Original Article

Effect of Difference in Fatty Acid Chain Lengths of Medium-Chain Lipids on Lipid/Surfactant/Water Phase Diagrams and Drug Solubility.

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Received: 12 July, 2011; *Accepted:* 17 August, 2011

ABSTRACT

Lipids consisting of medium chain fatty acids are commonly used in the development of lipid-based self-emulsifying and self-microemulsifying drug delivery systems. However, no systematic approach to selecting one lipid over another has been reported in the literature. In this study, propylene glycol (PG) monoester (PG monocaprylate, Capmul PG-8®) and PG diester (PG dicaprylocaprate, Captex 200P®) of C_8-fatty acids were compared with PG monoester (PG monolaurate, Capmul PG-12®) and PG diester (PG dilaurate, Capmul PG-2L®) of C_{12}-fatty acids with respect to their phase diagrams, and especially for their ability to form microemulsions in the presence of a common surfactant, Cremophor EL®, and water. The solubility of two model drugs, danazol and probucol, in the lipids and lipid/surfactant mixtures were also compared. The effect of the chain length of medium-chain fatty acids (C_8 versus C_{12}) on the phase diagrams of the lipids was minimal. Both shorter and longer chain lipids formed essentially similar microemulsion and emulsion regions in the presence of Cremophor EL® and water, although the C_{12}-fatty acid esters formed larger gel regions in the phase diagrams than the C_8-fatty acid esters. When monoesters were mixed with their respective diesters at 1:1 ratios, larger microemulsion regions with lower lipid particle sizes were observed compared to those obtained with individual lipids alone. While the solubility of both danazol and probucol increased greatly in all lipids studied, compared to their aqueous solubility, the solubility in C_{12}-fatty acid esters was found to be lower than in C_8-fatty acid esters when the lipids were used alone. This difference in solubility due to the difference in fatty acid chain length, practically disappeared when the lipids were combined with the surfactant.

KEY WORDS: Lipid-based drug delivery, medium chain lipid, propylene glycol monoester, propylene glycol diester, phase diagram, danazol, probucol, drug solubility

INTRODUCTION

During the past decade the pharmaceutical industry has shown great interest in the development of lipid-based drug delivery systems to increase the bioavailability of poorly water-soluble active pharmaceutical ingredients (APIs) (1-13). Since a lipid-based oral delivery system presents the API to the gastrointestinal (GI) tract in a solubilized state, it is also known to reduce the 'food effect' for poorly water-soluble

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drugs as it eliminates the dissolution step that is often influenced by the presence of food (14-17). In addition, it is also possible that the lipid-based oral delivery system may reduce the first-pass metabolism of certain APIs, although to a very limited extent, by channeling them to the lymphatic drug transport system (18). However, despite many potential advantages of lipid-based drug delivery systems in overcoming absorption and bioavailability issues with poorly water-soluble drugs, only a limited number of drug products utilizing such systems have been brought to the market (13, 19, 20). Although there has been a great deal of progress regarding the basic understanding of the principles of how lipid-based drug delivery systems function, a rational basis for the selection of lipid-based excipients necessary for the development of pharmaceutical dosage forms has not yet been established and the prediction of *in vivo* performance of these excipients is poor (6). According to Porter *et al.* (6) and Serajuddin *et al.* (21) much more work is needed in order to understand the basic physicochemical and biopharmaceutical characteristics that may result in more lipid-based products being brought to the market.

For the rational selection of lipid-based excipients for dosage form development, a proper understanding of how such formulations work *in vivo* is necessary. Lipid-based formulations are usually developed as preconcentrates consisting of lipid, surfactant, and, if necessary, co-surfactant and/or co-solvent, which form emulsions or microemulsions upon dilution with gastrointestinal fluids. The formulations are called self-emulsifying drug delivery system (SEDDS) or self-microemulsifying drug delivery system (SMEDDS) depending on whether they form emulsions or microemulsions, respectively, when dispersed in aqueous media. For a long time there was no general agreement over what constitutes a microemulsion (22, 23) but it has generally been recognized as a thermodynamically stable micellar or swollen micellar system of lipid, surfactant and water that is usually clear or translucent to the naked eye and where the particle size of the dispersed phase is generally less than 200 nm (24, 25). In recent years, the term self-nanoemulsifying drug delivery system (SNEDDS) has also been used in the pharmaceutical literature to describe certain lipid-based systems (18). However, there is no practical difference between a SMEDDS and a SNEDDS as the prefix 'micro' in the term microemulsion usually refers to 'small' particles and the actual size is indeed in the nanometer range (usually <200 nm). To distinguish between various lipid-based formulations, Pouton (6, 26) introduced a lipid formulation classification system (LFCS) that categorizes them as Type I, Type II or Type III based on typical compositions and possible effects of dilution and digestion on the precipitation of the API. Type III has been further divided into Types IIIA or Type IIIB having, respectively, a lipid content of 40-80% or <20% w/w. There are different schools of thought on how an API is released from a self-emulsifying lipid-based formulation. According to LFCS, Type III formulations, which produce particles in the range of 50 nm to 250 nm in contact with water, may be able to release the API without undergoing digestion in the GI tract, while digestion of lipids is necessary for formulations that produce a coarse emulsion. There are also reports indicating that the digestion of lipids and surfactants could be a factor in the release of APIs from self-microemulsifying formulations (13, 27). Even if the lipids and surfactants are digested, it is possible that the API released from a microemulsion could remain suspended in a finely divided state in the GI tract and have a high dissolution rate. Thus, the ability to form a microemulsion in contact with aqueous media plays a critical role in the *in vivo* performance of lipid-based systems. This may be exemplified by the performance of Sandimmune® and Neoral®, two products manufactured by Novartis. While Sandimmune® forms milky emulsions with large globules after dispersion in aqueous media, Neoral® forms translucent microemulsions with very fine globules of <150

nm (28). As a result, Neoral® provides superior bioavailability (29) and a reduced food effect (16) compared to Sandimmune®.

Based on the above analysis, it is important for 'a rational basis for the selection of lipid-based excipients' (6) to determine whether certain lipid/surfactant systems will form microemulsions and, if microemulsions are not formed, what particle sizes are produced in the emulsions. Many different lipids are available for the development of oral lipid-based formulations including long chain and medium chain triglycerides, propylene glycol esters, mono and diglycerides of medium chain and long chain fatty acids, various lipid mixtures, and so on (13, 30). Adding to the diversity, the fatty acid components of the lipids can be either saturated or unsaturated, increasing the field even more. According to Cannon and Long (30) lipids that have fatty acid chains of 14-20 carbons are considered long chain, while those with 6-12 carbons are medium chain. Unless they consist of unsaturated fatty acid chains, the long-chain glycerides are usually solid at room temperature and, therefore, may not be suitable for dissolving drugs (30). Further, long-chain glycerides which exist as liquids at room temperature (e.g., corn oil, sesame oil, peanut oil, olive oil, soybean oil, etc.) have been reported to have lower drug solubilites than medium-chain glycerides (31, 32). As part of an ongoing research to characterize various lipids for their suitability as excipients for drug products in our laboratory, we investigated the effect of the degree of esterification of medium chain lipids on their physicochemical properties relevant to pharmaceutical dosage form development (33). Since all lipids within a particular class (e.g., medium-chain, long-chain, and so on) are not the same and may have different chain lengths of constituent fatty acids, in this study we investigated the effects of the difference in chain length of various medium chain lipids on drug solubility, as well as, lipid/surfactant/water phase diagrams since they are important aspects of the development of self-emulsifying lipid-based formulations. In particular, drug solubility and phase diagrams of propylene glycol (PG) monocaprylate and PG dicaprylocaprate, which are, respectively, mono- and di-esters of PG with predominantly caprylic acid (C_8) were compared with respect to drug solubility and phase diagrams of PG monolaurate and PG dilaurate, which are, respectively, mono- and di-esters of PG with lauric acid (C_{12}). Since some of these esters are available commercially as mixtures of mono- and di-esters, we also used mixed lipids in constructing the phase diagrams.

MATERIALS AND METHODS

Materials

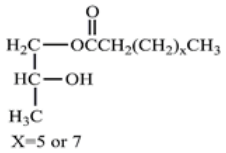
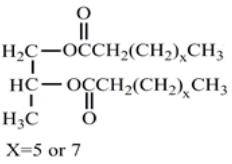
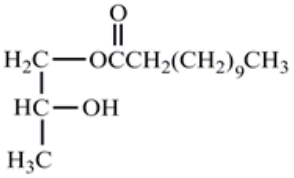
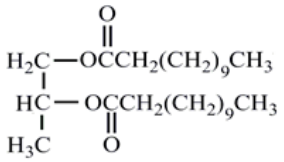
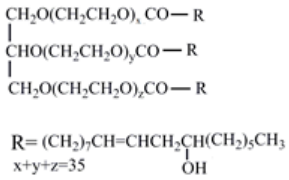
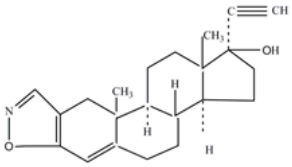
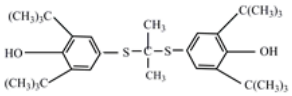
Table 1 lists trade names, suppliers, and chemical structures of various lipids, the surfactant, and the model APIs used. Compositions of lipids and the surfactant used are also given in Table 1 as they are usually available commercially as mixtures rather than single species. However, only structures of the most common components are shown. Distilled water was used throughout the study. All other chemicals and reagents used were of analytical grade or better.

Methods

Determination of phase diagram

Since the objective of the present study was to compare different lipids, phase diagrams of the lipids were prepared with the addition of a common surfactant, Cremophor EL®, according to the method described previously (33). Essentially, the method consisted of preparing mixtures of a particular lipid and the surfactant at ratios of 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2 and 9:1 w/w in different 100 ml volumetric flasks keeping the initial weight of each mixture constant at 4 grams. Water was then added to each flask in increments of 5% w/w. The concentration of water represents the percentage of the total weight of lipid, surfactant and water. Therefore, as the total weight of lipid and surfactant was kept constant at 4 grams, the weight of water added increased with the increase in the concentration

Table 1 Generic name, trade name, structure and composition of lipids, surfactant and drugs used

GENERIC NAME	TRADE NAME	STRUCTURE	COMPOSITION/DESCRIPTION
Propylene glycol monocaprylate	Capmul PG-8® (ABITEC Corp., Columbus, O, USA)	 X=5 or 7	Mixture of monoester (>90%) and di-ester (<10%) of propylene glycol with mainly caprylic acid.
Propylene glycol dicaprylocaprate	Captex 200P® dicaprylocaprate (ABITEC Corp.)	 X=5 or 7	Propylene glycol diesters of saturated fatty acids, mainly of caprylic (50-80%) and capric (20-50%) acids. Diester content > 90%.
Propylene glycol monolaurate	Capmul PG-12® (ABITEC Corp.)		Propylene glycol monoester and diester of medium chain fatty acids (mainly lauric) containing a minimum of 90% monoesters and a maximum of 10% diesters.
Propylene glycol dilaurate	Capmul PG-2L® (ABITEC Corp.)		Mixture of propylene glycol diester (75%) and monoester (25%).
PEG-35 Castor oil	Cremophor EL® (BASF, Tarrytown, NY, USA)		The main component (83%) is polyethylene glycol ester of ricinoleic acid
Danazol	Various (Donated by a major gneric pharmaceutical cmpany)		Derivative of the synthetic steroid ethisterone, a modified testosterone. Aqueous soubility: 0.59 µg/mL; logP : 4.53.
Probucol	Various (Sigma Aldrich, St. Louis, MO)		It is used as a lipid regulating agent to treat hyperlipidaemias, particularly in type IIa hyperlipoproteinaemia. Aqueous solubility: 0.002-0.005 µg/mL; logP: 11.

of water in the mixture. For example, the addition of 0.21 grams of water was necessary for the initial 5% water (increase from 0 to 5% w/w) so that the added water is 5% w/w of the total weight of 4.21 grams, while 0.889 grams of water was added to raise the concentration of water from 50 to 55% w/w (the amount of added water increased from 4 grams to 4.889 grams, making the total weight 8.889 grams). When combinations of two lipids were used in

the construction of pseudo-ternary phase diagrams, the weight of lipid represented the total weight of both lipids combined. After each addition of water, the mixture was shaken using a wrist-action shaker (Burrell Wrist Action Shaker, Burrell Scientific, Pittsburgh, PA, USA) for 15 minutes at the highest speed setting until the mixtures appeared as non-viscous fluids. When the mixtures appeared to form gels after the addition of water, the shaking was continued for at least 40 minutes. The shaking time was established through preliminary experiments, which showed that there was no further change in the mixtures when the shaking was continued beyond 40 minutes. The bottoms of the flasks were kept immersed in a water bath at 25°C during equilibration. The phase boundaries were established by visual observation of the different mixtures. There were three main phases in the phase diagram: 'clear' regions that included clear and translucent liquid consisting of microemulsion (ME), cloudy liquids apparently consisting of a coarse emulsion and a viscous gel or liquid crystalline phase. The particle size and viscosity were determined for selected mixtures.

Particle size measurement

Particle size analysis was performed for selected lipid/surfactant mixtures in the phase diagrams with water contents of 70%, 80%, 90% or 99% w/w by means of a dynamic light scattering at 25°C using Delsa Nano C particle size analyzer (Beckman Coulter Inc., Irvine, CA, USA). An approximately 2-3 ml aliquot was withdrawn from the volumetric flask containing the equilibrated mixture of lipid, surfactant and water for each particle size measurement. No additional dilution of the aliquot for the purpose of particle size analysis was necessary and, it was placed back into the volumetric flask after the determination of particle size for further use in constructing the phase diagram, if necessary. Disposable plastic cuvettes (Beckman Coulter disposable Cell, Beckman Coulter Inc., Irvine, CA, USA) were used to hold the aliquots during analysis. It should be noted that a phase diagram was first constructed by visual observation, as mentioned above, without particle size analysis. The last dilution (99% w/w) was made in a 500 ml beaker as the total volume of liquid was ~400 ml. Phase analysis for 9:1, 7:3, 1:1, 3:7 and 1:9 lipid/surfactant ratios and water contents of 70% to 99% w/w were then repeated twice and checked by visual observation and measurement of particle size.

Viscosity determination

The viscosity of the gel phase was measured using Brookfield RVDV III Ultra CP viscometer (Brookfield Engineering Laboratories, Inc. Middleboro, MA, USA) fitted with a CPE-52 cone and operated at the speed of 150 rpm. For viscosity determination, fresh mixtures (one sample for each measurement) with three different ratios of lipid to surfactant (5:5, 4:6 and 2:8 w/w) were prepared according to the procedure described earlier for the determination of a phase diagram by adding water, usually in the range of 20% to 60% w/w, and the mixtures were vortexed for approximately 1 minute and equilibrated at 25°C for ~40 minutes using the wrist action shaker. After equilibration, an approximately 0.5 ml aliquot was withdrawn and the viscosity was measured.

Solubility determination

Danazol, which has a solubility of 0.59 μg/ml in water and a logP value of 4.53 (34), and probucol, which has a solubility of 0.002-0.005 μg/ml and a logP value of 11 (35, 36), were selected as model APIs for comparison of their solubilities in different lipids and with added surfactants. The APIs were added individually to each lipid, lipid-lipid mixture or lipid/ surfactant mixture and then equilibrated at 25°C in a water bath by shaking for 24 hours using a the wrist action shaker at the maximum shaking speed. The aliquots were filtered through 0.45 μm polypropylene filters. Samples were taken by weight for dilution and drug

concentration was determined by HPLC analysis.

HPLC Analysis of Drugs

The HPLC analysis of danazol was carried out using a Waters HPLC system (Waters Corp., Milford, MA, USA) which consisted of a Waters 1515 Isocratic HPLC pump, Waters 717 Plus Autosampler, Waters 486 Absorbance Detector. The chromatographic conditions were as follows: 4.6 mm x 150 mm C18 Waters XBridge column (3.5 µm), acetonitrile-water (65:35 v/v) mobile phase (flow rate 0.5ml/minute), and a detection wavelength of 280 nm. For the HPLC analysis of probucol, a quaternary pump, an Agilent 1100 autosampler and a diode array detector (HP1100 series, Agilent Technologies, Wilmington, DE, USA) were used. The chromatographic column used was C_8 Waters XBridge column (3.5µm), 4.6 mm x 150 mm. A methanol-water mixture (95:5, v/v) at the rate of 1 ml/minute was used as the mobile phase and the detection wavelength was set at 243 nm.

RESULTS AND DISCUSSION

Phase diagrams

Comparative phase diagrams of propylene glycol monoesters with C_8 and C_{12} fatty acids

Phase diagrams of PG monocaprylate-Cremophor EL®-water and PG monolaurate-Cremophor EL®-water systems are presented in Figures 1A and 1B, respectively. PG monocaprylate (Capmul PG-8®) is a monoester of PG with caprylic acid, which is a C_8-fatty acid, and PG monolaurate (Capmul PG-12®) is the monoester of PG with lauric acid, a C_{12}-fatty acid. Although the fatty acid chain length of medium-chain lipids may have 6 to 12 carbon atoms (30), only 8 and 12-carbon fatty acid lipids are compared in this study as no 6-carbon fatty acid lipids are commonly used in pharmaceutical dosage forms. Since the primary objective of the present study was to compare lipids with differences in fatty acid chain length, the same surfactant (Cremophor EL®, PG-35 castor oil) was used for both phase diagrams.

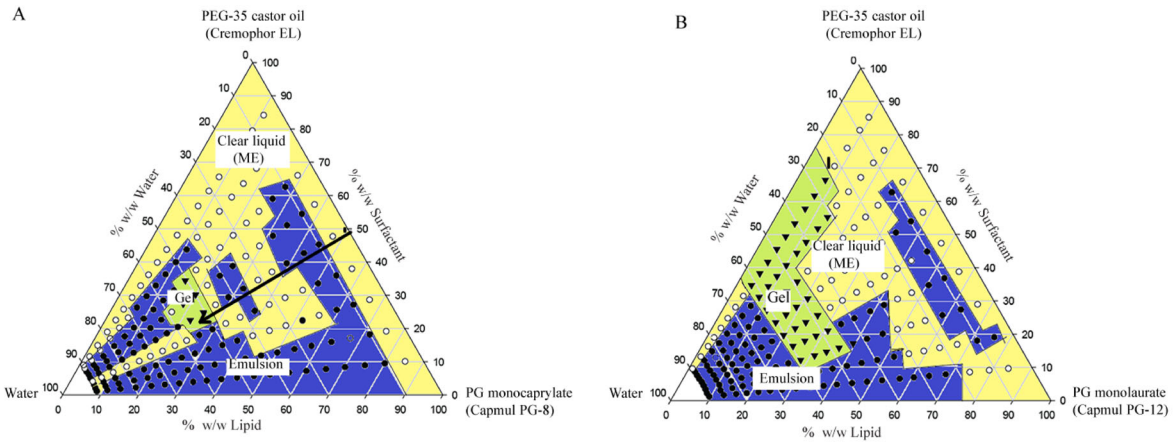


Figure 1 Phase diagram of (A) propylene glycol monocaprylate, (B) propylene glycol monolaurate with PEG-35 castor lipid and water. The arrows indicate the direction of the addition of water to lipid/surfactant mixtures.

As can be observed in Figures 1A and 1B, both C_8- and C_{12}-monoesters gave very similar phase diagrams. When mixed with Cremophor EL®, both lipids provided clear solutions, and the solutions remained clear when 5% w/w water was added to various lipid surfactant mixtures ranging from 9:1 to 1:9 w/w. The arrow in Figure 1A indicates the direction of the addition of water to lipid/surfactant mixtures. Water was added similarily in constructing the other phase diagrams. Upon further addition of water, some cloudiness of mixtures was observed visually at lipid content >30% w/w. However, the mixtures became clear again when the water content exceeded ~15-20% w/w. With continued addition of water to lipid/surfactant mixtures, patches of gels or cloudy emulsions were formed and ultimately, at a water content of 70% w/w and above, oil-in-water emulsions (turbid phase) were formed, except for the 1:9 w/w ratio of lipid to surfactant, where a micromemulsion or micellar solution (clear or translucent phase) was formed. It was interesting to note that there was also a clear or translucent zone in the phase diagram at around the 6:4 ratio in the monocaprylate phase diagram at relatively high water content. This anomalous behavior in the phase diagram was not investigated further in this study.

The results of particle size analyses of selected lipid/surfactant mixtures (9:1, 7:3, 1:1, 3:7 and 1:9 w/w) at 70% to 99% w/w are presented in Table 2. Only the mean particle sizes are listed and there was a normal distribution of particles around each mean. In general, particle size decreased for both lipids with an increase in water content. For a particular lipid, the greatest particle size was observed for the 9:1 w/w lipid/surfactant mixture and lowest particle size was observed for the 1:9 lipid/surfactant mixtures.

A comparison between the two lipids shows that the particle sizes obtained using of C_8-medium chain lipid (PG monocaprylate) at 7:3, 1:1, 3:7 and 1:9 w/w mixtures with Cremophor EL® were smaller than those obtained with C_{12}-medium chain lipids (PG monolaurate) at similar ratios with the surfactant.

A comparison of the particle size after 1 to 100 dilution with water (99% w/w water), which could be a good reflection of the dilution of a lipid-based preconcentrate in the gastrointestinal fluid, demonstrates that PG monocaprylate produced a microemulsion (particle size <200 nm) at lipid/surfactant ratios of 7:3 and lower, while PG monolaurate produced a microemulsion only with 1:9 lipid/surfactant mixture. Despite this difference, particle sizes for both PG monocaprylate and PG monolaurate after dilutions of their 7:3, 1:1, 3:7 and 1:9 w/w lipid/surfactant mixtures with water (90 and 99% w/w content) were still about 500 nm or less, indicating that both lipids produce fine emulsion particles and their performance in pharmaceutical dosage forms may not be significantly different (37).

The difference in particle size at the 9:1 w/w lipid/surfactant ratios may not be of practical significance for the development of self-microemulsifying dosage forms as emulsions with relatively large particle sizes in the micron range were formed for both lipids. The values ranged from 1 to 4 μm, except for PG monolaurate with 99% w/w water, where the particle size was ~500 nm.

Comparative phase diagrams of propylene glycol diesters with C_8 and C_{12} fatty acids

Figures 2A and 2C show the phase diagrams of the diesters of medium chain lipids with mostly C_8-fatty acids, while Figures 2B and 2D, respectively, provide phase diagrams of similar compositions of diesters of lipids with C_{12}-fatty acid chains. There were several considerations taken into account when selecting lipid diester components for these phase diagrams based on their chemical compositions.

As shown in Table 1, PG dicaprylocaprate (Captex 200P®) used in the present study is commercially available as the mixed ester of

both C_8-fatty acid (caprylic acid) and C_{10}-fatty acid (capric acid), although more of the caprylate than the caprate. On the other hand, PG dilaurate (Capmul PG-2L®) contained mostly the C_{12}-fatty acid (lauric acid). Thus, in the true sense, it is a comparison between a C_8/C_{10}-lipid and a C_{12}-lipid. However, since the larger fraction of PG dicaprylocaprate is C_8-fatty acid, it is regarded here as a C_8-fatty acid lipid. Another important consideration is that PG dilaurate is not available commercially in a pure diester form.

The Capmul PG-2L used as the dilaurate contained 75% dilaurate and 25% monolaurate, and thus it was essentially a 1:3-mixture of monoester and diester. On the other hand, the dicaprylocaprate, Captex 200P, was available with a higher diester concentration of approximately 90% w/w. Therefore, a comparison between commercially available C_8/C_{10}-fatty acid lipid (Captex 200P) with commercially available C_{12}-fatty acid lipid (Capmul PG-2L) was not meaningful as their monoester-to-diester ratios varied. It has been previously shown (33) that the degree of esterification of lipids has a major impact on phase diagrams.

For these reasons, Capmul PG-8 was mixed with Captex 200P at a 1:3 ratio to produce a degree of esterification of C_8/C_{10}-fatty acid lipid similar to the C_{12}-fatty acid lipid (Capmul PG-2L). Their respective phase diagrams are shown in Figures 2A and 2B.

A comparison of Figures 2A and 2B shows that lipids (1:3 mixture of mono- and di-ester) with the fatty acid chain lengths of C_8 (with some C_{10} present) versus C_{12} gave similar phase diagrams when mixed with Cremophor EL® and water. As water was added to the lipid/surfactant mixtures, both lipids initially gave a clear lipid phase of water-in-oil (w/o) microemulsion, which was then followed by gel formation and ultimately the formation of an oil-in-water (o/w) microemulsion or emulsion depending on the lipid-to-surfactant ratios.

The changes shown in the phase diagrams in Figure 2A may be followed in the direction of the arrow in Figure 1A. One significant difference between the two phase diagrams (Figures 2A and 2B) is that the gel phase, which was formed during phase transition from w/o microemulsion to o/w microemulsion or emulsion, was larger for the longer chain lipid (Figure 2B).

Table 2 Particle size (nm) after dilution of lipid/surfactant mixtures with water at different lipid to surfactant ratios. Individual values after two different determinations of particle sizes are given within parentheses

LIPIDS AND % WATER	LIPID/SURFACTANT RATIO				
	9:1	7:3	1:1	3:7	1:9
Propylene glycol monocaprylate (Capmul PG-8)					
70	4504 (3996,5011)	502 (464,539)	2115 (2549,1680)	4625 (4894,4355)	65 (44,85)
80	2847 (2488,3205)	371 (326,415)	579 (696,461)	931 (817,1044)	14 (13,14)
90	2507 (2570,2443)	222 (227,216)	81 (75,86)	327 (318,335)	12 (12,12)
99	1645 (1675,1615)	160 (157,163)	85 (81,89)	148 (144,151)	13 (13,14)
Propylene glycol monolaurate (Capmul PG-12)					
70	1358 (1316,1400)	1373 (1240,1505)	1516 (1453,1578)	1291 (1234,1348)	199 (200,198)
80	1110 (1076,1144)	650 (661,638)	558 (528,588)	885 (809,960)	127 (123,130)
90	1073 (1063,1083)	348 (396,300)	369 (373,365)	515 (546,484)	25 (24,25)
99	532 (508,555)	426 (447,405)	220 (222,218)	237 (264,200)	18 (18,17)
1:3 mixture of propylene glycol monocaprylate and propylene glycol dicaprylate					
70	399 (298,500)	510 (436,583)	389 (519,259)	419 (437,400)	199 (200,198)
80	345 (259,430)	304 (220,387)	174 (178,169)	31 (32,30)	127 (123,130)
90	398 (415,318)	188 (145,230)	71 (99,42)	24 (26,22)	25 (24,25)
99	2529 (3134,1924)	207 (214,199)	55 (70,40)	24 (23,25)	18 (18,17)
1:3 mixture of propylene glycol monolaurate and propylene glycol dilaurate (Capmul PG-2L)					
70	523 (469,577)	587 (455,521)	96 (81,110)	42 (37,46)	60 (57,63)
80	415 (374,456)	342 (383,300)	73 (66,80)	30 (32,27)	15 (15,14)
90	325 (301,348)	240 (200,280)	40 (39,40)	22 (21,23)	16 (15,16)
99	296 (266,325)	150 (110,189)	38 (36,40)	22 (19,24)	17 (17,17)

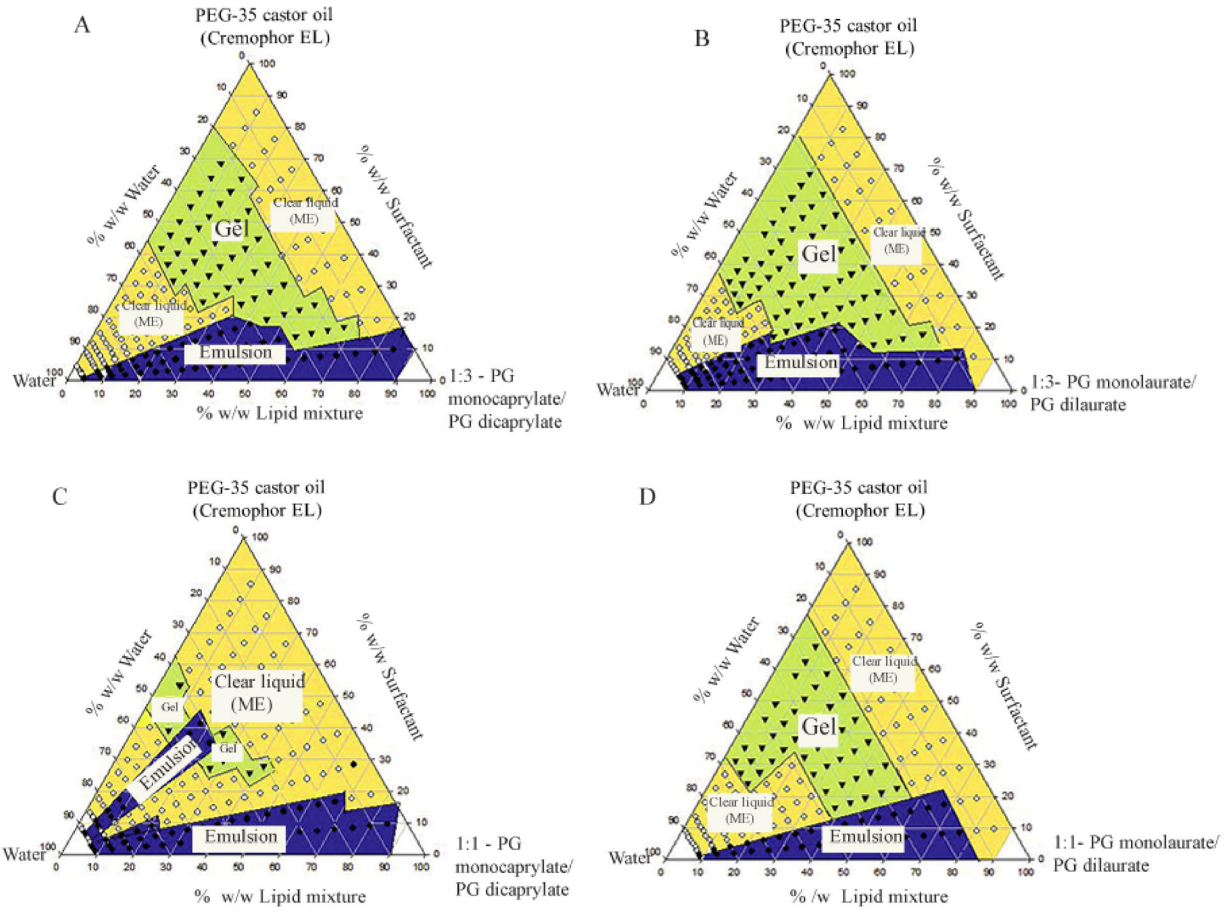


Figure 2 Phase diagram of (A) 1:3 mixture of propylene glycol monocaprylate to propylene glycol dicaprylate, (B) 1:3 mixture of propylene glycol monolaurate to propylene glycol dilaurate, (C) 1:1 mixture of propylene glycol monocaprylate to propylene glycol dicaprylate, and (D) 1:1 mixture of propylene glycol monolaurate to propylene glycol dilaurate, with PEG-35 castor lipid and water.

A comparison of particle size of the 1:3-mixture of PG monocaprylate and PG dicaprylate with that of the 1:3-mixture of PG monolaurate and PG dilaurate shows that the particle sizes at relatively high water contents (80% w/w and higher) were similar (Table 2), suggesting no major difference in performance of C_8 and C_{12} fatty acid in producing microemulsions or emulsions. One anomalous value in particle size was observed when the 1:3-mixture of PG monocaprylate and PG dicaprylate with 9:1 lipid-to-surfactant ratio was diluted 100 times with water (99% w/w water). The large particle size observed may be because there was insufficient surfactant present to emulsify the lipid mixture in presence of such an excess of water.

Since different mixtures of monoesters and diesters may be used in the development of lipid-based formulations, the effect of chain length (C_8 versus C_{12}) on phase diagrams was also studied by using 1:1 mixtures of monoester and diester. To prepare the 1:1-mixture of PG monoester and diester of predominantly C_8-fatty acid, Capmul PG-8 and Captex 200P were mixed at 1:1 w/w ratios, and to prepare the 1:1 mixture of PG monoester and diester of C_{12}-fatty acid, Capmul PG-12 and Capmul PG-2L were mixed at 2:3 w/w ratios. A comparison of Figures 2C and 2D and the particle size analysis data in Table 3 indicates that the general nature of the two phase diagrams is similar, with the major exception that the C_{12}-esters produced a large gel phase, which disappeared upon dilution with 50-70% w/w water. For the C_8-

esters, the gel phase was either minor or nonexistent. One interesting observation made in the phase diagram of the C_8-fatty acid esters (Figure 2C) by particle size analysis was that the particle sizes at high water content (~60% w/w and higher) initially decreased with the increase in surfactant concentration and then unexpectedly increased at around 3:7 ratios of lipid to surfactant (Table 3). The particle size again decreased upon further increases in surfactant concentration. The reason for such an effect of surfactant concentration on particle size was not investigated in the present study.

A direct comparison of the particle size produced by various lipids used in Figure 1 and 2 is shown graphically in Figure 3. A direct comparison between particle sizes of PG monocaprylate with PG monolaurate was possible in Figure 3 as both exist in relatively pure forms (> 90% monoester). However, to compare diesters, 1:3 mixtures of mono- and diesters were used as they were not available in pure forms. Lipid/surfactant mixtures at ratios of 7:3, 1:1 and 3:7 were used and particle size was determined after dilution of 1 gram of the mixture with 99 grams of water.

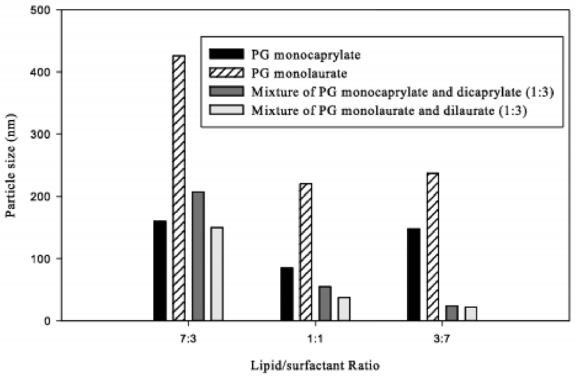


Figure 3 Particle size comparison of PG esters of C8 and C12 fatty acid at 99% w/w water anddifferent lipid to surfactant ratios.

The PG monoesters of both C_8 and C_{12} fatty acids gave larger particle size than those of the corresponding 1:3 mixture of mono- and diester. Comparing only the monoesters, the C_{12} fatty acid ester (PG monolaurate) appeared to provide a larger particle size than the C_8 fatty acid ester (PG monocaprylate). There was no major impact of lipid/surfactant ratio on the particle size of monoesters.

Table 3 Particle size (nm) after dilution of lipid/surfactant mixtures with water at different lipid to surfactant ratios

LIPIDS AND % WATER	LIPID/SURFACTANT RATIO				
	9:1	7:3	1:1	3:7	1:9
1:1 mixture of propylene glycol monocaprylate and propylene glycol dicaprylocaprate					
70	348 (315,380)	432 (507,356)	222 (225,218)	3980 (3947,4012)	19 (16,22)
80	274 (247,300)	228 (242,214)	38 (35,41)	1450 (1510,1390)	13 (12,13)
90	240 (220,259)	144 (145,143)	32 (24,39)	322 (311,333)	13 (12,13)
99	248 (225,270)	112 (108,116)	18 (18,18)	118 (109,127)	13 (11,15)
1:1 mixture of propylene glycol monolaurate and propylene glycol dilaurate					
70	284 (280,287)	176 (177,174)	192 (196,187)	98 (85,110)	194 (188,199)
80	214 (205,222)	114 (105,123)	158 (147,168)	64 (57,70)	21 (21,10)
90	250 (228,272)	85 (76,94)	86 (90,82)	41 (41,40)	16 (17,14)
99	387 (368,406)	75 (71,79)	56 (65,46)	53 (58,48)	16 (18,13)

On the other hand, there was no appreciable difference between particle sizes of the two diesters of C_8 and C_{12} fatty acids (containing 1:3 mixtures of mono- and diesters) and the particle size decreased with the increase in surfactant content of the mixtures.

The phase diagrams in the present investigation were determined and the particle size analysis was performed in absence of any dissolved drug. There were two reasons behind taking this approach. First, the main focus of the present investigation was to develop a basic understanding of the effect of carbon chain length on the phase behavior of medium chain lipids. It was more appropriate that this was ascertained in the absence of drug. Second, we demonstrated in a previous study that the presence of drug (2% w/w danazol) did not have any significant impact on the microemulsion regions of medium chain mono- and diglycerides with respect to both the area of the microemulsion phase and the size of lipid particles (33). It was, therefore, expected that the presence of drug would not have significant effect on the microemulsion regions of PG esters in the present investigation. Further, the effect of drug on the emulsion phase could not be studied as any phase separation of drug could not be distinguished from the cloudy emulsion.

Viscosity and significance of gel phase

The viscosities of the various gel regions shown in Figures 1 and 2 are presented in Table 4. Considering that the viscosities of water and glycerol under identical temperature are approximately 1mPa.s and 1000 mPa.s, respectively, the observed viscosity values <150 mPa.s may be considered low. Presumably due to their low viscosities, the gel phases easily converted to clear liquid (microemulsion) or milky white liquid (emulsion) upon further dilution with water in excess of around 60% w/w water. The viscosity of the gel phase initially increased with the increase in water content and then it decreased until the gel completely disappeared.

The tendency for gel formation was higher for lipids with C_{12}-fatty acid chains (larger gel region) than those for lipids with C_8-fatty acid chains, as evident from the larger gel region in Figures 1 and 2. However, as shown in Table 4, the gels formed were of low viscosity and the difference between lipid with C_8 and C_{12} fatty acids was not appreciable, except at the high surfactant content (2:8 lipid/surfactant ratios).

As we have reported previously (33), the gels were formed during the transition of mixtures from initial w/o microemulsion to o/w microemulsion or emulsion. There are several reports in the literature showing how various lipid/surfactant mixtures successively form a w/o microemulsion, a bicontinuous gel phase, and then an o/w microemulsion upon the addition of water (38-43). The gel, which is an intermediate phase occurring at the phase inversion from w/o to o/w microemulsions,

Table 4 Viscosity (mPa.s) of gels formed by lipid/surfactant mixtures following addition of water

LIPIDS USED AND% WATER	VISCOSITY AT DIFFERENT LIPID/Cremophor EL® RATIOS		
	5:5	4:6	2:8
PG monolaurate			
20	-[a]	-	73 (72,73)[b]
30	-	-	74 (77,71)
40	-	-	93 (97,89)
50	65 (64,66)	67 (63,71)	71 (76,66)
60	64 (67,60)	62 (67,57)	56 (54,57)
3:1 mixture of PG dicaprylocaprate and PG monocaprylate			
20	84 (85,83)	88 (85,91)	66 (64,68)
30	102 (105,99)	105 (106,103)	115 (116,114)
40	85 (87,83)	78 (79,77)	123 (124,121)
50	73 (77,69)	-	80 (84,76)
60	-	-	-
3:1 mixture of PG dilaurate and PG monolaurate			
20	89 (87,91)	75 (71,79)	124 (120,127)
30	97 (95,99)	95 (90,99)	148 (142,153)
40	109 (104,113)	117 (111,122)	131 (128,133)
50	88 (85,91)	122 (115,129)	116 (112,119)
60	92 (88,95)	99 (97,101)	94 (90,98)

[a](-) indicates that no gel was formed.
[b]Results of duplicate determinations of viscosity are given in parentheses

may be comprised of different structures beginning with a lamellar bilayer that may evolve into hexagonal phases in which surfactant molecules aggregate into circular cylindrical micelles and/or bicontinuous cubic phases (38, 42). A detailed investigation of the microstructure of the gel phase has not been conducted in the present study. However, it should be recognized that because of their low viscosities the formation of gels may not be an impediment for the conversion of preconcentrates into o/w microemulsions or emulsions after oral administration. Since certain lipid/surfactant mixtures form gels when in contact with water, the gel formation may also be advantageous in prolonging contact time and drug release, when applying the preconcentrates locally in the eye, nose or other non-oral body cavities.

Solubility

The solubilities of danazol and probucol in different lipids individually and in mixtures with each other or with the surfactant (Cremophor EL®) are given in Table 5. As stated in Table 1, danazol, a synthetic steroid, has low aqueous solubility and high octanol-water logP value, which makes it a good candidate for lipid-based formulations. Probucol is even less soluble in water, by a factor of at least 100, making it extremely insoluble in water. It also has a very high logP value. These properties suggest that enhancing solubility and dissolution using SEDDS/SMEDDS may lead to improved absorption and bioavailability of these compounds.

Since, as stated earlier, oral lipid-based drug delivery systems are usually developed as microemulsion preconcentrates primarily comprising of mixtures of lipids and surfactants, an adequate solubility of the API in lipids, surfactants and their mixtures is critically important for the successful development of such products.

Table 5 shows that both danazol and probucol had solubilities that were two and three orders of magnitude greater respectively than their water solubilities in the medium chain lipids used in the present study as well as in their mixtures with Cremophor EL®. The solubility of the compounds in lipids alone, however, decreased with the increase in fatty acid chain length. For example, solubilities of danazol and probucol in PG monocaprylate (C_8-fatty acid lipid) are 30 mg/g and 130 mg/g, respectively, as compared to 18 mg/g and 105 mg/g, respectively, in PG monolaurate (C_{12}-fatty acid lipid). As mentioned earlier, the solubility in PG dilaurate alone could not be determined as it was available only as a mixture with PG monolaurate. Therefore, the solubility of the compounds was determined in the 1:3-mixture of monolaurate and dilaurate and compared with those with the 1:3-mixture of monocaprylate and dicaprylate. These results with predominantly diesters also indicate that there is a trend of a decrease in the solubility of the compounds with the increase in chain length of diesters. This trend appears to be counterintuitive as one might expect that the longer the hydrophobic chain the greater is the

Table 5 Solubility of danazol and probucol at different ratios of lipid and surfactant (n=2)

	SOLUBILITY OF DANAZOL (mg/g)		SOLUBILITY OF PROBUCOL (mg/g)	
LIPID/ SURFACTANT	C_8 fatty acid	C_{12} fatty acid	C_8 fatty acid	C_{12} fatty acid
Monoester: Cremophor EL®[a]				
1:0[b]	30 (31,28)[d]	18 (17,19)	130 (127,133)	105 (103,106)
7:3	27 (28,26)	23 (24, 21)	128 (129,126)	113 (115,112)
1:1	28 (28,27)	26 (23,28)	129 (128,130)	94 (95,93)
3:7	27 (27,27)	28 (28,27)	88 (85,92)	99 (98,99)
0:1[c]	32 (31,32)	32 (31,32)	61 (59,62)	61 (59,62)
Mixture of mono/diester (1:3):Cremophor EL®				
1:0[b]	16 (16,15)	9 (8,10)	179 (177,181)	163 (163,166)
7:3	21 (22,20)	17 (16,18)	120 (122,118)	129 (126,131)
1:1	26 (28,23)	21 (20,21)	128 (129,126)	107 (109,105)
3:7	29 (30,28)	28 (28,27)	107 (109,104)	105 (103,108)
0:1[c]	32 (31,32)	32 (31,32)	61 (59,62)	61 (59,62)

[a]Cremophor EL®= PEG-35 Castor oil [b]Lipid only [c]Surfactant only [d]Values for duplicate determination of solubility are given in parenthesis

affinity of lipids for hydrophobic or poorly water-soluble drugs. However, as stated by Rane and Anderson (44), it is difficult to predict drug solubility in lipids and lipid/surfactant mixtures. It is possible that a certain degree of polarity that is present in a lipid with lower chain lengths is necessary to solubilize drugs as there could be an interaction between hydrophilic head groups of the lipids and the polar moieties in the APIs. The trend of decreasing solubility of both danazol and probucol with increasing fatty acid chain length diminished or practically disappeared when the relatively polar surfactant (Cremophor EL®) was mixed with the lipids. More studies are needed to examine complex interactions between APIs, lipids and surfactants.

Formulation development considerations

Many investigators have studied lipid/surfactant/water phase behavior of medium- and long-chain fatty acid lipids commonly used in pharmaceutical dosage forms (44-48). However, in most of the studies, the primary focus was on the identification of regions of microemulsion, and not the full determination and characterization of different phases within individual phase diagrams. The results of the present investigation provide a more complete picture of how mixtures of different medium chain lipids would behave when they are diluted with water, especially in the GI tract after oral administration. A formulation scientist will thus be able to select appropriate lipid/surfactant combination for a particular formulation. The particle size determination indicated whether an emulsion or a microemulsion would be formed when a lipid/surfactant mixture or preconcentrate was diluted with water (or the GI fluid). Although the formation of a microemulsion upon dilution with water is often desired, it is possible that a formulation scientist may not always require the formation of a microemulsion, that is, that the particle size of an emulsion may be small enough to meet the requirements of the dosage form (37). Thus, the results of the present investigation offer multiple options to the formulation scientist to make informed decisions when developing a dosage form.

Medium chain lipids with different fatty acid chain lengths are available commercially for application in the pharmaceutical industry. Even a particular lipid used in drug product development may be a mixture of two (or more) lipids with different chain lengths (e.g., PG dicaprylocaprate used in the present study). The results of the present study show that the difference in chain lengths of medium chain lipids may not have major impact on the performance of drug formulations when they are combined with surfactants in self-emulsifying formulations. On the other hand, the degree of esterification of lipids, as well as, the mixing of two lipids with different degrees of esterification greatly influences drug formulation. One of the most significant findings of the present investigation is the effect of combining PG monocaprylate with PG dicaprylocaprate. When the two lipids were combined at a 1:3 ratio, the area of the clear o/w microemulsion region increased considerably. When the two lipids were mixed at a 1:1 ratio further increase in the microemulsion region was observed and the gel region practically disappeared. As shown in Figures 2C and 2D, the initial lipid/surfactant mixture may contain at least 70% lipid and yet a microemulsion may be obtained after dilution with water. Thus, combining a monoester of medium chain fatty acid with a diester provides a unique opportunity for developing LFCS Type IIIA formulations by increasing lipid content. The increase in lipid content in a self-microemulsifying formulation may also reduce any propensity for the precipitation of drug after dilution of a formulation with aqueous media in the GI tract (27, 49, 50).

CONCLUSION

In recent years, much attention has been focused on lipid based drug delivery systems, and especially on self-emulsifying and self-

microemulsifying drug delivery systems. However, there is only limited literature on basic physicochemical properties of various available lipids to enable the selection of optimal lipids for drug formulation. In this study, we compared the effect of chain length of medium-chain fatty acid esters (C_8 versus C_{12}) of propylene glycol on phase diagrams and drug solubility. Both the PG monoesters and diesters were used. Microemulsions and emulsion regions formed by the relatively shorter (C_8) and the longer (C_{12}) chain lipids in combination with a surfactant (Cremophor EL®) and water were essentially similar. One major difference between them was that the gel regions of the C_{12}-fatty acid esters in the phase diagrams were larger than those with C_8-fatty acid esters. The formed gels were, however, of low viscosity and converted to microemulsions and emulsions at high water content (>60% w/w). The solubility of the two model APIs, danazol and probucol, increased significantly in all lipids studied as compared to their aqueous solubility, and a noticeable trend was that the aqueous solubility of drugs in lipids alone decreased with an increase in fatty acid chain length. This difference in solubility either diminished or practically disappeared when the lipids were mixed with the surfactant, Cremophor EL®. Thus, the length of medium chain lipids may have only a minor influence on dosage form development. In contrast, the degree of esterification, as well, as combining two lipids (mono- and diesters) may have a more pronounced effect. When the monoesters were mixed with their respective diesters, larger microemulsion regions with smaller lipid particle sizes were observed as compared to those with individual lipids.

ACKNOWLEDGMENTS

This study was supported, in part, with a generous research grant from the ABITEC Corporation, 501 W. 1st Avenue, Columbus, OH 43215. The authors wish to thank Dr. Donald Kelemen and Dr. Jim Williams from the ABITEC Corporation for helpful discussions and valuable suggestions.

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