In vitro dispersion test that could serve as a predictive method for assessing performance of lipid-based drug delivery systems.

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Original Article

ABSTRACT

A relatively simple *in vitro* dispersion test using the USP Dissolution Apparatus II filled with 250 ml of dispersion fluid (0.01M HCl) at 37°C and a rotation speed of 50 RPM was used to assess the performance of lipid-based formulations. Solutions of probucol in mixtures with the surfactant Cremophor® EL with four different medium chain lipids (glyceryl monocaprylocaprate, Capmul® MCM EP; glyceryl dicaprylate; glyceryl tricaprylate, Captex® 8000 EP/NF; caprylic/capric triglyceride, Captex® 355 EP/NF) were formulated and filled into Size 00 hard gelatin capsules (~1 g/capsule) for dispersion testing. Drug concentration in the dispersion fluid and the particle size of the dispersed phase as a function of time were measured with, and without, filtration through 0.45 micron filters. All the lipid/surfactant mixtures dispersed in <1 hour (>80%), indicating suitability for their use in immediate-release formulations. The particle size of the unfiltered samples confirmed whether a microemulsion (<250 nm), a very fine emulsion (250-1000 nm) or an emulsion with relatively larger globule sizes (>1000 nm) was formed. The dispersion test developed here could be used to screen different lipid-based formulations for *in vitro* performance. Justification for using an *in vitro* dispersion test to predict *in vivo* performance of lipid-based drug delivery systems has been provided.

KEY WORDS: Lipid-based drug delivery system, SEDDS, medium chain lipid, monoglyceride, diglyceride, triglyceride, probucol, drug solubility, drug dispersion

INTRODUCTION

Lipid based formulations are well known for increasing the absorption and bioavailability of poorly water-soluble lipophilic drugs (1-8). Such formulations range from simple oil solutions to complex self-emulsifying drug delivery systems (SEDDS) or self-microemulsifying drug delivery systems (SMEDDS). SEDDS and SMEDDS are generally preferred for developing pharmaceutical dosage forms as the drug is dissolved into a mixture of lipids and surfactants. Co-surfactants can be added optionally to improve emulsification and organic solvents may be further added to increase solubility. Such formulations are often referred to as the preconcentrate as they do not contain water. They form an emulsion or

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microemulsion only when they come in contact with the aqueous environment of the gastrointestinal (GI) tract after oral ingestion.

Despite the predominance of poorly water-soluble drugs in the discovery and development pipeline of the pharmaceutical industry (9) and much interest in lipid-based formulations in recent years, there is only a limited number of lipid-based formulations available on the market (4, 10-11). One major challenge in the development of lipid-based systems is that there are no definitive *in vitro* tests to predict the *in vivo* performance of different lipid-based formulations for a particular drug (6,12). However, there is a good understanding of what is expected from a lipid-based formulation to achieve good *in vivo* performance. Examination of two formulations of cyclosporine A (Neoral® and Sandimmune®; Novartis Pharmaceuticals Corp., Basel, Switzerland) available on the market showed that the formulation that dispersed in aqueous media as a microemulsion (Neoral®) rather than an emulsion (Sandimmune®) provided superior bioavailability with less food effect (13-14). To explain such a finding, Pouton (8) introduced a 'lipid formulation classification system' (LFCS) categorizing the lipid-based systems based on the particle size of the emulsion or microemulsion formed when the formulations are mixed with aqueous media. The Type III LFCS formulations that form microemulsions with particle sizes ranging from 50 to 250 nm provided the optimal *in vivo* performance of poorly water-soluble drugs. Since the drug is in a dissolved state within the lipid-surfactant globules of colloidal dimensions, it can be released without requiring further lipid digestion in the GI tract. Therefore, it is essential that any *in vitro* test employed to assess the performance of lipid-based formulations considers the rate and efficiency of emulsification and the resulting droplet size of the globules.

Serajuddin *et al.* (15) demonstrated in the early 1980s that the particle size of the resulting dispersed phase of the dispersion of a poorly water-soluble drug, i.e., Gelucire 44/14 (a lipid-like ester of polyethylene glycol and glycerin with long-chain fatty acids, Gattefosse Corp, Paramus, NJ, USA) had a major impact on the oral bioavailability of the drug. Instead of using dissolution media with sink conditions produced by the artificial enhancement of solubility using surfactants, co-solvents, pH adjustment and so on, the dispersion tests were conducted in physiologically relevant aqueous media under non-sink conditions. It was observed that if the drug dispersed in the aqueous media under non-sink conditions as submicron particles, the absorption of the drug improved in a more predictable manner (16). This is because the dissolution rate of the drug in the GI fluid, especially in the presence of bile salts, lecithin, lypolytic products and so on, increases greatly due to the very high surface area of the dispersed submicron particles. As a consequence of the redissolution step, it is not necessary that the drug be liberated from the lipid-based formulation as a solution, it could release the drug as fine particulates as long as they are dispersed (17-18). A similar dispersion test was later applied successfully in the development of optimal dosage forms of poorly water-soluble drugs dissolved in PEG-polysorbate 80 (19-21) and PEG-lipid-Cremophor® EL mixtures (22). Several other studies indicate the use of dispersion tests in assessing *in vitro* self-emulsifying carriers consisting of lipid-surfactant mixtures (2, 23-27). Ditner *et al.* (28) described a method where only the particle size after 1:100 and 1:1000 dilutions of SEDDS with artificial intestinal fluid was measured. There was no analysis of the drug in the aqueous dispersions.

Despite a clear demonstration of a relevant correlation of an *in vitro* dispersion to the *in vivo* performance of lipid-based or lipid-like formulations, the dispersion test has not yet been fully adopted by the pharmaceutical industry to evaluate such products. There are two primary reasons for this.

First, several studies suggested that, *in vitro* digestion rather than the *in vitro* dispersion, is a better indicator of the *in vivo* performance of lipid-based systems (29-32). Since there is a potential for the precipitation of the drug after lipolysis, they suggested that this could impede the bioavailability of the formulation. Other studies indicate that the precipitates produced after digestion exist in amorphous or finely divided states, which redissolve rapidly and may not impact drug absorption (33, 34). In most reported digestion tests, the precipitated drug remained dispersed in the aqueous media after digestion and was separated from the bulk liquid only by ultracentrifugation (32, 35).

Second, there is no commonly accepted method for conducting an *in vitro* dispersion test. While Serajuddin *et. al.* (15, 16) used the USP Dissolution Apparatus II to conduct the dispersion test, Nielsen *et. al.* (25) described a dispersion test method wherein a preconcentrate was added dropwise into an aqueous medium (0.7% w/v NaCl in water) whilst stirring (100 RPM) until it reached 1% of the medium. Emulsification, turbidity and apparent stability of the resultant emulsion were observed. Zaghloul *et al.* (36) also described a dispersion testing method, where 1 ml of a lipid-based formulation containing probucol was added to 900 or 1000 ml of water in an Erlenmeyer flask at room temperature and shaken by hand. The spontaneity of emulsification was characterized by visual observation. It was judged 'good' when a transparent emulsion was formed, 'bad' when there was poor or no emulsification, and 'fair' when in between the two states. In addition, the turbidity and droplet sizes of the various emulsions were determined. In yet another method, Buyukozturk *et. al.* (37) investigated the ability of different formulations to emulsify in aqueous media by adding 10 or 100 μl of the drug solution in lipid/surfactant mixture to 10 ml of water at 37°C, followed by vortexing for 30 seconds before assessing the emulsions formed.

To develop a systematic approach for selecting appropriate lipids for the development of lipid-based oral formulations for poorly water-soluble drugs, various lipid-surfactant mixtures were studied for their ability to form microemulsions or emulsions and a dispersion test was applied to evaluate several formulations (38-41). The USP Apparatus II dissolution method at 50 RPM using 250 ml of a pH 2 aqueous dispersion medium was usually used in these studies. The primary objectives of the present investigation were to apply the dispersion test to compare *in vitro* performance of several lipid-based formulations and to demonstrate that the method could be easily adapted for a general evaluation of different formulations during research and development providing a potential predictor of *in vivo* performance. Lipid-based formulations of a very poorly water-soluble drug, probucol, were developed by mixing four medium chain lipids at varying ratios with a common surfactant, Cremophor® EL® (PEG-35 castor oil). The lipids used were a monoglyceride (glyceryl monocaprylocaprate; Capmul® MCM EP), a diglyceride (glyceryl dicaprylate) and two triglycerides (glyceryl tricaprylate; Captex® 8000® and caprylic/capric triglyceride; Captex® 355 EP/NF®). The dispersion of the formulations in aqueous medium as a function of time was studied and the particle size of the dispersed lipid phase determined. Since there was a possibility that the solvent capacities of the preconcentrates could change upon dilution with the aqueous media resulting in drug precipitation (42-43), the study included the investigation of drug precipitation after the dispersion.

MATERIALS AND METHODS

Materials

Probucol, a neutral compound with extremely low aqueous solubility of 2-5 mg/ml (44) and a log P value of 11 (25), was selected as the model drug for developing the dosage form and testing the dispersion. Glyceryl mono-caprylocaprate (Capmul® MCM EP), glyceryl

dicaprylate, glyceryl tricaprylate (Captex® 8000 and caprylic/capric triglyceride (Captex® 355 EP/NF) were supplied by ABITEC Corp., Columbus, Ohio, USA. The structures of the lipids used have been reported previously (39). Glyceryl dicaprylate is not commercially available and it was prepared by ABITEC Corp. specifically for the present study. Cremophor® EL was supplied by BASF Corp., Tarrytown, NY, USA. All reagents and chemicals used were of analytical grade or better. The distilled water used was of the USP grade.

Solubility study

An excess amount of probucol was added to each 25 ml volumetric flask containing approximately 4 grams of lipid, surfactant or the lipid/surfactant mixture. The combination was vortexed for adequate wetting and distribution of the suspended drug, and the suspension was then shaken (24 hours at 25°C, maximum speed, Burrell Wrist Action Shaker, Burrell Scientific, Pittsburgh, PA, USA). Aliquots from different flasks were filtered through polypropylene filters (0.45 µm) and the filtrates were analyzed, after suitable dilution with methanol, for the drug concentration using HPLC. It was established at the outset of the study that a shaking period of 24 hours was sufficient to establish equilibration of drug as there was no further change in concentration when the shaking was continued up to 7 days.

Analysis of probucol

The HPLC analysis system for probucol consisted of a quaternary pump, an autosampler, and a diode array detector (HP1100 series, Agilent Technologies, Wilmington, DE). The chromatographic column used was a C_8 Waters X-Bridge column (3.5µm, 4.6 mm x 150 mm). A mixture of methanol and water (95:5 v/v) at a flow rate of 0.5 ml/min was used as the mobile phase and the UV detector wavelength was set at 243 nm.

Preparation of the test formulations

The drug was dissolved in lipid/Cremophor® EL mixtures at approximately 80% of the equilibrium solubility of each mixture. Approximately 1 g of each preconcentrate thus prepared was filled into a hard gelatin capsule (size 00). The lipid/surfactant mixtures without the drug were also filled in capsules for use as controls. Exact compositions of the capsule fill materials are given in Table 1.

Table 1 Compostions of test capsules

Lipid-surfactant mixture used	Weight of lipid surfactant mixture per capsule	Weight of drug per capsule
Capmul® MCM (glyceryl monocaprylocaprate): Cremophor® EL, w/w		
7:3	1.027	64
1:1	1.001	59
3:7	1.013	62
Glyceryl dicaprylate: Cremophor® EL, w/w		
7:3	---	---
1:1	1.000	77
3:7	0.977	73
Captex® 8000 (glyceryl tricaprylate):Cremophor® EL, w/w		
7:3	1.004	98
1:1	1.006	107
3:7	0.982	81
Captex® 355 (caprylic/capric triglyceride):Cremophor® EL, w/w		
7:3	0.980	99
1:1	0.976	120
3:7	1.018	80

The dispersion test

The dispersion test was performed using the USP dissolution apparatus II (paddle method, 50 RPM) with 250 ml of 0.01N HCl (pH~2.1) at 37° C. Capsules with different lipid/surfactant ratios (7:3, 1:1 and 3:7 w/w) were used as shown in Table 1. Aliquots were withdrawn (3 ml) from each vessel at 10, 15, 30, 60 and 120 minutes for particle size analysis using a DelsaNano C particle size analyzer (Beckman Coulter Inc., Brea, CA, USA), as well as, for the drug assay (HPLC). The particle size was analyzed without filtration, and the samples

were then filtered (0.45 μm) for drug analysis. In the particular case of 7:3 lipid/surfactant mixtures, the dispersion test was continued for up to 24 hours and the drug analysis performed both before and after filtration. Similar dispersion tests were employed for the control capsules (without probucol) and the particle sizes analyzed without filtration to determine the effect of the presence of the drug, if any, on the particle size. All experiments were carried out in triplicate.

Particle size determination

The particle sizes of the aliquots collected during the the dispersion test were measured using a dynamic light scattering technique with a DelsaNano C particle size analyzer. Approximately 2-3 ml of the sample was taken in a disposable plastic cuvette (Beckman Coulter disposable cell, Beckman Coulter Inc., Brea, CA, USA) for analysis.

RESULTS AND DISCUSSION

Solubility

To ascertain how much probucol should be incorporated into the various formulations, the solubility of probucol in lipids, surfactant and different lipid/surfactant mixtures was determined and is shown in Table 2. The lipids exhibited high probucol solubility in the order glyceryl tricaprylate (Captex® 8000) = caprylic/caprate triglyceride (Captex® 355 EP/NF) > glyceryl dicaprylate > glyceryl monocaprylocaprate (Capmul® MCM EP). The highest solubility of approximately 133 mg/g was obtained for the two triglycerides (Captex® 8000 and Captex® 355 EP/NF) which would be expected as both lipids are structurally similar, the only difference being the partial presence of C_{10}-fatty acids instead of C_8-fatty acids in Captex® 355 EP/NF. The solubility of probucol in the two glycerides was also similar. The drug solubility decreased as the hydrophilicity of the lipids increased with

Table 2 Solubility of probucol in lipids, surfactant and lipid-surfactant mixtures. Each solubility value is the average of two determinations, and individual numbers are given in parenthesis.

Lipid:Surfactant Mixture Used	Solubility of probucol (mg/g)
Capmul® MCM EP (glyceryl monocaprylocaprate):Cremophor® EL	
1:0[a] (neat lipid)	52 (51,52)
7:3	78 (79,77)
1:1	74 (74,73)
3:7	76 (79,73)
0:1[b]	61 (59,62)
Glyceryl dicaprylate: Cremophor® EL	
1:0[a]	97 (92,102)
7:3	106 (100,114)
1:1	96 (95,96)
3:7	94 (96,92)
0:1[b]	61 (59,62)
Captex® 8000 (glyceryl tricaprylate):Cremophor® EL	
1:0[a]	134 (132,136)
7:3	127 (126,129)
1:1	133 (131,135)
3:7	103(104,101)
0:1[b]	61 (59,62)
Captex® 355 EP/NF (caprylic/capric triglyceride):Cremophor® EL	
1:0[a]	133 (136,129)
7:3	134 (131,137)
1:1	154 (153,154)
3:7	98 (95,101)
0:1[b]	61 (59,62)

[a]Neat lipid [b]Neat surfactant

structural change from triester to diester and then to monoester, with Capmul® MCM EP having the lowest drug solubility of 52 mg/ml. The order of change in solubility of probucol with the structural change in lipids observed in the present study is understandable since the drug is extremely hydrophobic (log P = 11), it had the highest solubility in the triglycerides, which were the most nonpolar lipids used. However, the order in the change of solubility observed in the present investigation does not agree with our observation with another hydrophobic drug, danazol (log P = 4.53), where the solubility varied in the opposite order of triglyceride < diglyceride < monoglyceride (40). These results highlight that the prediction of solubility in various lipids is still difficult and further studies are needed to understand drug/lipid interactions (45).

The solubility of probucol in the surfactant,
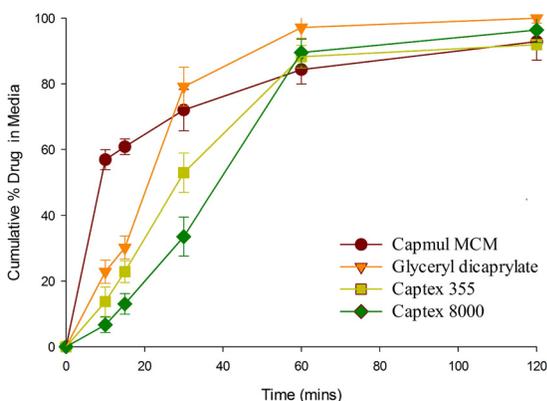
Figure 1 Cumulative % of probucol dispersed in 250 ml of 0.01N HCl at 37°C in formulations containing 3:7 w/w ratio of lipid and surfactant.

Drug concentrations in dispersion fluids as a function of time were initially determined by filtering samples through filters of 0.45 μm pore size. Preliminary experiments showed that the drug concentration in an unfiltered sample at each data point was approx. 2-5% higher. The primary consideration behind filtering samples was that it would filter out any precipitated drug. While over 90% of drug could be found in the filtered samples with 3:7 and 1:1 w/w lipid/surfactant ratios, the recovery of drug after dispersion for some of the formulations with lipid/surfactant (7:3) was only around 50%. For this reason, the dispersion test of 7:3 w/w lipid/surfactant was repeated by taking aliquots with, and without, filtration and the results are shown in Figure 3A with filtration and 3B without filtration. The particle size of the dispersed oil globules, with and without the presence of the drug, was measured only for the unfiltered dispersions and the results are shown in Table 3. The following is a more detailed description of the results of the dispersion test of the different formulations.

Cremophor® EL, was 61 mg. Although this value is much lower than the solubility of ~133 mg/g in the triglycerides (Captex® 8000 and Captex® 355 EP/NF), Table 2 shows that there was no appreciable decrease in the probucol solubility in the lipid/surfactant mixtures from that in the lipid alone, except when the ratio of lipid to surfactant was very low (3:7). Indeed, at 1:1 ratio of Captex® 355 EP/NF to Cremophor® EL, the solubility was much higher than that in the lipid alone. Additionally, for the monoglyceride and the diglyceride, there was no decrease in solubility when mixing the lipid with the surfactant, although the solubility in the surfactant was less than half that in the lipid. Rather, there appears to be a synergistic increase in solubility when glyceryl monocaprylocaprate (Capmul® MCM EP) was combined with Cremophor® EL.
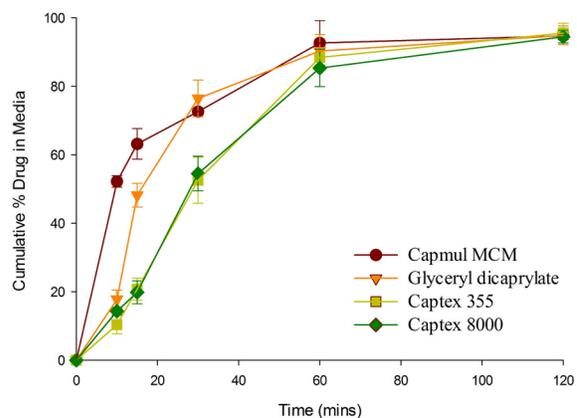
Dispersion test

The dispersion profiles of lipid/surfactant formulations (3:7, 1:1 and 7:3 w/w) in HCl (0.01N) are shown in Figures 1, 2 and 3, repsectively. All formulations were dispersed in <1 hour, which is much shorter than the typical residence time of 3 to 5 hours in the small intestine, where the drug is expected to be absorbed (46).

Dispersion of 3:7 and 1:1 lipid/surfactant mixtures

As shown in Figures 1 and 2, more than 80% of the drug dispersed in 60 minutes from the 1:1 and 3:7 lipid/surfactant mixtures, and the dispersion was more than 90% in 120 minutes.

Figure 2 Cumulative % of probucol dispersed in 250 ml of 0.01N HCl at 37°C in formulations containing 1:1 w/w ratio of lipid and surfactant.

Table 3 Particle size lipid globules at different time intervals of dispersion test from different lipid-surfactant mixtures (test formulations) in 250 ml of 0.01N HCl at 37°C.

LIPIDS USED & TIME (mins)	PARTICLE SIZE (nm)*					
	3:7 Lipid:Surafactant Mixture		1:1 Lipid:Surafactant Mixture		7:3 Lipid:Surafactant Mixture	
	Control	With drug	Control	With drug	Control	With drug
Capmul® MCM EP(Glyceryl monocaprylocaprate)						
10	428 ± 1	418 ± 31	420 ± 30	420 ± 6	-	-
15	471 ± 1	481 ± 24	397 ± 30	387 ± 28	338 ± 62	344 ± 62
30	502 ± 6	484 ± 14	475 ± 20	407 ± 12	709 ± 77	364 ± 57
60	502 ± 10	488 ± 12	442 ± 16	406 ± 22	792 ± 51	919 ± 46
120	538 ± 24	522 ± 31	505 ± 22	479 ± 30	1142 ±63	1123 ± 16
180	-	-	-	-	1055± 65	1099 ± 44
240	-	-	-	-	884 ± 44	1184 ± 73
Glyceryl dicaprylate						
10	74 ± 9	68 ± 5	111 ± 20	110 ± 9	-	-
15	77 ± 4	75 ± 1	118 ± 6	124 ± 9	-	-
30	76 ± 9	84 ± 6	120 ± 4	127 ± 4	-	-
60	90 ± 7	89 ± 1	128 ± 3	133 ± 10	-	-
120	89 ± 1	80 ± 9	124 ± 1	134 ± 10	-	-
Captex® 8000 (Glyceryl tricaprylate)						
10	32 ± 2	32 ± 2	123 ± 8	121 ± 2	-	-
15	30 ± 1	32 ± 1	161 ± 7	134 ± 1	278 ± 8	329 ± 19
30	31 ± 2	32 ± 2	179 ± 6	168 ± 7	283 ± 7	295 ± 14
60	28 ± 1	32 ± 3	178 ± 10	180 ± 2	293 ± 14	309 ± 19
120	28 ± 1	31 ± 1	172 ± 13	181 ± 5	301 ± 5	288 ± 14
180	-	-	-	-	306 ± 7	278 ± 22
240	-	-	-	-	321 ± 6	322 ± 80
Captex® 355 EP/NF (Caprylic/capric triglyceride).						
10	33 ± 5	38 ± 5	145 ± 1	126 ± 1	-	-
15	33 ± 7	33 ± 1	194 ± 6	165 ± 6	359 ± 5	339 ± 44
30	32 ± 5	35 ± 1	215 ± 4	178 ± 9	357 ± 26	296 ± 23
60	41 ± 12	32 ± 1	220 ± 2	178 ± 1	349 ± 21	294 ± 20
120	31 ± 6	32 ± 1	214 ± 6	196 ± 10	336 ± 21	291 ± 26
180	-	-	-	-	334 ± 22	278 ± 22
240	-	-	-	-	318 ± 21	283 ± 9

*n = 3
(-) = not determined

From the approximate slopes of the profiles observed in these figures, the rate of dispersion was found to be glyceryl monocaprylocaprate > glyceryl dicaprylate > glyceryl tricaprylate = caprylic/capric triglyce-ride. The difference in the rate of dispersion may be correlated with the possible gel formation by lipid/surfactant mixtures upon initial contact with aqueous media (40). Therefore the delay in the dispersion of the triglyceride/surfactant and diglyceride/ surfactant mixtures compared to the monoglyceride/surfactant mixtures is attributed to gel formation when the capsule contents came in contact with the dispersion medium. It was observed that as soon as the capsules containing the mixtures of a diglyceride or a triglyceride with the surfactant opened during the dispersion and the content came in contact with the dilute HCl, gels formed. The gels initially remained at the bottom of the vessel and dispersed gradually over time. In contrast, there was practically no
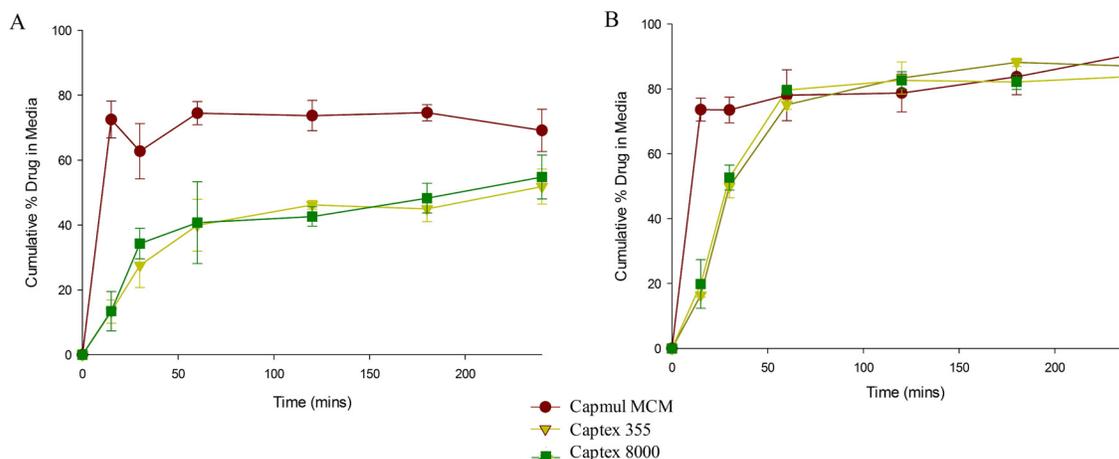
Figure 3 Cumulative % of probucol dispersed in 250 ml of 0.01N HCl at 37 °C in formulations with 7:3 w/w ratio of lipid and surfactant when filtered (A) and unfiltered (B) samples were used. No dispersion test for formulations containing glyceryl dicaprylate was performed as the results were expected to fall in between monocaprylate and tricaprylate.

gel formation with the monoglyceride/surfactant mixture. A faster dispersion rate was observed for the diglyceride compared to the triglycerides, which can be attributed to the difference in the viscosity of the gels formed, the gel formed by a diglyceride was reported to be less viscous than that of a triglyceride (40).

Filtered dispersions were used to determine the drug concentrations shown in Figures 1 and 2 with over 90% of drug recovered from filtered glyceryl monocaprylocaprate dispersions, thus indicating no precipitation of drug during the test period of 2 hours. As shown in Table 3, the 3:7 and the 1:1 lipid/surfactant mixtures containing the diglyceride and the two triglycerides formed microemulsions with particle sizes ~200 nm or less, and therefore the dispersions could pass through the filter (0.45 μm) and most of the drug could be measured in the filtrate. These results, therefore, indicate that both filtered and unfiltered media may be used during the dispersion test when the particle size of the dispersed phase is less than the pore size of the filter used. As mentioned previously, the difference in the drug content between filtered and unfiltered samples is approximately 1-2%. It is interesting to note that although the

average particle size of glyceryl monocaprylocaprate dispersions was around 400 to 500 nm, approximately 90% of the drug could still be recovered from the filtered dispersion fluid despite the cut-off particle size for filtration being 450 nm. These results indicate that oil globules with a size >450 nm may still pass or 'squeeze' through the filters. This would not be the case if the particles were solid, and it may, therefore, be assumed that few, if any, solid drug particles with particle sizes greater than 450 nm were formed during the dispersion testing within a period of 2 hours.

Dispersion of 7:3 lipid/surfactant mixtures

For the 7:3 w/w lipid/surfactant mixtures, formulations containing the monoglyceride (Capmul® MCM EP) and the two triglycerides (Captex® 355 EP/NF and Captex® 800) were used for the dispersion test. The diglyceride was excluded from this part of the study as it was expected, based on the results for the 3:7 and 1:1 w/w mixtures, that they would be in between those of the monoglyceride and the triglyceride. When the drug was analyzed in unfiltered dispersions, approximately 80% of the drug could be observed in the dispersion

fluids from all three formulations (Figure 3A). Since the surfactant content of the formulations was relatively low, an oily layer was observed on the inner surface of the dispersion vessels and inside the pipettes used to transfer fluids, which could be responsible for less than 100% drug concentrations in the dispersion media. In contrast to the unfiltered samples, incomplete drug dispersion from the two formulations containing triglycerides was observed when the samples were filtered through 0.45 μm filters, where only approximately 40-50% drug could be measured in the dispersion fluids. One possibility for the difference between filtered and unfiltered samples could be that there was precipitation of the drug. However, no such precipitates of drugs could be observed by microscopic examination of the unfiltered samples under cross-polarized light, and there was no separation of any drug crystals even when the samples were centrifuged (4000 RPM). The dispersion test was continued for up to 4 hours to investigate the possibility of any precipitation of the drug. If there was any precipitation at all, the particle size of the precipitate would increase with time and the drug concentration in the dispersion medium after filtration would further decrease. However, the concentration of drug in the dispersion remained practically constant and no crystalline drug could be identified in the dispersion fluid. Therefore, the possibility of drug precipitation was eliminated.

Unlike the triglycerides, approximately 80% of the drug concentration was observed in the filtered dispersion fluids of the monoglyceride formulation. Although the monoglyceride (Capmul® MCM EP) gave the largest globule sizes among the three lipids (Table 3), it did not exhibit any appreciable difference in the drug concentration between the filtered and unfiltered samples. At the 7:3 ratio of Cremophor® EL, all three lipids formed emulsions rather than microemulsions. However, being liquid, the lipid phase could in theory pass or 'squeeze' through the filters irrespective of globule size. It is apparent that while the relatively more hydrophilic

monoglyceride (HLB 6) could pass through the polypropylene filter that was used to filter aqueous solutions, the hydrophobic triglyceride (HLB 2) was partially retained on filter. These results indicate that although the dispersion test of lipid-based formulations in aqueous media may be performed by using both filtered and unfiltered samples when microemulsions (<250 nm) are formed, the dispersion test for formulations forming emulsions in aqueous media should preferably be conducted using unfiltered aliquots.

Particle size

The results of the particle size determination during the dispersion test indicate that 1:1 and 3:7 w/w lipid/surfactant mixtures of diglyceride and triglyceride produced microemulsions upon dispersion in aqueous media (<250 nm), while the monoglyceride produced emulsions with relatively larger particle sizes (400 to 500 nm). At the higher lipid/surfactant ratio of 7:3 w/w, the three lipids (one monoglyceride and two triglycerides) produced emulsions (>250 nm), although the particle sizes were still relatively low (approximately 1 μm or lower). In general, the particle size of the lipid globules decreased with an increase in the surfactant concentration in the formulations. Table 3 also shows that the particle sizes in the dispersion tests were highly reproducible, there was practically no effect of the drug on particle size, and the size did not change appreciably over a test period of 2 hours.

Significance of the dispersion test

The significance of the dispersion test on the potential *in vivo* performance of self-emulsifying drug delivery systems is illustrated in Figure 4. Once a self-emulsifying drug delivery system (SEDDS), which is also called an emulsion or microemulsion preconcentrate, comes into contact with the fluids of the gastrointestinal tract, it could form (1) a microemulsion with a particle size of <250 nm, (2) a very fine emulsion with a particle size in the range of 250
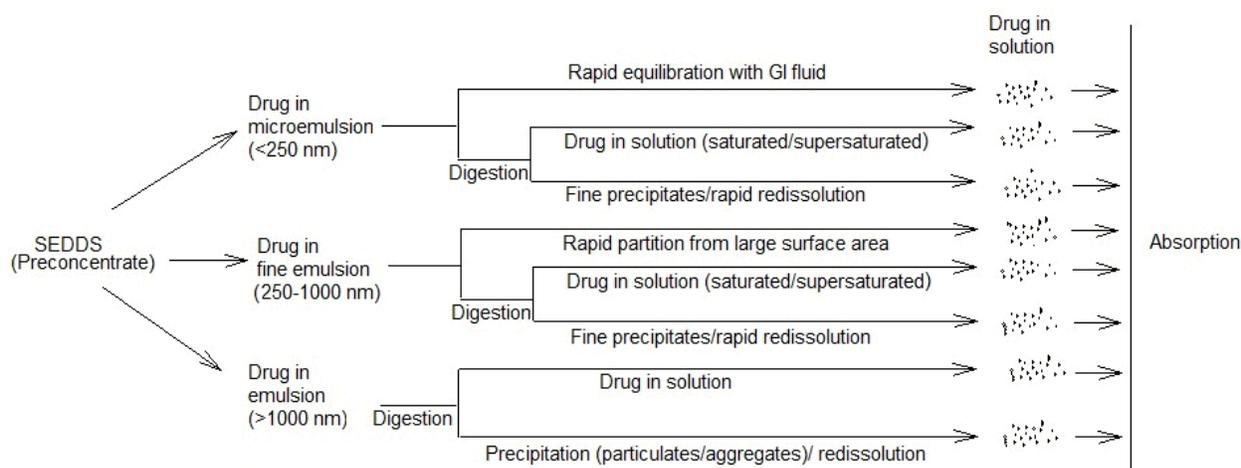
Figure 4 Schematic representation of the fate of self-emulsifying drug delivery systems (SEDDS) after the dispersion in the gastrointestinal fluids with different particle sizes forming microemulsion (<250 nm), fine emulsion (250-1000 nm), and emulsion with relatively larger particle size (>1000 nm).

to 1000 nm or, (3) a coarse emulsion with a particle size >1000 nm. The possible impacts of these alternatives on the *in vivo* performance of lipid-based formulations are discussed below.

Microemulsion

Although there has been some controversy over whether microemulsions are emulsions or a micelles (47, 48), it is now well recognized that they are thermodynamically stable micellar or swollen micellar systems containing a mixture of a lipid, surfactant and water with a particle size of <250 nm (49). Because of the micellar structure, various components of micro-emulsions, such as a surfactant, lipid and drug, are in dynamic equilibrium with individual molecules in the bulk phase. In addition, microemulsions are continuously disintegrating and reassembling within milliseconds to seconds (50). Due to these phenomena, drug release from microemulsions during the intestinal drug absorption process could be extremely rapid. As indicated by Pouton (8), no digestion of the lipid is necessary to facilitate drug release from microemulsions during absorption. Even if the lipid is digested in the GI fluid, as shown by several other studies, it is expected that the drug will exist in an extremely dispersed state and will not re-aggregate. For

example, if is assumed that 1 g of the preconcentrate containing 100 mg of drug is taken orally and it forms a microemulsion in 250 ml of the stomach fluid, the drug will be distributed in the total volume of fluid as extremely fine packets of lipid-surfactant mixtures with a particle size less than 250 nm. The likelihood is, that in most microemulsion systems, the particle size will be <100 nm. Now, if the microemulsion of such a small size is subsequently digested in the intestine, the particle size of any drug precipitating from individual microemulsion particles will be extremely low. Under the dynamic environment of the GI tract, the potential for the agglomeration of such particles before they are dissolved is remote. The agglomeration may happen only under static condition of the *in vitro* digestion, and even there the precipitates can be separated from the aqueous phase only after centrifugation at a high speed.

Fine Emulsion (250-1000 nm)

Instead of forming microemulsions, in many cases the SEDDS result in fine emulsions with a particle size in the range of 250 to 1000 nm (38-40). For such particles, the drug may be released from the lipids in two different ways: (1) the drug may rapidly partition from the lipid

phase to the aqueous phase due to the very high surface area of such emulsion particles, or (2) the lipids may be digested in the GI fluid and liberate the drug in a molecular or finely dispersed state. Due to the very small size of the emulsion globules, any drug liberated upon digestion of the lipids would also be very small and finely dispersed. It is expected that such a nanoparticulate drug would be rapidly dissolved and absorbed. Thus, the fine emulsion and the microemulsion may behave alike. Indeed, Nielsen *et al.* (51) showed that when the particle size of the lipid globules is very small, any differences in particle size is unlikely to have a practical significance on the bioavailability of the dissolved drug.

Coarse Emulsion (>1000 nm)

As reported by Pouton (8), lipid digestion may play a major role in the drug release from larger lipid globules in coarse emulsions, which may have a wide range of globule sizes above 1000 nm. Depending on the globule size and drug load, there is a potential that any drug precipitating from such systems may aggregate and redissolve relatively slowly. Therefore, one objective of developing SEDDS would preferably be to produce emulsions with droplet sizes in the submicron range as opposed to phase separated large emulsion globules.

Based on the above considerations, the rate of dispersion of the SEDDS and the globule sizes of the dispersed phase may serve as useful indicators of the *in vivo* performance of the different formulations. This study provides a practical method for carrying out dispersion tests. A volume of 250 ml of 0.01M HCl at 37°C was used to approximate the gastric fluid volume after the oral intake of a capsule with a glass of water. If necessary, other fluids of the same volume may be used. The USP Dissolution Apparatus II, which is commonly available in most pharmaceutical development laboratories, was used for the dispersion test with a moderate agitation speed of 50 RPM. The method can be easily standardized and if

necessary, sampling and analysis could be automated.

CONCLUSIONS

Several formulations of probucol using medium chain lipids together with a surfactant, Cremophor® EL, were developed and then tested for *in vitro* performance. The solubility of a poorly water-soluble drug, probucol, in medium chain lipids differed greatly depending on the degree of esterification of the glyceryl moiety. The solubility of the drug in the two triglycerides used (~133 mg/g) was 2.5 times that in the monoglyceride (52 mg/g), and the solubility in the diglyceride (97 mg/g) was in between those of triglycerides and the monoglyceride. With the addition of Cremophor® EL (having low drug solubility, 61 mg/g) to di- and triglycerides having higher drug solubility at 7:3 and 1:1 ratios of lipid to surfactant, there was no decrease in drug solubility in the mixture, rather, the solubility increased in some cases. The rate of dispersion of lipid/surfactant mixtures in aqueous media and the particle size of microemulsions and emulsions formed may also differ depending upon the nature of the lipids used. The di- and triglyceride formulations produced slower dispersion rates than that of the monoglyceride due to gel formation when they came in contact with water. However, all lipid/surfactant mixtures still dispersed in <1 hour, indicating their suitability for the development of immediate-release formulations. In contrast to the rate of dispersion, di- and triglycerides exhibited better emulsification and smaller particle sizes than the monoglyceride. It is expected that the results presented will help in selecting medium chain glycerides for the development of lipid-based drug delivery systems for poorly water-soluble drugs. The *in vitro* dispersion test used was capable of differentiating between various formulations

and the test could serve as a predictive tool to assess *in vivo* performance of such formulations.

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