20)	15 (14,16)
PG monocaprylate + PG dicaprylocaprate (1:1 w/w)					
70	701 (736,666)	368 (386,351)	61 (89,34)	32 (28,36)	38 (55,22)
80	496 (517,476)	175 (174,175)	31 (29,34)	45 (41,49)	13 (14,12)
90	568 (355,972,538,405)[a]	94 (108,80)	26 (25,28)	20 (19,21)	13 (13,13)
99	844 (530,1032,1290,543)[a]	88 (92,84)	28 (27,30)	21 (21,21)	14 (14,15)
PG monocaprylate + Glycerol tricaprylocaprate (1:3 w/w)					
70	329 (371,287)	276 (261,291)	223 (200,246)	45 (39,51)	38 (35,41)
80	282 (324,241)	180 (149,212)	71 (67,76)	26 (25,27)	12 (10,14)
90	270 (317,224)	134 (105,164)	42 (42,43)	20 (21,20)	19 (27,12)
99	924 (429,1491,527,1247)[a]	127 (93,162)	48 (41,55)	21 (22,20)	15 (18,13)
PG monocaprylate + Glycerol tricaprylocaprate (1:1 w/w)					
70	560 (310,514,959,456)[a]	287 (341,234)	74 (80,68)	50 (70,30)	75 (85,65)
80	309 (253,366)	123 (148,98)	28 (29,28)	18 (18,18)	14 (14,14)
90	262 (232,293)	94 (101,87)	27 (28,26)	16 (16,17)	13 (13,14)
99	583 (470,696)	79 (81,77)	29 (28,30)	18 (18,19)	14 (15,14)

[a] Particle sizes of emulsions formed at 9:1w/w lipid/surfactant rations were highly variable. For several mixtures, two additional particle size determinations were performed by preparing new samples.

Solubility of danazol in different lipids and lipid-surfactant mixtures

Solubilities of danazol in the individual lipids, mixtures of lipids and the surfactant used (Cremophor® EL) are given in Table 3. Rane and Anderson (27) reported that it is difficult to predict solubility of drugs in different lipids.

For danazol, the solubility was found to be considerably higher in PG monocaprylate than in the dicaprylocaprate or the tricaprylocaprate. This is contrary to the general perception that the more hydrophobic a lipid is, the easier a hydrophobic drug can be solubilized into it. It appears that the free polar -OH group in the monocaprylate somehow affects the solubility of danazol. The solubility of danazol in relatively more hydrophilic Cremophor® EL was comparable to that in PG monocaprylate.

The solubilities of danazol in several lipid-surfactant mixtures are provided in Table 4. It was observed in lipid-lipid, as well as, lipid-surfactant mixtures, that the effect on the

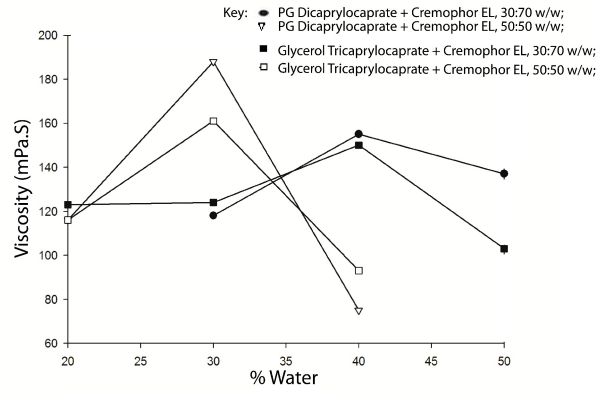

Figure 3 Comparison of viscosities of gels formed by lipid-surfactant mixtures at room temperature in presence of increasing amounts water. Key: ● PG dicaprylocaprate + Cremophor EL, 30:70 w/w; ▿ PG dicaprylocaprate + Cremophor EL, 50:50 w/w; ■, glycerol tricaprylocaprate + Cremophor EL, 30:70 w/w; □, glycerol tricaprylocaprate + Cremophor EL, 50:50 w/w.

Table 3 Solubility of danazol in individual lipids, lipid mixtures and surfactant (± s.d., n=3).

LIPID	SOLUBILITY OF DANAZOL (mg/g)
Glycerol tricaprylocaprate	7 ± 1
Propylene glycol (PG) dicaprylocaprate	9 ± 1
PG monocaprylate	31 ± 1
PG monocaprylate + Glycerol tricaprylocaprate (1:3 w/w)	12 ± 1
PG monocaprylate + PG dicaprylocaprate (1:3 w/w)	14 ± 1
PG monocaprylate + Glycerol tricaprylocaprate (1:1 w/w)	18 ± 1
PG monocaprylate + PG dicaprylocaprate (1:1 w/w)	19 ± 1
Cremophor® EL (surfactant)	26 ± 1

solubility of danazol when two or more components were combined, was additive and there was no synergistic increase (or decrease) in the solubility. In other words, when two lipids or a lipid and a surfactant were mixed in different ratios, the solubility of danazol was found to be similar to those calculated based on the solubilities of individual components and the ratios between them. Although certain drugs may have lower solubilities in lipids containing di- and tri-esters of medium chain fatty acids than those in monoesters or surfactants, the results of the present investigation indicate that the solubility of a drug may be optimized by combining lipids and

Table 4 Solubility of danazol at different ratios of lipid (or lipid mixtures) to surfactant (n=2).

LIPID : SURFACTANT RATIO	SOLUBILITY(mg/g)
Glycerol tricaprylocaprate: Cremophor® EL	
7:3	14 (13,14)*
1:1	17 (17,17)
3:7	19 (18,19)
PG dicaprylocaprate : Cremophor® EL	
7:3	15 (15,15)
1:1	19 (18,19)
3:7	22 (22,21)
PG monocaprylate : Cremophor® EL	
7:3	28 (28,28)
1:1	28 (28,27)
3:7	27 (27,27)
PG monocaprylate + PG dicaprylocaprate (1:3) : Cremophor® EL	
7:3	19 (18,19)
1:1	21 (21,21)
3:7	23 (23,23)
PG monocaprylate + PG dicaprylocaprate (1:1) : Cremophor® EL	
7:3	22 (21,22)
1:1	24 (24,24)
3:7	25 (25,25)
PG monocaprylate + Glycerol tricaprylocaprate (1:3) : Cremophor® EL	
7:3	17 (16,17)
1:1	20 (20,20)
3:7	23 (23,23)
PG monocaprylate + Glycerol tricaprylocaprate (1:1) : Cremophor® EL	
7:3	21 (21,20)
1:1	22 (22,22)
3:7	24 (24,24)

* Individual values are given in parentheses

surfactants of different chemical structures and hydrophilic-lipophilic properties.

Dispersion of lipid-based systems

Dispersion tests were performed to simulate how rapidly the lipid-based formulations would dilute in aqueous stomach fluids after oral ingestion, whether the dilution would be complete, what the particle sizes of the lipid globules would be, and whether there would be any precipitation of the drug from the lipid globules after mixing with an aqueous medium. As some of the lipid-surfactant mixtures have a tendency to form gels in contact with water, the emulsification time, i.e., the time for the complete mixing of the preconcentrates with the aqueous phase, was determined.

Figure 4A shows the dispersion profiles of danazol for 50:50 mixtures of lipid and

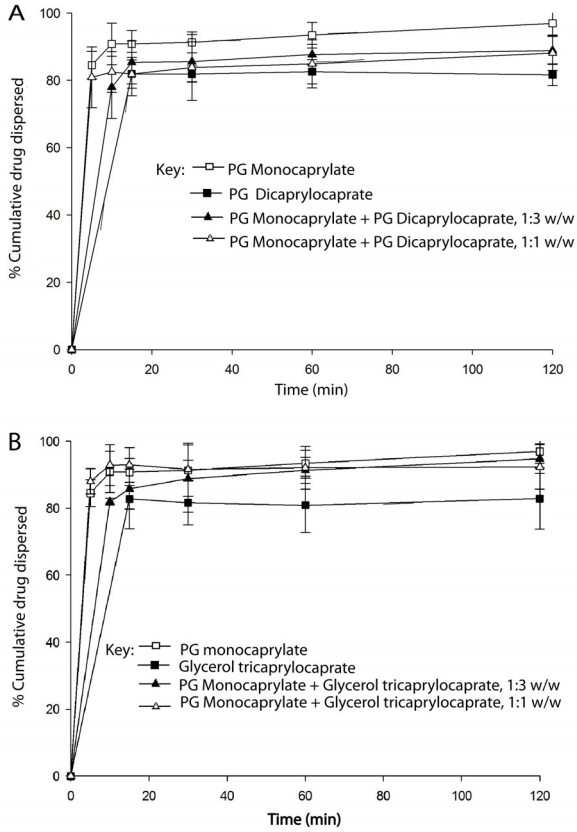

Figure 4 Dispersion profiles of danazol from 50:50 mixtures of lipids and Cremophor EL in 250 mL of 0.01M HCl at 37 °C (n=3). One g of preconcentrate containing 15 mg of danazol was used for each test. Lipids used (A): —□— , PG monocaprylate; —■— , PG dicaprylocaprate;—▲— , PG monocaprylate plus PG dicaprylocaprate, 1:3 w/w; and—△— , PG monocaprylate plus PG dicaprylocaprate, 1:1 w/w. (B): —□— , PG monocaprylate; —■— , Glycerol tricaprylocaprate; —▲— , PG monocaprylate plus glycerol tricaprylocaprate, 1:3 w/w; and—△— , PG monocaprylate plus glycerol tricaprylocaprate, 1:1 w/w.

Cremophor® EL when the PG monoester alone, PG diester alone, and the combinations of the monoester and the diester were used as the lipid components. A visual observation showed that preconcentrates containing PG dicaprylocaprate alone and the 1:3-mixture of the monocaprylate and the dicaprylocaprate took 13 and 9 minutes, respectively, for complete dispersion or emulsification in the aqueous medium. The formulations containing the PG monocaprylate or the 1:1-mixture of monocaprylate and dicaprylocaprate dispersed in <3 minutes. It was also observed that gels were formed with PG dicaprylocaprate alone and the 1:3-mixture of PG monocaprylate and dicaprylocaprate at the bottom of dissolution vessels, and the time required for complete disappearance of the gels (emulsification time) was responsible for the lag time observed in Figure 4A. The formation of the gel also corresponds with the phase diagrams in Figures 1b and 2a. Nevertheless, more than 80% w/w of drug dispersed from all formulations in 15 minutes. There was no decrease in drug concentration for up to 2 hours, indicating that there was no precipitation of drug following dispersion in an aqueous medium.

Similar results of the dispersion tests were obtained when PG monocaprylate was mixed with glycerol tricaprylocaprate (Figure 4B). Since glycerol tricaprylocaprate and the 1:3-mixture of PG monocaprylate and glycerol tricaprylocaprate were observed in their phase diagrams to form gels (Figures 1c and 2c, respectively), their complete dispersion in aqueous media took 14 and 8 minutes, respectively. The dispersion profile of the PG monocaprylate formulation is also shown in Figure 4B for comparison. Both PG monocaprylate and the 1:1-mixture of PG monocaprylate and glycerol tricaprylocaprate dispersed in <3 minutes. In all cases, the dispersion of danazol was >80%.

It was observed for all formulations that although >80% of the drug dispersed (shown in in Figures 4A and b), the drug concentration leveled off at <100% concentration. Therefore, drug concentrations were also measured without filtration through the 0.45 μm-pore filter. In the absence of filtration, more than 90% of the drug dispersed within 15 minutes in all formulations. Drug concentrations after both 15 and 120 minutes were found to be 5-10% higher in the unfiltered aliquots than in those filtered. The lower concentration of the drug after filtration was attributed to the adsorption of dispersed lipids onto the filter and not due to the retention of any large particles on filters as all particle sizes were

Table 5 Particle size following the dispersion of 1:1 lipid-surfactant mixtures without (control) and with danazol present (preconcentrate), where 1 gram of each mixture was added to 250 ml of 0.01N HCl (n=3).

	LIPID USED IN PRECONCENTRATE	PARTICLE SIZE (nm)			
		Control (without danazol)		Preconcentrate (with danazol)	
		15 min	2 h	15 min	2 h
1	PG monocaprylate	85	115	83	104
2	PG dicaprylocaprate	92	91	87	85
3	PG monocaprylate + PG dicaprylocaprate, 1:3	34	38	36	34
4	PG monocaprylate + PG dicaprylocaprate, 1:1	42	43	42	55
5	Glyceryl tricaprylocaprate	135	137	123	121
6	PG monocaprylate + Glyceryl tricaprylocaprate, 1:3	38	37	38	36
7	PG monocaprylate + Glyceryl tricaprylocaprate, 1:1	32	30	31	32

much below the 0.45 μm pore size of the filter used. Particle sizes after dispersion of different preconcentrates, as well as, for the control (no drug present) at 15 minutes and 2 hours are presented in Table 5. They were found to be practically unchanged from 15 minutes to 2 hours for both the preconcentrates and the controls. Particle sizes (Table 5) and particle size distribution (not shown) after the dispersion of the preconcentrates were found to be very similar to that of the control (with no drug). These observations led to the conclusion that the presence of drug did not influence dispersion of preconcentrates in aqueous media and there was no precipitation of the drug since there was no separate particle size distribution for any precipitated drug.

DISCUSSION

Relevance of microemulsion formation to the performance of lipid-based formulations

Meinzer *et al.* (28) reported that a lipid-based cyclosporine A formulation that produced a microemulsion upon dilution with aqueous media had superior bioavailability compared to a formulation that formed emulsions. This is indeed the primary reason why Neoral® is clinically better and has been commercially more successful, than Sandimmune®, both cyclosporine A products manufactured by Novartis. At least some animal models indicate that lipid-based formulations may exhibit good bioavailability even when they do not form microemulsions (29). The latter is possible because gastrointestinal fluids contain bile salts, lecithin, etc., that have surface activity and may cause or influence emulsification of lipids in the GI fluids after oral administration [8]. However, such effects may vary between individuals, and they may also vary depending on the GI environment at different times of the day. A well-formulated lipid-based dosage form that produces a microemulsion would not be affected by such physiological variables. The present report describes strategies to formulate microemulsion preconcentrates by combining a fatty acid monoester with a di- or tri-ester. As the drugs solubilized in microemulsions are in dynamic equilibrium with aqueous media, the drug would be released from microemulsions without the need for digestion of the lipids. Thus there would be less propensity for phase separation or the precipitation of the drug as reported for certain lipid-based systems (25, 26, 30). Even if there is some phase separation of the drug due to the digestion of lipids and/or surfactants, the drug is likely readily dispersed and dissolved without compromising bio-availability (31).

Possible mechanism for the observed phase behavior

Scriven (32) reported that the transition from the oil-rich w/o microemulsion phase to the water-rich o/w microemulsion phase occurs through an intermediate bicontinuous structure. Subsequently Ezrahi *et al.* (33) reported that the bilayer divides the space into two interwoven continuous networks of water and a combined

lipid and surfactant. It is possible that a similar mechanism of transition from w/o to o/w phases occurred during this present study. The bicontinuous structure may not necessarily lead to gel formation. It has been reported that multiple liquid crystalline structures may exist during the phase transition from w/o to o/w microemulsions or emulsions (34) and the gel formation could be related to the existence of such liquid crystalline structures. Further studies are necessary to clarify the mechanism of the gel formation. The primary focus of the present investigation was to determine how a dilution of lipid-surfactant mixtures could lead to the formation of microemulsion or emulsion to assist in developing an oral dosage form. It is, however, also recognized that the gel structures identified here may also be used for the preparation of oral and local drug delivery systems for proteins and small molecules (35), as well as, for controlled release systems (36).

The dramatic effect, observed here, of mixing PG monocaprylate with PG dicaprylocaprate or glycerol tricaprylocaprate on the formation of microemulsion may be explained by the work of Kuneida *et al.* (37). The authors reported that amphiphilic oils, such as the ones used here, influence the surfactant layer curvature, which, in turn, dictates the placement of solubilized oils in surfactant aggregates. Being more hydrophilic than PG dicaprylocaprate and glycerol tricaprylocaprate (HLB of both ~2), PG monocaprylate (HLB~7) will penetrate the surfactant (Cremophor® EL) palisade layer at the oil-water interface, making the surfactant layer curvature in micelles or microemulsions less positive, where the curvature was defined as positive when the surfactant film was convex toward water (37). Such a penetration of PG manocaprylate into the interfacial surfactant layer and, in other words, its activity as a relatively more lipophilic cosurfactant appears to be responsible for the swelling of micelles, leading to greater solubilization of lipids and an increase in the o/w microemulsion regions in the phase diagrams. A similar model has also been proposed by Prajapati *et al.* (16).

The phase diagrams of PG monocaprylate (Figure 1a) and the 1:3-mixture of PG monocaprylate and PG dicaprylocaprate (Figure 2a) constructed here are essentially similar to those reported earlier (24), where lipids from a different manufacturer was used. There is only a minor difference in the phase diagram of the 1:1-mixture of PG monocaprylate and PG dicaprylocaprate (Figure 2b) from that reported previously (24). These results, therefore, indicate that although neat lipids are not commercially available for pharmaceutical use, the materials from different manufacturers may behave similarly. However, this should be verified on a case by case basis.

CONCLUSION

The present study provides a systematic approach to selecting PG esters of medium chain fatty acids for the development of lipid-based drug delivery systems. PG dicaprylocaprate and glycerol tricaprylocaprate were found to form large gel regions in their phase diagrams. The gel region was minimal in the case of PG monocaprylate. Mixtures of PG monocaprylate and PG dicaprylocaprate or glycerol tricaprylocaprate had positive effects on the formation of microemulsions upon dilution with water and in the dispersion of preconcentrates in aqueous media, thereby reducing the need for a surfactant in the formulation. Complete dispersion of the drug in aqueous media was obtained either immediately (<5 minutes) if there was no gel formation or in <15 minutes if a gel was formed upon the addition of water to the lipid-surfactant mixtures. No precipitation of danazol was observed during the dispersion testing indicating that the drug remained solubilized in microemulsions. The particle size analysis during dispersion testing indicated that microemulsions with finer particle sizes may be obtained from a combination of PG monoester with either a PG diester or triglyceride.

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REFERENCES

1 Shah N.H., Carvajal M.T., Patel C.I., Infeld M.H., Malick A.W., Self-emulsifying drug delivery systems (SEDDS) with polyglycolized glycerides for improving in vitro dissolution and oral absorption of lipophilic drugs, Int. J. Pharm., 106:15-23, 1994

2 O'Driscoll C.M., Griffin B.T., Biopharmaceutical challenges associated with drugs of low aqueous solubility – the potential impacts of lipid-based formulations, Adv. Drug Deliv. Rev., 60:617-624, 2008

3 Charman W.N., Lipids, Lipophilic drugs and oral drug delivery - Some emerging concepts, J. Pharm. Sci., 89:967-978, 2000

4 Li P., Ghosh A., Wagner R.F., Krill S., Joshi Y.M., Serajuddin A.T.M., Effect of combined use of nonionic surfactant on formation of oil-in-water microemulsion, Int. J. Pharm., 288:27-34

5 Porter C.J.H., Trevaskis N.L., Charman W.N., Lipids and lipid based formulations: Optimizing oral delivery of lipophilic drugs, Nat. Rev. Drug Disco., 6:231-248, 2007

6 Spernath A., Aserin A., Microemulsions as carriers for drugs and nutraceuticals, Adv. Colloid Interface Sci., 128-130:47-64, 2007

7 Serajuddin A.T.M., Li P., Haefele T., Development of lipid-based drug delivery systems for poorly water-soluble drugs as viable oral dosage forms – Present status and future prospects, Am. Pharm. Rev., 11:34-42, 2008

8 Porter C.J.H., Pouton C.W., Cuine J.F., Charman W.N., Enhancing intestinal drug solubilisation using lipid-based delivery systems, Adv. Drug Deliv. Rev., 60:673-691, 200

9 Li P., Pudipeddi M., Hynes S.R., Royce A.E., Serajuddin A.T.M., Development and characterization of a solid microemulsion preconcentrate system for oral delivery of poorly water soluble drugs, J. Pharm. Sci., 98:1750-1763, 2009

10 Mueller E.A., Kovarik J.M., van Vree J.B., Influence of a fat-rich meal on the pharmacokinetics of a new oral formulation of cyclosporine in a crossover comparison with the market formulation, Pharm. Res., 11:151-155, 1994

11 Mueller E.A., Kovarik J.M., Kutz K., Minor influence of a fat-rich meal on the pharmacokinetics of a new oral formulation of cyclosporine, Transplant. Proc., 26:2957-2958, 1994

12 Woo J.S., Kyoung Y., Hong J.Y., Lim S.J., Reduced food-effect and enhanced bioavailability of a self-microemulsifying formulation of itraconazole in healthy volunteers, Eur. J. Pharm. Sci., 33:159-165, 2008

13 Rosano H.L., Introduction, in: Rosano H.L. and Clausse M. (eds.), Microemulsion System, Informa, New York, USA, pp. xv-xix, 1987

14 Shah D.O., Micelles, microemulsions and monolayers: Quarter century progress at the University of Florida, in: Shah D.O. (eds.), Micelles, microemulsion and monolayers : Science and technology, Informa, New York, USA, pp. 1-52, 1999

15 Lawrence M.J., Rees G.D., Microemulsion-based media as novel drug delivery systems, Adv. Drug Deliv. Rev., 45:89-121, 2000

16 Prajapati H.N., Dalrymple D.M., Serajuddin A.T.M., A comparative evaluation of mono-, di- and triglyceride of medium chain fatty acids by lipid/surfactant/water phase diagram, solubility determination and dispersion testing for application in pharmaceutical dosage form development, Pharm. Res., 29:285-305, 2012

17 Pouton C.W., Lipid formulations for oral administration of drugs: Non-emulsifying, self-emulsifying and self-microemulsifying drug delivery systems, Eur. J. Pharm. Sci., 11:S93-S98, 2000.

18 Pouton C.W., Formulation of poorly water-soluble drugs for oral administration: Physicochemical and physiological issues and the lipid formulation classification system, Eur. J. Pharm. Sci., 29:278-287, 2006

19 Jannin V., Musakhanian J., Marchaud D., Approaches for the development of solid and semi-solid lipid-based formulations, Adv. Drug Deliv. Rev., 60:734-746, 2008

20 Cannon J.B., Long M.A., Emulsions, microemulsions, and lipid-based drug delivery systems for drug solubilization and delivery – Part II: Oral applications, in: Liu R. (dd), Water-Insoluble Drug Formulation, CRC Press, Boca Raton, Florida, USA, pp. 227-253, 2008

21 Mullertz A., Ogbonna A., Ren S., Rades T., New perspectives on lipid and surfactant based drug delivery systems for oral delivery of poorly soluble drugs, J. Pharm. Pharmacol., 62:1622-1636, 2010

22 Small D.M., A classification of biological lipids based upon their interaction in aqueous systems, J. Am. Oil Chemists Soc., 45:108-119, 1968

23 Fahy E., Subramaniam S., Brown H.A., Glass C.K. , A comprehensive classification system for lipids, J. Lipid Res., 46:839-861, 2005

24 Prajapati H.N., Patel D.P., Patel N.G., Dalrymple D.M., Serajuddin, A.T.M., Effect of difference in fatty acid chain lengths of medium-chain lipids on lipid/surfactant/water phase diagrams and drug solubility, J. Excipients and Food Chem., 2:73-88, 2011

25 Cuine J.F., Charman W.N., Pouton C.W., Edwards G.A., Porter C.J.H., Increasing the proportional content of surfactant (Cremophor® EL) relative to lipid in self-emulsifying lipid-based formulations of danazol reduces oral bioavailability in beagle dogs, Pharm. Res., 24:748-757, 2007

26 Mohsin K., Long M.A., Pouton C.W., Design of lipid based formulations for oral administration of poorly water-soluble drugs: Precipitation of drug after dispersion of formulations in aqueous solution, J. Pharm. Sci., 98:3582-3595, 2009

27 Rane S.S., Anderson B.D., What determines drug solubility in lipid vehicles: Is it predicatable? Adv. Drug Deliv. Rev., 60:638-656, 2008

28 Meinzer A, Mueller E, Vondersher J., Microemulsion – A suitable galenical approach for the absorption enhancement of a low soluble compound? B.T. Gattefosse 88:21-2, 1995

29 Nielsen F.S., Petersen K.B., Mullertz A., Bioavailability of probucol from lipid and surfactant based formulations in minipigs: Influence of droplet size and dietary state. Eur. J. Pharm. Biopharm. 69:553–562, 2008

30 Porter C.J.H., Kaukonen A.M., Boyd B.J., O'Connor J.M., Edwards G.A., Charman W.N., Susceptibility to lipid-mediated digestion reduces the oral bioavailability of danazol after administration as a medium-chain lipid-based microemulsion formulation, Pharm. Res., 21:1405-1412, 2004

31 Sassene P.J., Knoff M.M., Hesselkilde J.Z., Koradia V., Larsen A., Rades T., Mullertz, A., Precipitation of a model drug during *in vitro* lipolysis: Characterization and dissolution of the precipitates, J. Pharm. Sci. 90:4982-4991, 2010

32 Scriven L.E., Equilibrium bicontinuous structure, Nature 263:123-125, 1976

33 Ezrahi S., Aserin A., Garti N., Aggregation behavior in one-phase (Winsor IV) microemulsion systems, in: Kumar P., Mittal K.L. (eds), Handbook of microemulsion science and technology, Informa Inc., New York, USA, pp 185-246, 1999

34 Sagalowicz L., Leser M.E., Watzke H.J., Michel M., Monoglyceride self-assembly structures as delivery vehicles, Trends in Food Sci. Technol., 17:204-214, 2006

35 Shah J.C., Sandhale Y., Chilukuri D.M., Cubic phase as drug delivery systems, Adv. Drug Del. Rev., 47:229-250, 2001

36 CarvaLeila F.C., Sarmento V.H.V., Chiavacci L.A., Barbi M.S., Gremiao M.P.D., Development and in vitro evaluation of surfactant systems for controlled release of zidovudine, J. Pharm. Sci., 99:2367-2374, 2009

37 Kuneida H., Horii M., Koyama M., Sakamoto K., Solubilization of polar oils in surfactant self-organized structures, J. Colloid Interface Sci., 236:78-84, 2001