AAPS PharmSciTech, Vol. 13, No. 2, June 2012 (© 2012)
DOI: 10.1208/s12249-012-9773-8

Research Article

The Studies of Phase Equilibria and Efficiency Assessment for Self-Emulsifying Lipid-Based Formulations

Ahmad Abdul-Wahhab Shahba,[1] Kazi Mohsin,[1,2] and Fars Kaed Alanazi[1]

Received 6 December 2011; accepted 6 March 2012; published online 23 March 2012

Abstract. The study was designed to build up a database for the evaluation of the self-emulsifying lipid formulations performance. A standard assessment method was constructed to evaluate the self-emulsifying efficiency of the formulations based on five parameters including excipients miscibility, spontaneity, dispersibility, homogeneity, and physical appearance. Equilibrium phase studies were conducted to investigate the phase changes of the anhydrous formulation in response to aqueous dilution. Droplet size studies were carried out to assess the influence of lipid and surfactant portions on the resulted droplet size upon aqueous dilution. Formulations containing mixed glycerides showed enhanced self-emulsification with both lipophilic and hydrophilic surfactants. Increasing the polarity of the lipid portion in the formulation leaded to progressive water solubilization capacity. In addition, formulations containing medium chain mixed glycerides and hydrophilic surfactants showed lower droplet size compared with their long chain and lipophilic counterparts. The inclusion of mixed glycerides in the lipid formulations enormously enhances the formulation efficiency.

KEY WORDS: droplet size analysis; lipid-based drug delivery systems; phase diagram study; self-emulsification assessment; self-emulsifying formulations.

INTRODUCTION

For drugs with sufficient lipophilicity, formulating the drug substance in a self-emulsifying lipid-based formulation has been recently introduced as an attractive option to enhance the oral bioavailability of poorly water soluble drugs (PWSD) (1–3).

A self-emulsifying/microemulsifying drug delivery system (SEDDS/SMEDDS) is an oral lipid dosage form which comprises a mixture of oils, surfactants and possibly cosolvents that has the ability to form fine oil in water (o/w) emulsion or microemulsion upon mild agitation following dilution with an aqueous phase (4,5). This property renders SEDDS/SMEDDS as good candidates for oral delivery of PWSD with adequate solubility in oil or oil/surfactant blends (6,7). These systems self-emulsify in the stomach and present the drug in small droplets of oil, thus they improve drug dissolution through providing a large interfacial area for partitioning of the drug between the oil and GIT fluid (8).

Upon dilution, SEDDS typically produce emulsion with a droplet size between 100 and 300 nm, while SMEDDS form transparent microemulsions with a droplet size of less than 50 nm (5).

The formulation of SEDDS sounds to be comparatively simple; all what is required is to incorporate the drug into a suitable oil–surfactant mixture, and then the mixture could be filled in a soft or hard gelatin capsules. However, the selection of the formulation components and their relative quantities in the formulation is very tricky. Only very specific pharmaceutical excipient combinations could lead to efficient self-emulsifying systems. Past studies have shown that the self-emulsification process is specific to the nature of the oil/surfactant pair, surfactant concentration, oil/surfactant ratio and temperature at which emulsification takes place (5).

The prime objective of the present study is to build a parameter for excipient selection to develop successful self-emulsifying formulations. Within the scope of the present studies, the recent lipid formulation classification system (LFCS) can be used to compare the performance of lipid formulations. LFCS was designed earlier by Pouton (9,10) according to the lipophilic content of the formulations. The fundamental differences between type I, II, III, and IV formulations are summarized in Table I (9–11). In practice, it is not always easy to physically distinguish between LFCS types II, III, and IV; except that the expectation is that particle size will be increasingly fine in the numerical order they are presented.

Within the requirement of the current project, it was planned to investigate various self-emulsifying lipid formulations using wide ranges of oils composed of medium chain as well as long chain mono-, di-, and triglycerides, lipid soluble cosolvents, and commonly used non-ionic surfactants, then most importantly to study the equilibrium phase behavior by constructing a series of phase diagrams of oil–surfactant–water systems. The particle size distribution of the formulations after

[1] Kayyali Chair for Pharmaceutical Industries, Department of Pharmaceutics, College of Pharmacy, King Saud University, P.O. Box 2457, Riyadh, 11451, Kingdom of Saudi Arabia.
[2] To whom correspondence should be addressed. (e-mail: mkazi@ksu.edu.sa)

Table I. The Lipid Formulation Classification System (LFCS), Adapted from Refs. (9–11)

			Type III		
	Type I	Type II	IIIA	IIIB	Type IV
Composition	Oils without surfactants	Oils and lipophilic surfactants	Oils, hydrophilic surfactants, and cosolvents		Hydrophilic surfactants and co-solvents (oil free)
Oils (%)	100	40–80	40–80	<20	–
Surfactants (%)	–	20–60 (HLB<12)	20–40 (HLB>12)	20–50 (HLB>12)	50–100 (HLB>12)
Cosolvents (%)	–	–	0–40	20–50	0–50
Drug delivery system	Digestible oils	SEDDS	SEDDS	SEDDS/SMEDDS	SMEDDS
Particle size of dispersion (nm)	Coarse	100–250	100–250	50–100	<50

Increasing hydrophilic content →

aqueous dispersion and the appearance of the emulsion droplets were also analyzed as a function of different lipid compositions in classification systems.

Thus, the overall studies were carried out to obtain a comprehensive understanding of how phase behavior varies for different lipid–surfactant compositions as they are diluted with water.

MATERIALS AND METHODS

Materials

Detailed information about the composition and characteristics of the utilized materials are summarized in Table II.

Self-Emulsification Assessment

A visual test to assess the self-emulsification properties reported earlier (12,13) was modified and adopted in the present study. The visual test is mainly designed to measure the apparent spontaneity of emulsion formation against time. Formulation (100.0 µl) was subjected to 1:400 aqueous dilution in a 50.0-ml glass beaker at room temperature (RT), and the contents were gently mixed using a magnetic stirrer (at ~500 rpm) (14).

The self emulsification test was a combined test used for the evaluation of the excipients miscibility, formulation spontaneity, homogeneity, dispersibility, and appearance after aqueous dilution as follows:

- The blends of different excipients such as oils, surfactants, and/or cosolvent mixtures were examined carefully to evaluate the mutual miscibility between the components.
- The spontaneity of the formulation was judged as "good" when the droplets easily spread in water, to form emulsion, within 1 min. It was judged as "moderate" when the droplets took 1–10 min to completely spread in water. Finally, the formulation was judged as "poor" when the droplets tend to coalesce, needed high shear mixing, and/or took >10 min to completely spread in water.
- The homogeneity of the formulation was judged as "good" when the formulation was able to spread in water without causing any phase separation. It was termed as "moderate" when the formulation tends to spread in water leaving some turbidity or flakes on the top. Finally, it was termed as "poor" when the formulation resulted in phase separation upon aqueous dilution.
- The dispersibility of the formulation was judged as "good" if the formulation globules were able to completely disperse in water without any suspended particles. It was termed as "moderate" if the formulation tends to leave few suspended particles which tend to disappear at higher temperature (above 37 °C). Finally, it was termed as "poor" if the formulation resulted in non-dispersible flakes.

For further characterization, the optical density (OD) of each diluted formulation was assessed at 600.0 nm by UV-visible spectrophotometer (UVD-3200, Labomed Inc.,USA) with 10 mm glass cuvette, using distilled water as blank (15,16). The formulation appearance and clarity were categorized according to the following OD_{600} ranges: (a) Transparent: (OD_{600}=0–0.05), (b) Bluish (semi-transparent): (OD_{600}=0.05–0.1), (c) Turbid: (OD_{600}=0.1–0.3) and (d) Milky: (OD_{600}>0.3).

Equilibrium Phase Studies

Ternary and pseudo-ternary phase diagrams of oils, surfactants, cosolvents, and water were constructed representing lipid formulations at various stages of aqueous dilution. Each phase diagram was constructed by preparing and screening the phase behavior of 81 different samples. The primary blends were prepared by varying the ratio of oil mixture to surfactant as 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9 (*w*/*w*). Each blend was thoroughly mixed and subsequently titrated with water at different percentages (10 %, 20 %, up to 90 %). Samples were stored in glass tubes (12×100 mm) with watertight closures for further examination. Samples were examined at RT (20±2 °C), and then equilibrated in water bath (SW22 Julabo, LABORTECHNIK, GMBH, Germany) at 37 °C for 48 h to provide sufficient time for all the temperature-related changes to take place (17,18). Each mixture was visually observed at both RT and 37 °C for phase clarity and flow ability.

The efficient self-emulsifying formulations were selected on the basis of their characteristic dilution profiles. It is reasonably desirable to select self-emulsifying systems that are able to solubilize large volumes of water during dispersion (18,19).

Table II. Materials List

Name	Function	Composition and description	Manufacturer
Miglyol 810 (M810)	Oil	Medium chain triglycerides (MCT, 72 % C_8 and 27.5 % C_{10})	Sasol GmbH, Witten, Germany
Captex 355 (Cap355)	Oil	Medium chain triglycerides (MCT, 56 % C_8 and 44 % C_{10})	Abitec, Columbus, USA
Imwitor 988 (I988)	Oil	Mixture of medium chain mono and di glycerides (MCDM)	Sasol GmbH, Witten, Germany
Imwitor 308 (I308)	Oil	Medium chain mono-glycerides (MCM, 90 % mono-caprylate)	Sasol GmbH, Witten, Germany
Soybean oil (Soy)	Oil	Long chain triglycerides (LCT, 25 % oleic acid, 54 % linoleic acid)	Croda, East-Yorkshire, England
Arachis oil (Ar)	Oil	Long chain triglycerides (LCT, 56 % oleic acid, 44 % linoleic acid)	Winlab, Gemini-house, England
Maisine 35-1 (M35-1)	Oil	Long chain mono-glycerides (LCM), mainly, with di-and tri-esters	Gattefossé, Saint-Priest, France
Oleic acid (OL)	Oil	Long chain fatty acid (LCFA) (mono-unsaturated, C18)	Avonchem, Cheshire, England
Linoleic acid (LN)	Oil	Long chain fatty acid (LCFA) (poly-unsaturated, C18)	Fluka Chemika AG, Switzerland
Span 80	Non-ionic surfactant	Sorbitan monooleate, water-insoluble (HLB=4.3)	Fluka Chemika AG, Switzerland
Span 20	Non-ionic surfactant	Sorbitan monolaurate, water-insoluble (HLB=8.6)	BDH, Prolabo, England
Tween 85 (T85)	Non-ionic surfactant	Polyoxyethylene (20) sorbitan trioleate, water-insoluble (HLB=11)	Merck-Schuchardt OHG, Germany
Tween 80 (T80)	Non-ionic surfactant	Polyoxyethylene (20) sorbitan monooleate, water-soluble (HLB=15)	BDH, Prolabo, England
HCO-30	Non-ionic surfactant	Polyoxyethylene hydrogenated castor oil, water-soluble (HLB=11)	Nikko Chemicals Co., Tokyo, Japan
Cremophor EL (Cr-EL)	Non-ionic surfactant	Polyoxyl 35 Castor Oil, water-soluble (HLB=12–14)	BASF, Ludwigshafe, Germany
Cremophor RH40 (Cr-RH40)	Non-ionic surfactant	Polyoxyl 40 hydrogenated castor oil, water-soluble (HLB=14–16)	BASF, Ludwigsha, Germany
Propylene glycol (PG)	Cosolvent	Water-soluble cosolvent	Winlab, Gemini-house, England

Different proportions of excipients were measured by weight rather than volume as some of the excipients are too viscous to dispense by volume. Accordingly, mixture compositions were expressed as percent *w*/*w*.

Droplet Size Analysis

The mean droplet size of the diluted self-emulsifying formulations was measured using Brookhaven particle size analyzer (90 plus Brookhaven, USA). The self-emulsifying formulations were diluted in a ratio of 1: 1,000 *v*/*v* (SEDDS: distilled water) and mixed for 1 min before testing (13,20).

Statistical Analysis

SPSS 18® software was used to analyze the data. One-way analysis of variance (ANOVA) followed by post hoc tests (LSD) were applied to compare the mean droplet size of diluted formulations. A value of $p<0.05$ was denoted as "significant" throughout the study.

RESULTS AND DISCUSSION

Self-Emulsification Assessment

Formulations containing 50 % oil and 50 % surfactant were used extensively in the current research. These formulations were not necessarily the most efficient emulsifying formulations for each combination of excipients, but they represented a common reference for each system (using 50 % surfactant) and ensuring that all the formulations were well dispersed to form colloidal systems (18).

According to the assessment criteria (mentioned in the "MATERIALS AND METHODS" section), the formulation was accepted as SEDDS/SMEDDS only if it shows complete excipient miscibility, as well as, at least moderate spontaneity, homogeneity, and dispersibility (Tables III, IV, and V). Formulation appearance and droplet size (after aqueous dilution) have been accounted as an assisting tool to differentiate between SEDDS and SMEDDS.

Formulations containing surfactants with higher (>12) HLB values (e.g., T80 and Cr-El, hydrophilic in nature) exhibited good self-emulsifying properties compared to formulations containing surfactants with lower (<10) HLB values (e.g., span 20 and span 80, lipophilic in nature). These findings are matching with previously reported data which suggested that the required HLB value of a surfactant for developing suitable self-emulsifying formulation should preferably be around 10 or higher (5).

Within the results of long chain glycerides (LCG): Arachis oil and soybean oil (long chain triglycerides, LCT) showed poor self-emulsifying properties with both lipophilic and hydrophilic surfactants (Tables III and IV). Maizine-35-1 (long chain monoglycerdies, LCM) showed good self-emulsifying properties with hydrophilic surfactant (Cr-El) (Table IV). While in case of medium chain glycerides (MCG), mostly all the oils including tri-, di-, or mono-glycerides showed good self-emulsifying behaviors with lipophilic surfactants (T85) but failed to show acceptable self-emulsifying properties with hydrophilic surfactants

Table III. Self-Emulsification Assessment of Type II Lipid-Based Formulations

Formulation	Type	Excipients miscibility	Spontaneity	Homogeneity	Dispersibility	Appearance (OD_{600}[a])	Overall performance
Ar/T85 (50/50)	II	Immiscible	–	–	–	Milky (1.34)	×
Ar/span 80 (30/70)	II	Miscible	Good	Poor	Poor	Milky (0.91)	×
Ar/span 20 (50/50)	II	Immiscible	–	–	–	Milky (0.49)	×
Soy/T85 (50/50)	II	Immiscible	–	–	–	Milky (1.49)	×
M810/T85 (50/50)	II	Miscible	Good	Good	Good	Milky (0.32)	√
I988/T85 (50/50)	II	Miscible	Good	Good	Good	Milky (1.47)	√
I308/T85 (50/50)	II	Miscible	Good	Good	Good	Turbid (0.16)	√
M810/I988/T85 (25/25/50)	II	Miscible	Good	Good	Good	Transparent (0.04)	√
M810/I308/T85 (25/25/50)	II	Miscible	Good	Good	Good	Transparent (0.02)	√
LN/T85 (50/50)	II	Miscible	–	Poor	–	Milky (1.32)	×
OL/T85 (50/50)	II	Miscible	Good	Poor	Good	Milky (1.34)	×
OL/M810/T85 (25/25/50)	II	Miscible	Good	Poor	Good	Milky (0.93)	×
OL/I988/T85 (25/25/50)	II	Miscible	Good	Moderate	Good	Milky (1.84)	√
OL/I308/T85 (25/25/50)	II	Miscible	Good	Good	Good	Milky (1.86)	√

[a] Data are expressed as mean of three replicates
(√): accepted as SEDDS/SMEDDS
(×): rejected as SEDDS/SMEDDS

(HCO-30 and Cr El) due to the formation of rigid flakes that took comparatively long time to dissolve (Tables III and IV). Interestingly, formulations containing mixed glycerides (mix of tri-, di-, and/or mono-glycerides), such as M810/I988 or M810/I308, exhibited excellent self-emulsifying properties with much more transparent appearance compared with formulations containing tri-, di-, or mono-glycerides alone (Table III). These results are in agreement with recently published data by Mohsin *et al.* (18).

On the other hand, formulations containing long chain fatty acids (LCFA) alone (e.g., OL or LN) showed poor self-emulsifying behaviors with either lipophilic or hydrophilic surfactants where adding cosolvents, mono- and/or di-glycerides with OL or LN (e.g. OL/PG, OL/I988, and OL/I308) significantly improved the self-emulsifying properties of the formulation (Tables III, IV, and V).

From the overall results of self-emulsification assessment studies, it can be stated that: the best self-emulsification efficiency could be achieved by the combination of mixed glycerides and hydrophilic surfactants.

Equilibrium Phase Studies

To conduct phase diagram studies, a range of self-emulsifying formulations were prepared using five oils (Cap355, M810, I988, I308, and OL) with the hydrophilic surfactant (Cr-EL). Ternary and pseudo-ternary phase diagrams were constructed

Table IV. Self-Emulsification Assessment of Type IIIA Lipid-Based Formulation

Formulation	Type	Excipients miscibility	Spontaneity	Homogeneity	Dispersibility	Appearance (OD_{600}[a])	Overall performance
Ar/Cr EL (50/50)	IIIA	Immiscible	–	–	–	Milky (1.25)	×
Soybean/T80 (50/50)	IIIA	Immiscible	–	–	–	Milky (0.73)	×
Soy/Cr RH40 (50/50)	IIIA	Miscible	Moderate	Poor	–	Milky (0.71)	×
Soy/HCO-30 (30/70)	IIIA	Miscible	Poor	Good	Moderate	Milky (0.42)	×
M-35/Cr EL (50/50)	IIIA	Miscible	Good	Good	Good	Milky (0.41)	√
M810/T80 (50/50)	IIIA	Miscible	Moderate	Good	Good	Milky (1.70)	√
M810/Cr El (50/50)	IIIA	Miscible	Poor	Good	Good	Bluish (0.08)	×
Cap355/HCO-30 (50/50)	IIIA	Miscible	Poor	Good	Good	Milky (0.39)	×
Cap355/Cr El (50/50)	IIIA	Miscible	Poor	Good	Good	Bluish (0.07)	×
LN/Cr EL (50/50)	IIIA	Miscible	Good	Poor	Moderate	Turbid (0.27)	×
LN/HCO-30 (50/50)	IIIA	Miscible	Good	Poor	Poor	Milky (1.08)	×
OL/Cr El (50/50)	IIIA	Miscible	Good	Poor	Poor	Milky (0.83)	×
OL/Cr RH40 (50/50)	IIIA	Miscible	Good	Poor	Poor	Milky (0.78)	×
OL/T80 (50/50)	IIIA	Miscible	Good	Poor	Good	Milky (0.60)	×
OL/M810/T80 (25/25/50)	IIIA	Miscible	Good	Good	Good	Milky (1.13)	√
OL/I988/T80 (25/25/50)	IIIA	Miscible	Good	Good	Good	Milky (0.78)	√
OL/M810/Cr El (25/25/50)	IIIA	Miscible	Moderate	Good	Moderate	Turbid (0.13)	√
OL/I988/Cr El (25/25/50)	IIIA	Miscible	Good	Good	Good	Turbid (0.19)	√

[a] Data are expressed as mean of three replicates
(√): accepted as SEDDS/SMEDDS
(×): rejected as SEDDS/SMEDDS

Table V. Self-Emulsification Assessment of Type IIIB and Type IV Lipid-Based Formulations

Formulation	Type	Excipients miscibility	Spontaneity	Homogeneity	Dispersibility	Appearance (OD_{600}[a])	Overall performance
OL/I308/T80 (25/25/50)	IIIB	Miscible	Good	Good	Good	Milky (1.06)	√
OL/I308/Cr El (25/25/50)	IIIB	Miscible	Good	Good	Good	Turbid (0.22)	√
OL/PG/Cr El (25/25/50)	IIIB	Miscible	Good	Good	Good	Turbid (0.16)	√
OL/I988/PG/Cr El (20/20/10/50)	IIIB	Miscible	Good	Good	Good	Turbid (0.17)	√
OL/I308/PG/Cr El (20/20/10/50)	IIIB	Miscible	Good	Good	Good	Turbid (0.20)	√
Cr El/PG (50/50)	IV	Miscible	Good	Good	Good	Transparent (0.00)	√
Cr EL (100)	IV	Miscible	Moderate	Good	Good	Transparent (0.0038)	√

[a] Data are expressed as mean of three replicates
(√): accepted as SEDDS/SMEDDS
(×): rejected as SEDDS/SMEDDS

to represent the corresponding phases formed at selected proportions of oil, surfactant, and water. The phase diagram illustrates the phase changes which occur when the oil/surfactant mixture are combined with water.

Conventional nomenclature was used to identify phase regions; clear isotropic aqueous phase (L1), clear isotropic oily phase (L2), milky emulsion phase (L1+L2), and liquid crystals dispersed within aqueous or oily phase (LC) (18). The presence of liquid crystals was examined by the use of polarizing plate fitted with cross-polarizing filter (EW-48404-62, Cole-Parmer, USA) where LC phase was differentiated from (L2) phase through the characteristic birefringence of the liquid crystals (Fig. 1). The L2 region was recognized as a transparent liquid which takes up large mass of water during dilution without inducing any detectable phase separation. Extensive absorption of water is generally associated with rapid emulsification and fine particle size (9). Thus, it has great influence on the SEDDS/SMEDDS efficiency. On the other hand, the presence of L1 region indicates the potential of the formulation to produce microemulsion upon aqueous dilution (considered as SMEDDS). The gel phase was expressed in some phase diagrams simultaneously with other phases (e.g., L2, LC, or L1+L2), and it was distinguished by the poor flowability or the stickiness of the formulation within the test tube (19).

Captex 355/Cremophor-El/Water System

Figure 2 depicts ternary phase diagrams for Cap355/Cr El/water system at (a) RT (20±2 °C) and (b) 37 °C. The anhydrous formulation consisted of MCT (56 % caprylic acid-C_8 and 44 % caproic acid-C_{10}) and water-soluble surfactant; thus the phase diagram (Fig. 2) represents a typical example for LFCS type IIIA lipid formulation.

At RT, the oil Cap355 showed partial miscibility with Cr-El up to 40/60 (%*w*/*w*), oil/surfactant ratio. However, starting from the ratio of 50/50 (%*w*/*w*), the oil/surfactant mixture showed complete miscibility. This anhydrous system solubilized a low mass of water as indicated by the small L2 region (Fig. 2a). Solubilization of water was slightly increased at very high Cr-El concentrations (90 %). Surfactant concentrations above 30 % were showing some LC phases upon aqueous dilution. L2 phase reappeared again but with higher viscosity gel phase at 40–50 % water and above 60 % surfactant. This phase diagram shows an extensive gel phase region which, due to its rigidity, is expected to affect the spontaneity of the formulation to self emulsify upon aqueous dilution. This was confirmed by the rigid flakes (poor spontaneity) recognized upon aqueous dilution of Cap355/Cr-El system (Table IV). At lower surfactant concentrations (<50 %), the system produced L1+L2 phase, which can be observed within the bottom half of the phase diagram. The higher surfactant

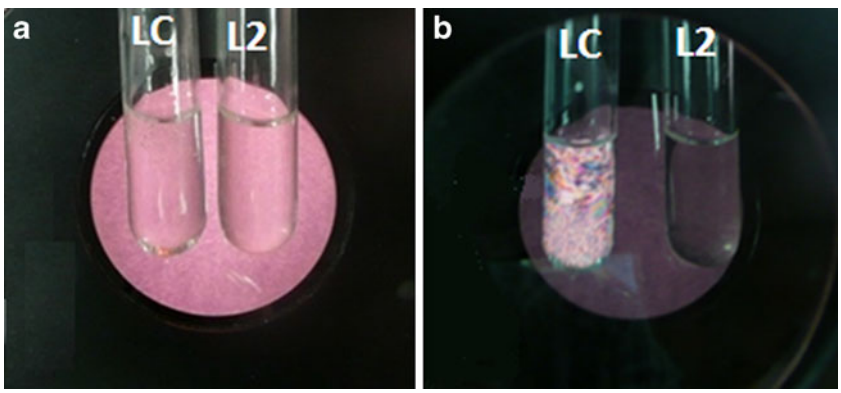

Fig. 1. L2 and LC phases of M810/Cr-El (40/60 % *w*/*w*) with 5 % and 10 % water, respectively, **a** without using polarizing filter, **b** under polarizing filter

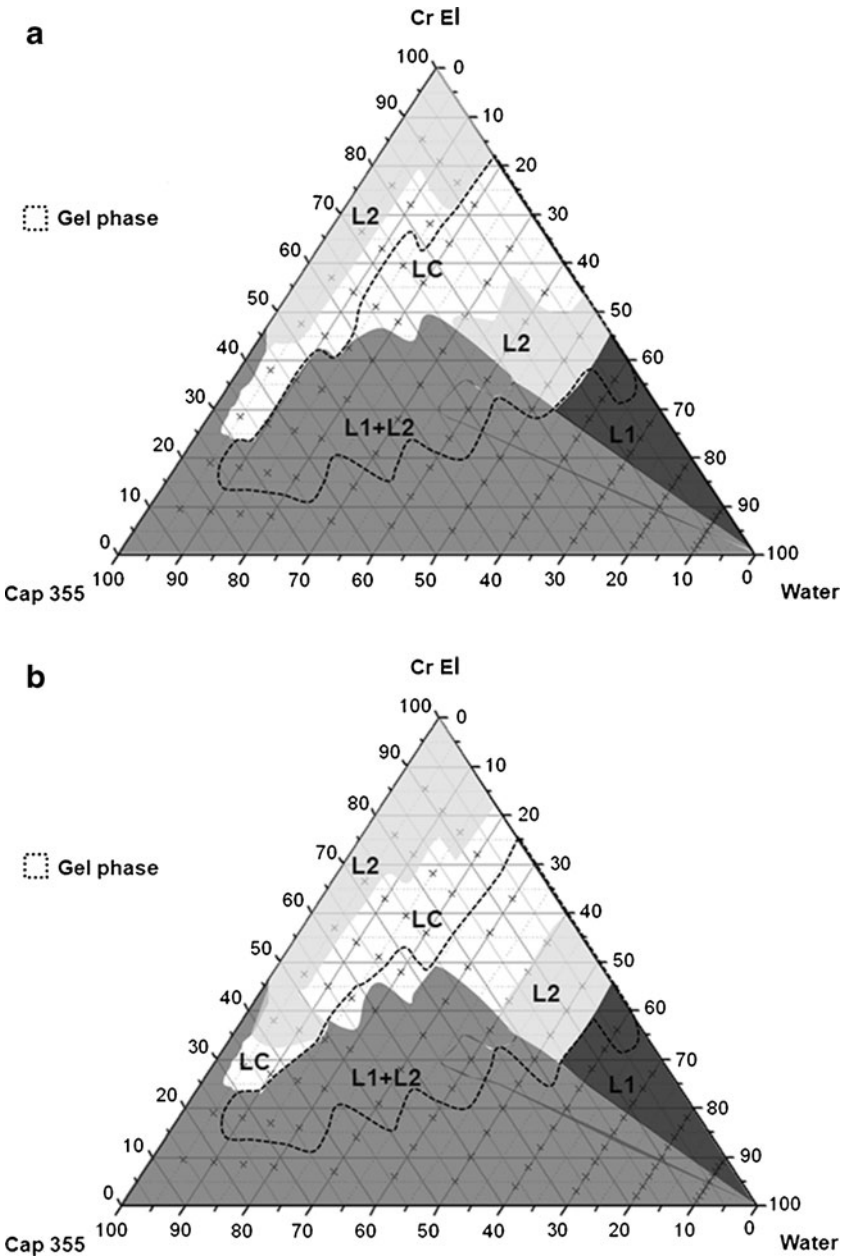

Fig. 2. Ternary equilibrium phase diagram of Cap355/Cr El/water system at **a** RT (20±2 °C) and **b** 37 °C, where (*L1*) denotes: clear isotropic aqueous phase, (*L2*): clear isotropic oily phase, (*LC*): liquid crystals with oily or aqueous phase, (*L1+L2*): milky emulsions, (*Cr-El*): Cremophor El and (*Cap355*): Captex 355

concentrations (from 60 % to 100 %) were able to provide clear L1 phase after 50 % aqueous dilution (Fig. 2a).

Increasing the temperature to 37 °C resulted in slight increase of L2 region and decrease of gel phase region (Fig. 2b). This indicates that the self-emulsifying properties of this system are expected to improve upon increasing the temperature.

Miglyol 810/Cremophor El/Water System

Figure 3 depicts ternary phase diagrams for M810/Cr El/water system at (a) RT (20±2 °C) and (b) 37 °C. This phase diagram presents another example of type IIIA lipid formulation. The formulation contains MCT but with higher proportion of caprylic acid (72 % caprylic acid-C_8 and 27.5 % caproic acid-C_{10}).

Similar to Cap355/Cr-El system, M810/Cr-El system showed complete miscibility at higher (above 40 %) surfactant concentrations (Figs. 2a and 3a). Most of the phase regions (such as LC, L1+L2, L1 and gel phase) and temperature effect were almost identical in the two systems (Figs. 2 and 3). Due to extensive gel-phase existence, the M810/Cr-El system also showed poor spontaneity upon aqueous dilution (Table IV).

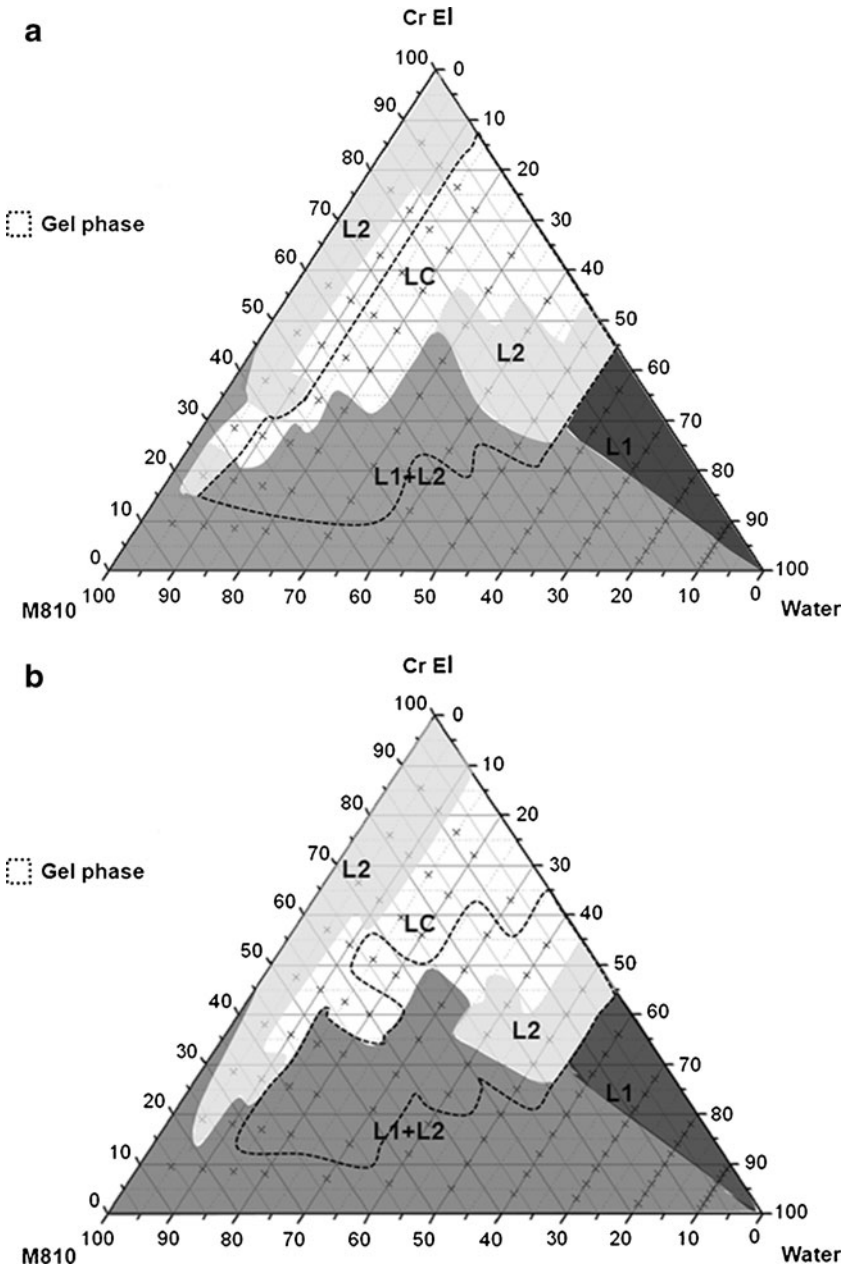

Fig. 3. Ternary equilibrium phase diagram of M810/Cr El/water system at **a** RT (20±2 °C) and **b** 37 °C, where (*L1*) denotes: clear isotropic aqueous phase, (*L2*): clear isotropic oily phase, (*LC*): liquid crystals with oily or aqueous phase, (*L1+L2*): milky emulsions, (*Cr-El*): Cremophor El and (*M810*): Miglyol 810

[Oleic Acid:Imwitor 988 (1:1)]/Cremophor-El/Water System

Figure 4 shows the pseudo-ternary phase behavior of [OL:I988 (1:1)]/Cr-El/water system which is expected to be LFCS type IIIA formulation. The replacement of MCT with the oil mixture of LCFA and medium chain mono/di-glycerides mixture (MCDM) resulted in a significant change in the phase regions of the system.

At RT, the oil mixture [OL:I988 (1:1,%*w*/*w*)] showed complete miscibility with Cr-El along all the oil/surfactant ratios. The solubilization of water was significant at surfactant concentrations between 60 % and 70 %, producing the characteristic finger-like projection of the L2 region towards the water axis (Fig. 4a). This phase behavior was quite similar to the previously reported system of [M812:I988 (7:3)]/T80/water (18).

LC phase was greatly pronounced at surfactant concentrations between 50 % and 60 %, up to 80 % water, producing another finger-like projection near the water axis. The L1+L2 region was extensively enlarged on the expense of L1 phase that could only appear at very high surfactant concentrations (90 %) starting from 60 % aqueous dilution (Fig. 4a).

The result from this system showed no gel phase existence, which positively influenced the spontaneity of the formulation. As a result, the [OL:I988 (1:1)]/Cr-El system showed good spontaneity (fast self-emulsifying ability) upon

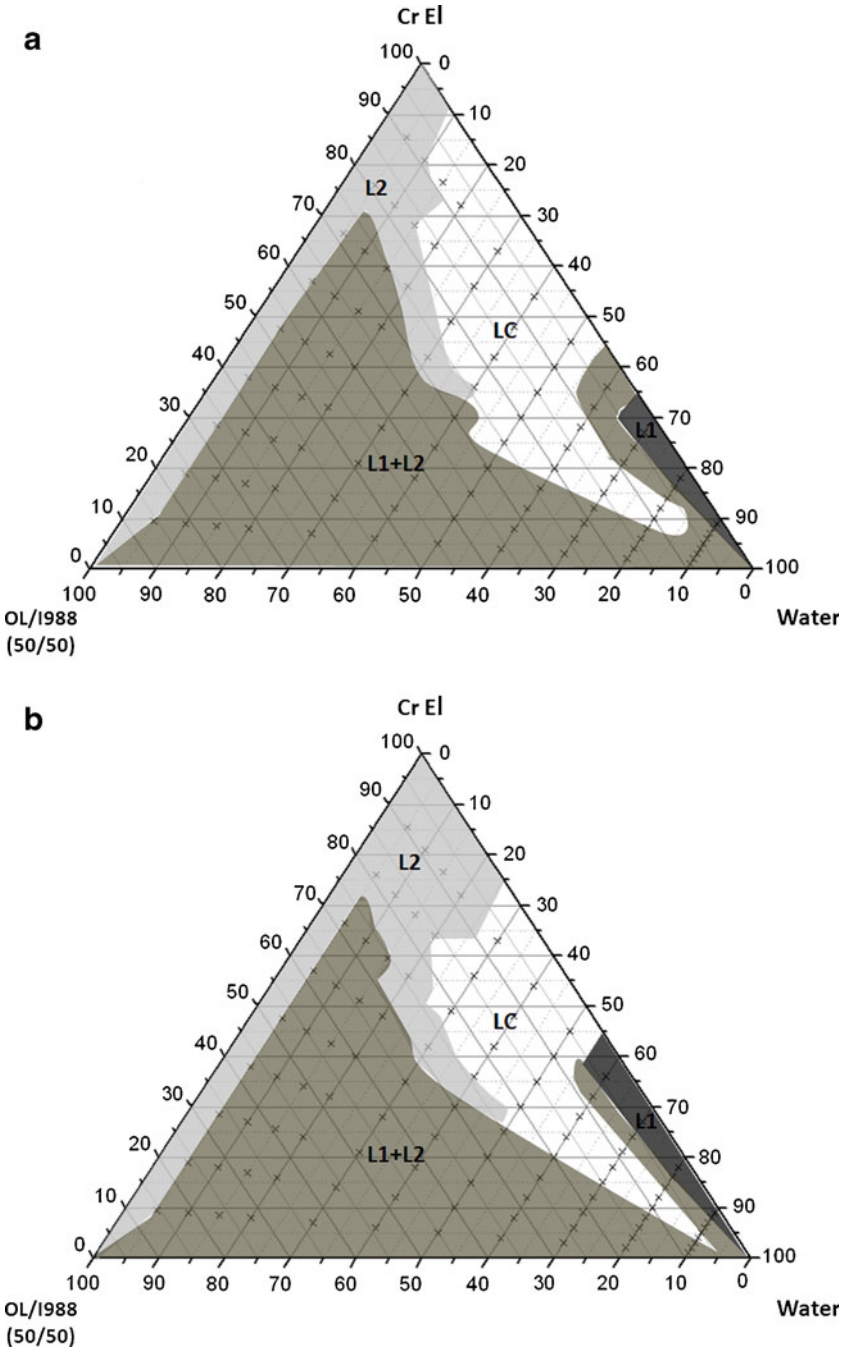

Fig. 4. Pseudo-ternary equilibrium phase diagram of [OL:I988 (1:1)]/Cr El/water system at **a** RT (20±2 °C) and **b** 37 °C, where (*L1*) denotes: clear isotropic aqueous phase, (*L2*): clear isotropic oily phase, (*LC*): liquid crystals with oily or aqueous phase, (*L1+L2*): milky emulsions, (*Cr-El*): Cremophor El and (*OL*): oleic acid and (*I988*): Imwitor 988

aqueous dilution (Table IV). Increasing the temperature to 37 °C resulted in slight increase of both L2 and L1 region (Fig. 4b).

[Oleic Acid:Imwitor 308 (1:1)]/Cremophor El/Water System

Figure 5 represents the pseudo-ternary phase diagram for [OL:I308 (1:1)]/Cr El/water system, which was chosen as an example of a type IIIB formulation in this study. The replacement of MCDM by medium chain mono-glycerides (MCM) resulted in a significant expansion in the L2 region.

At RT, the oil mixture [OL:I308 (1:1),%*w*/*w*] showed complete miscibility with Cr-El along all the oil/surfactant ratios. The solubilization of water was pronounced at surfactant concentrations between 50 % and 60 %, up to 60 % water by weight at the maximum, producing a larger finger-like projection of the L2 region towards the water axis (Fig. 5a). This system also fairly resembled the previously reported phase diagram of I308/T80/water (18).

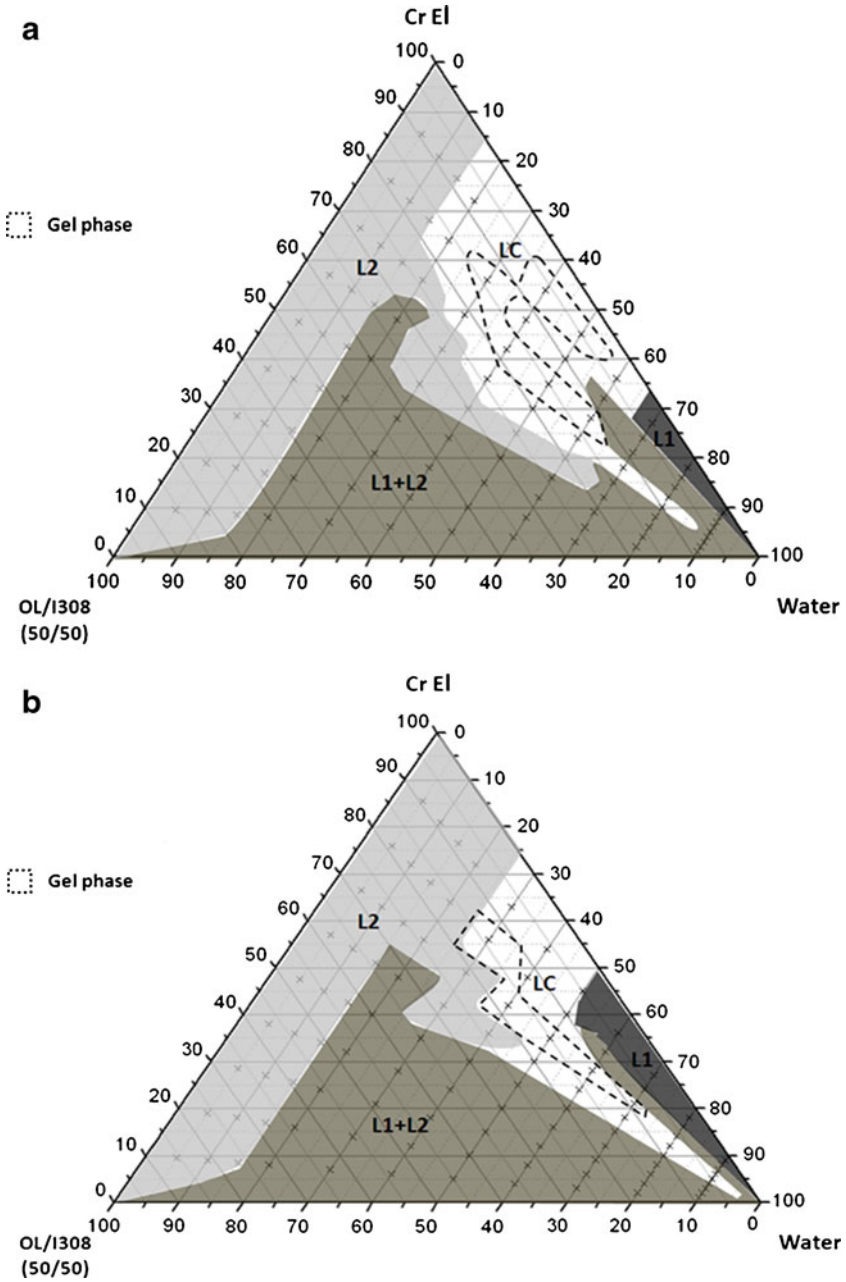

Fig. 5. Pseudo-ternary equilibrium phase diagram of [OL:I308 (1:1)]/Cr El/water system at **a** RT (20±2 °C) and **b** 37 °C, where (*L1*) denotes: clear isotropic aqueous phase, (*L2*): clear isotropic oily phase, (*LC*): liquid crystals with oily or aqueous phase, (*L1+L2*): milky emulsions, (*Cr-El*): Cremophor El and (*OL*): oleic acid and (*I308*): Imwitor 308

In this phase diagram, LC phase also appeared as a small finger-like projection near the water axis. The L1+L2 region was reduced due to L2 region enlargement. L1 phase appeared at very high surfactant concentrations (90 %) starting from 60 % aqueous dilution (Fig. 5a). Gel phase was recognized only at higher surfactant concentrations of 70–90 % with 30–60 % aqueous dilution. The gel phase region was relatively small to cause significant influence on the spontaneity of the system, since [OL:I308 (1:1)]/Cr-El system was able to show good spontaneity upon aqueous dilution (Table V).

Similarly, increasing the temperature to 37 °C resulted in a considerable increase of L2 region and decrease of gel phase region (Fig. 5b), which is expected to enhance the self-emulsification efficiency of the system.

The studies of equilibrium phase diagrams assisted to identify the self-emulsifying regions and also to establish the optimum concentrations of oil, surfactant, and/or cosurfactant required for efficient formulations development (19). But generally, the efficiency of emulsification was good when the surfactant concentration was ≥50 %. In the current studies, it was observed that increasing the concentration of the monoglycerides (I988 to I308) improved the water solubilization in the system (Figs. 4a and 5a), which is expected to enhance the efficiency of the self-emulsification process (21,22).

Table VI. Mean droplet size of the diluted self-emulsifying formulations

Formulation	Mean droplet size (nm)[a]
I988/T85 (50/50)	287.5±18.0
I308/T85 (50/50)	210.9±17.5
M810/T85 (50/50)	128.4±6.0
M810/I988/T85 (25/25/50)	50.4±4.8
M810/I308/T85 (25/25/50)	29.3±2.7
OL/I988/T85 (25/25/50)	467.1±23.4
OL/I308/T85 (25/25/50)	449.6±39.4
OL/I988/T80 (25/25/50)	294.8±37.0
OL/I308/T80 (25/25/50)	226.4±21.4
OL/I988/Cr EL (25/25/50)	77.2±5.4
OL/I308/Cr EL (25/25/50)	108.3±5.0
OL/I308/PG/Cr EL (20/20/10/50)	107.8±3.6

[a] Data are expressed as mean±S.D, $n=3$

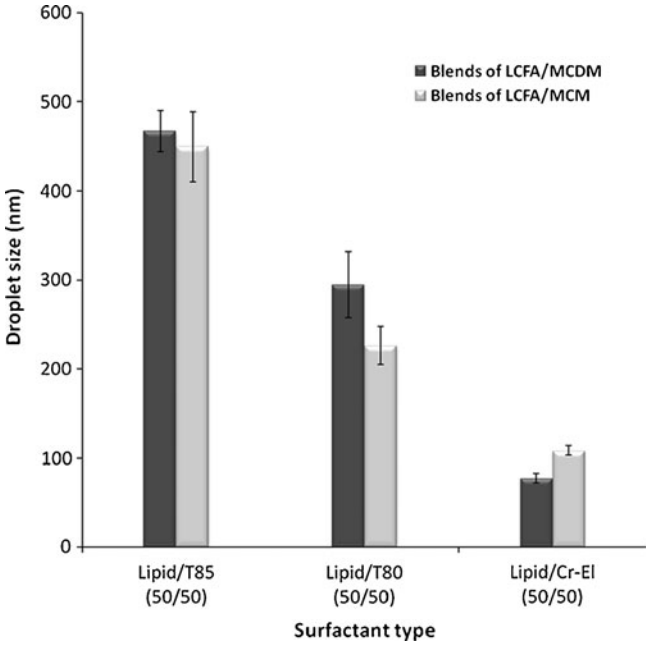

Fig. 7. Influence of surfactant type on the mean droplet size of the diluted self-emulsifying formulations, where LCFA is represented by (OL), MCDM: (I988), MCM: (I308). Data are expressed as mean±S.D, $n=3$

Droplet Size Analysis

The self-emulsification efficiency is strongly associated with the mean droplet size of the produced emulsion (23). Various components in the formulation are known to influence the droplet size. Lipid chain length, glycerides type, and degree of the formulation saturation are proven to affect the droplet size of the resulted diluted formulation (18,19). Table VI shows the mean droplet size resulted after (1:1,000) aqueous dilution of anhydrous self-emulsifying formulations.

Influence of Lipid Chain Length on the Mean Droplet Size

The lipid chain length had been reported to enormously affect the droplet size of the diluted self-emulsifying formulations (20). Providing that the surfactant portion is fixed, the formulations containing MCG (e.g., M810) showed significantly lower ($p<0.05$) mean droplet size compared with formulations containing LCFA (e.g., OL) (Table VI). These results are strongly matching with previously reported data (13) where larger mean droplet size was observed with formulations containing Myvacet 9–45 (LCG) compared to the formulations containing Captex-200 (MCG). This might be owing to the differences in the penetration of LCG and MCG into the tail region of the surfactant and their subsequent influence on the curvature of the interfacial film (13).

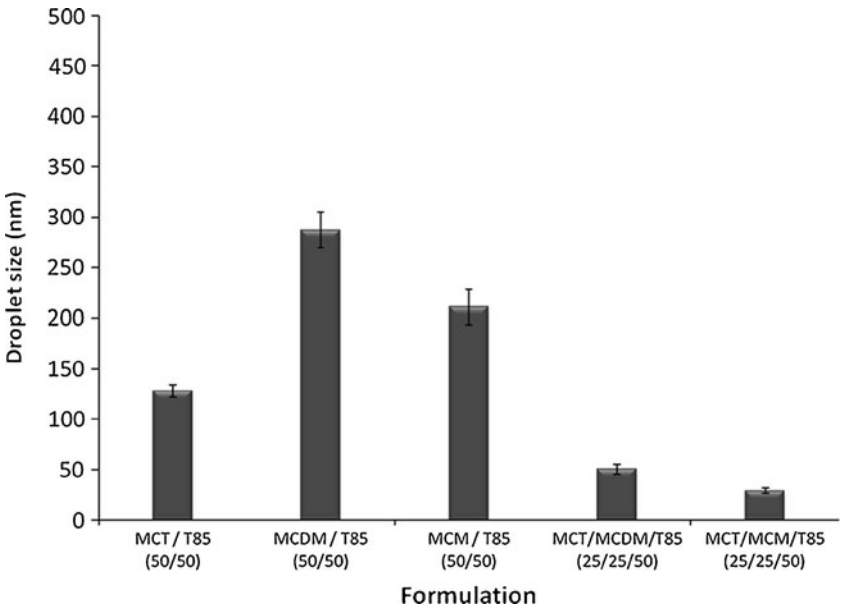

Fig. 6. Influence of glycerides composition on the mean droplet size of the diluted self-emulsifying formulations, where MCT is represented by (M810), MCDM: (I988) and MCM: (I308). Data are expressed as mean±S.D, $n=3$

Influence of Glycerides Composition on the Mean Droplet Size

The glycerides composition in lipid excipients was found to have remarkable effect on the mean droplet size of the diluted self-emulsifying formulations. The current study was involved to have better comparison between the use of MCT, MCDM, or MCM alone *versus* using the combination of MCT with MCDM or MCM which is known as (mixed glycerides). The mixed glycerides (represented by two formulations: MCT/MCDM/T85 and MCT/MCM/T85) showed significantly lower ($p<0.05$) mean droplet size (50 and 29 nm, respectively) compared with MCT/T85 (128 nm), MCDM/T85 (288 nm), and MCM/T85 (211 nm) (Fig. 6). These results are strongly toning with previous data that showed lower droplet size and enhanced water solubilization upon blending MCT with MCDM (18). This was thought to be due to the enhanced dispersion and water penetration into the formulation in case of using mixed glycerides. Despite of using a lipophilic surfactant, both MCT/MCDM/T85 (25/25/50, %*w*/*w*/*w*) and MCT/MCM/T85 (25/25/50, %*w*/*w*/*w*) produced extremely low droplet size (50 and 29 nm, respectively) (Fig. 6), isotropic and transparent appearance upon aqueous dilution (Table III). Accordingly, both formulations can be categorized as SMEDDS (5). These results indicate the vital role of the lipid component in the formulation, since formulations containing mixed glycerides were able to produce SMEDDS even with using water-insoluble surfactants.

Influence of Surfactant Type on the Mean Droplet Size

The surfactant component is known to have significant influence on the self-emulsifying efficiency and in turn the mean droplet size resulted after aqueous dilution of self-emulsifying formulations (24). Having a fixed lipid composition in the system, the mean droplet size showed significant decrease ($p<0.05$) upon increasing the surfactant hydrophilicity by moving from T85 to T80 and Cr-El (Fig. 7). The low droplet size observed with Cr-El blended formulations could be explained with the extensive water uptake (L2 and LC regions) within the corresponding phase diagrams (Figs. 4 and 5). These results are consistent with the well-established theories emphasizing the importance of using high HLB surfactants to obtain fine and efficient self-emulsification (9).

CONCLUSION

Lipid-based formulations introduce a vital option to improve the oral bioavailability of PWSD compounds. However, the selection of the formulation components and their relative quantities in the formulation is very critical and requires lot of considerations. To assess the formulation performance, thus select the efficient formulations, it is necessary to establish a standard assessment criteria as well as ternary phase diagram studies. The combined self-emulsification test revealed that the best formulation efficiency could be achieved by using the combination of mixed glycerides and hydrophilic surfactants. The equilibrium phase studies showed that combining two different glycerides oils resulted in significant expansion of the L2 region. Formulations containing mixed glycerides showed significantly lower droplet size compared with formulation containing tri-, di-, or mono-glycerides alone. The overall data in the current studies proved that formulations containing medium chain mixed glycerides have shown the best performance in terms of their excellent self-emulsification efficiency, extensive L2 region, lack of gel phase existence, lower droplet size, and thus can be considered as SMEDDS.

ACKNOWLEDGMENTS

The authors would to like to acknowledge the SABIC graduate student fund (No: MED-30-44). The authors are grateful to the distinguished scientific support from Prof. Adel Sakr.

REFERENCES

1. Rai A, Tiwari G, Tiwari R. Self-emulsifying drug delivery system: an approach to enhance solubility. Syst Rev Pharm. 2010;1:133–40.
2. Porter CJH, Trevaskis NL, Charman WN. Lipids and lipid-based formulations: optimizing the oral delivery of lipophilic drugs. Nat Rev Drug Discov. 2007;6:231–48.
3. Rahman MA, Harwansh R, Mirza MA, Hussain S, Hussain A. Oral lipid based drug delivery system (LBDDS): formulation, characterization and application: a review. Curr Drug Deliv. 2011;8:330–45.
4. Kohli K, Chopra S, Dhar D, Arora S, Khar RK. Self-emulsifying drug delivery systems: an approach to enhance oral bioavailability. Drug Discov Today. 2010;15:958–65.
5. Gursoy RN, Benita S. Self-emulsifying drug delivery systems (SEDDS) for improved oral delivery of lipophilic drugs. Biomed Pharmacother. 2004;58:173–82.
6. Gershanik T, Benita S. Self-dispersing lipid formulations for improving oral absorption of lipophilic drugs. Eur J Pharm Biopharm. 2000;50:179–88.
7. Pouton CW. Formulation of self-emulsifying drug delivery systems. Adv Drug Del Rev. 1997;25:47–58.
8. Pouton CW. Self-emulsifying drug delivery systems: assessment of the efficiency of emulsification. Int J Pharm. 1985;27:335–48.
9. Pouton CW. Lipid formulations for oral administration of drugs: non-emulsifying, self-emulsifying and "self-microemulsifying" drug delivery systems. Eur J Pharm Sci. 2000;11:S93–8.
10. Pouton CW. Formulation of poorly water-soluble drugs for oral administration: physicochemical and physiological issues and the lipid formulation classification system. Eur J Pharm Sci. 2006;29:278–87.
11. Porter C, Pouton C, Cuine J, Charman W. Enhancing intestinal drug solubilisation using lipid-based delivery systems. Adv Drug Del Rev. 2008;60:673–91.
12. Craig DQM, Barker SA, Banning D, Booth SW. An investigation into the mechanisms of self-emulsification using particle size analysis and low frequency dielectric spectroscopy. Int J Pharm. 1995;114:103–10.
13. Kommuru TR, Gurley B, Khan MA, Reddy IK. Self-emulsifying drug delivery systems (SEDDS) of coenzyme Q10: formulation development and bioavailability assessment. Int J Pharm. 2001;212:233–46.
14. Nekkanti V, Karatgi P, Prabhu R, Pillai R. Solid self-microemulsifying formulation for candesartan cilexetil. AAPS PharmSciTech. 2010;11:9–17.
15. Thakkar H, Nangesh J, Parmar M, Patel D. Formulation and characterization of lipid-based drug delivery system of raloxifene-microemulsion and self-microemulsifying drug delivery system. J Pharm Bioallied Sci. 2011;3(3):442–8.
16. Date AA, Nagarsenker MS. Design and evaluation of self-nanoemulsifying drug delivery systems (SNEDDS) for cefpodoxime proxetil. Int J Pharm. 2007;329:166–72.
17. Kossena GA, Charman WN, Boyd BJ, Dunstan DE, Porter CJ. Probing drug solubilization patterns in the gastrointestinal tract after administration of lipid-based delivery systems: a phase diagram approach. J Pharm Sci. 2004;93:332–48.

18. Mohsin K, Long MA, Pouton CW. 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. 2009;98:3582–95.
19. Kang BK, Lee JS, Chon SK, Jeong SY, Yuk SH, Khang G, *et al*. Development of self-microemulsifying drug delivery systems (SMEDDS) for oral bioavailability enhancement of simvastatin in beagle dogs. Int J Pharm. 2004;274:65–73.
20. Atef E, Belmonte AA. Formulation and *in vitro* and *in vivo* characterization of a phenytoin self-emulsifying drug delivery system (SEDDS). Eur J Pharm Sci. 2008;35:257–63.
21. Wakerly MG, Pouton CW, Meakin BJ, Morton FS. Self-emulsification of vegetable oil-nonionic surfactant mixtures. In: Scamehorn JF, editor. Phenomena in mixed surfactant systems. Washington: American Chemical Society; 1986. p. 242–55.
22. Charman SA, Charman WN, Rogge MC, Wilson TD, Dutko FJ, Pouton CW. Self-emulsifying drug delivery systems: formulation and biopharmaceutic evaluation of an investigational lipophilic compound. Pharm Res. 1992;9:87–93.
23. Shah NH, Carvajal MT, Patel CI, Infeld MH, Malick AW. Self-emulsifying drug delivery systems (SEDDS) with polyglycolyzed glycerides for improving *in vitro* dissolution and oral absorption of lipophilic drugs. Int J Pharm. 1994;106:15–23.
24. Pouton CW, Porter CJ. Formulation of lipid-based delivery systems for oral administration: materials, methods and strategies. Adv Drug Deliv Rev. 2008;60:625–37.