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Article

Optimization of self-nanoemulsifying formulations for weakly basic lipophilic drugs: role of acidification and experimental design

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Formulators face great challenges in adopting systematic approaches for designing self-nanoemulsifying formulations (SNEFs) for different drug categories. In this study, we aimed to build-up an advanced SNEF development framework for weakly basic lipophilic drugs, such as cinnarizine (CN). First, the influence of formulation acidification on CN solubility was investigated. Second, formulation self-emulsification in media with different pH was assessed. Experimentally designed phase diagrams were also utilized for advanced optimization of CN-SNEF. Finally, the optimized formulation was examined using cross polarizing light microscopy for the presence of liquid crystals. CN solubility was significantly enhanced upon external and internal acidification. Among the various fatty acids, oleic acid-based formulations showed superior self-emulsification in all the tested media. Surprisingly, formulation turbidity and droplet size significantly decreased upon equilibration with CN. The design was validated using oleic acid/Imwitor308/Cremophor El (25/25/50), which showed excellent self-nanoemulsification, 43-nm droplet size (for CN-equilibrated formulations), and 88 mg/g CN solubility. In contrast to CN-free formulations, CN-loaded SNEF presented lamellar liquid crystals upon 50% aqueous dilution. These findings confirmed that CN-SNEF efficiency was greatly enhanced upon drug incorporation. The adopted strategy offers fast and accurate development of SNEFs and could be extrapolated for other weakly basic lipophilic drugs.

Uniterms: Self-nanoemulsifying formulations/solubility. Cinnarizine/lipophilic drugs. Acidification/experimental design. Solubility enhancement.

INTRODUCTION

As a result of modern drug discovery practices, there has been a consistent rise in the number of newly discovered chemical entities that are considered poorly water-soluble drugs (PWSDs). Owing to their poor dissolution, these PWSDs often present low and erratic bioavailabilities. Pharmaceutical experts are therefore facing great challenges in formulating these entities into oral dosage forms with adequate bioavailability (Dahan, Hoffman, 2008). Among the various lipid-based formulations, self-nanoemulsifying formulations (SNEFs) represent one of the most promising candidates in terms of enhancing the *in-vivo* performance of orally administered PWSD (Bahloul *et al.*, 2015). Compared with other drug delivery systems, SNEFs offer great advantages, including ease of manufacture and scaling-up, improved physical stability, and maximized drug entrapment capacity. Upon introduction to physiological media, no dissolution step would be required owing to the formation of a nano-droplet size emulsion. This facilitates a higher rate and extent of absorption and therefore high and reproducible bioavailability values (Balakumar *et al.*, 2013; Gupta, Kesarla, Omri, 2013).

Current development approaches in the SNEF area are mostly empirical and consume large amounts of time and money (Dahan, Hoffman, 2008; Bahloul *et al.*, 2015). SNEFs are commonly developed using the "trial and error approach" which involves changing one parameter at a time or the conventional "ternary phase diagrams" technique. These two methods require several experiments, hence resulting in high cost and long development periods (Bahloul, Lassoued, Sfar, 2014). In addition, some formulations might be mischaracterized

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owing to inadequate testing conditions, as further mentioned in the "self-emulsification assessment section" of the current study. Many other limitations still restrict the potential usage of SNEFs, including the lack of predictive *in-vitro* tests, insufficient *in-vivo* drug precipitation studies, and lack of *in vitro–in vivo* correlations (IVIVC) (Bahloul *et al.*, 2015). SNEF optimization for each drug is not an easy process. Only very specific excipient combinations will produce efficient self-nanoemulsifying systems. This could be confirmed by the vast amount of research performed in this area leading to only limited numbers of SNEF products in the pharmaceutical market (Gursoy, Benita, 2004; Elnaggar, El-Massik, Abdallah, 2009; Bahloul *et al.*, 2015).

Therefore, regarding *in-vitro* prospects, there is a need for an advanced approach to accelerate the design, characterization, and optimization of SNEFs and adopt a formulation design platform that suits similar drug models (Bahloul *et al.*, 2015).

The aim of the current study is to build-up an advanced SNEF design framework for weakly basic lipophilic drugs. Cinnarizine (CN), a highly lipophilic compound (*log P* = 5.8) (Loftsson, Hreinsdóttir, Másson, 2005), was selected as the model drug for the current study. CN is a weak base with Pk_{a1} = 1.94 and Pk_{a2} = 7.47 (Tokumura *et al.*, 1987). It shows a pH-dependent solubility that is 0.29 mg/mL at pH 2, 0.017 mg/mL at pH 5, and 0.002 mg/mL at pH 6.5 (Gu *et al.*, 2005; Raghuvanshi, Pathak, 2014). Therefore, it is vital to consider the influence of formulation acidity and pH variation during the design of CN-SNEF (Shahba, Mohsin, Alanazi, 2012a). In the current study, we aim to bridge the gaps regarding critical characterization issues, explore the role of formulation acidification, and utilize the experimental design for a time-effective and accurate optimization of SNEFs for weakly basic lipophilic drugs.

MATERIAL AND METHODS

Material

Miglyol 810 (M810, medium chain triglycerides), Miglyol 812 (M812, medium chain triglycerides), Imwitor 308 (I308, medium chain monoglycerides), and Imwitor 988 (I988, medium chain mono/diglycerides) were kindly supplied by Sasol GmbH, Germany. Oleic acid (OL, long chain fatty acid C18) and Tween 80 (T80) were obtained from Avonchem, UK. Tween 85 (T85) was purchased from Merck-Schuchardt OHG, Germany. Tween 20 (T20) was purchased from BDH, UK. Cremophor EL (Cr-El) and Cremophor RH40 (Cr-RH40) were supplied by BASF, Germany. CremerAc Capric acid (C10) 98/100 and Cremer AC distilled coconut oil fatty acids (COFA) were kindly supplied by Cremer Oleo GmbH & Co. KG, Germany. Caprylic acid (C8) was purchased from Wuji Xinhui Chemical Co. Ltd, China. Sefsol 218 (S218), HCO-30, and HCO-60 were kindly provided by Nikko Chemicals, Japan. Lauroglycol 90 and Capmul PG-12 were kindly supplied by Gattefosse, France and Abitec, USA, respectively. CN was supplied by FDC Limited, India.

Equilibrium solubility studies

The equilibrium CN solubility in diverse formulations was determined according to previously reported studies (Baka, Comer, Takács-Novák, 2008) with some modifications. After at least a three-day equilibration period, samples were withdrawn, centrifuged, and assayed using ultra-performance liquid chromatography (UPLC) (Abdel-Hamid *et al.*, 2012; Shahba, Mohsin, Alanazi, 2012a).

Self-emulsification assessment

A previously reported (Kommuru *et al.*, 2001; Shahba, Mohsin, Alanazi, 2012b) visual test, used to assess self-emulsification efficiency, was modified and adopted for the current study. In accordance with routine practice, formulation dispersion in water was utilized as a fast and general tool to assess formulation self-emulsification efficiency. However, in the current study, it was proven that "formulation dispersion in water" was not sufficient alone because some formulations may show variable emulsification behaviors at different pH.

To assess such formulations accurately, drug-equilibrated formulations were dispersed under the following conditions:

Primarily, formulations were subjected to 1:400 aqueous dilution in a 50.0 mL glass beaker, and the contents were gently mixed (~500 rpm) using a magnetic stirrer (Nekkanti *et al.*, 2010; Shahba, Mohsin, Alanazi, 2012b). Alternatively, formulations (20–30 mg) were diluted with 50.0 mL of 0.1 N HCl (pH 1.2) in order to mimic gastric pH. The contents were then gently mixed as previously described. Finally, 25.0 mL of ≈120 mM tribasic sodium phosphate solution was added to the latter media to reach a pH of 6.8, mimicking intestinal pH. The contents were again gently mixed as previously described. This step was very critical, particularly for weakly basic drugs whose behavior varies greatly depending on media pH.

The formulations were evaluated on the basis of performance indicator tools, such as excipient miscibility, spontaneity, and homogeneity/dispersibility (Shahba, Mohsin, Alanazi, 2012b).

Optical density

For further exploration of assessment results, the optical density (OD) of selected diluted formulations were examined at 600.0 nm using an UV-visible spectrophotometer (UVD-3200, Labomed Inc., USA) (Date, Nagarsenker, 2007; Thakkar *et al.*, 2011). Prior to OD measurement, drug free and/or drug equilibrated formulations were subjected to 1:400 aqueous dilutions in a 50.0 mL glass beaker, and the contents were gently mixed using a magnetic stirrer (~500 rpm) (Nekkanti *et al.*, 2010; Shahba, Mohsin, Alanazi, 2012b). Formulation clarity was categorized according to the following OD_{600} ranges: transparent: OD_{600} = 0.00-0.05, bluish: OD_{600} = 0.05-0.1, turbid: OD_{600} = 0.1-0.3, and milky: OD_{600} > 0.3 (Shahba, Mohsin, Alanazi, 2012b).

Droplet size analysis

The average droplet size of the diluted SNEF was examined using a Zetasizer Nano ZS (Malvern, UK). The formulations were diluted in distilled water at a ratio of 1:1000 v/v and mixed for 1 min prior to examination (Kommuru *et al.*, 2001; Atef, Belmonte, 2008).

Experimentally designed phase diagrams

Phase diagram studies often require large numbers of sample preparations, thereby requiring extensive time. In the current study, all phase diagrams were constructed using advanced experimental design in order to reduce the number of experiments, save time, and obtain a comprehensive analysis of the data. Design Expert® (version 9, Stat-Ease, Inc., USA) was used to construct the D-optimal mixture design (Mukherjee, Plakogiannis, 2010) of CN-SNEFs. The mixture study included three components: the oil portion (represented by free fatty acid, A), the co-surfactant portion (represented by I308, B), and the surfactant portion (represented by Cr-El, C).

COFA/I308/Cr-El system

This system was chosen as a model formulation to study the influence of time and drug loading on formulation turbidity upon aqueous dilution. Formulation turbidity was assessed by measuring the optical density at 600 nm (Date, Nagarsenker, 2007; Thakkar *et al.*, 2011). On the basis of preliminary self-emulsification and solubility data, the range for each component was selected as follows:

$$10 \leq \text{COFA} \leq 50$$
$$0 \leq \text{I308} \leq 60$$
$$30 \leq \text{Cr-El} \leq 70$$
$$\text{Total components} = 100$$

The base design suggested 17 runs for the fitting of a special cubic model, a check for lack of fit, and an estimate of experimental error in formulation OD_{600} (Mukherjee, Plakogiannis, 2010).

The design included the assessment of five responses as follows:

R1: OD_{600} after 5 min of aqueous dilution of CN-free formulation; R2: OD_{600} after 1 h of aqueous dilution of CN-free formulation; R3: OD_{600} after 5 min of aqueous dilution of CN-equilibrated formulation; R4: OD_{600} after 1 h of aqueous dilution of CN-equilibrated formulation; R5: OD_{600} after 2 h of aqueous dilution of CN-equilibrated formulation.

OL/I308/Cr-El system

On the basis of preliminary self-emulsification and solubility data, the range of each component was selected as follows:

$$10 \leq \text{OL} \leq 30$$
$$10 \leq \text{I308} \leq 40$$
$$30 \leq \text{Cr-El} \leq 70$$
$$\text{Total components} = 100$$

The base design suggested U_Pseudo coding with 16 runs for the fitting of a quadratic model, a check for lack of fit, and an estimate of experimental error (Mukherjee, Plakogiannis, 2010).

The design included the assessment of three responses as follows:

R1: droplet size after 1 h of aqueous dilution of drug-free formulation (nm); R2: droplet size after 1 h of aqueous dilution of drug-equilibrated formulation (nm); R3: equilibrium solubility (mg/g).

Cross polarizing light microscopy

Liquid SNEF samples were transferred onto microscope slides and coverslipped a few minutes prior to examination. Samples were then examined using

crossed polarized light microscopy (Carl Zeiss, Axio lab. A1 (equipped with camera), Jena, Germany) (Mohsin, Long, Pouton, 2009; Mohsin, Pouton, 2012). Samples were examined for the existence of birefringence, a characteristic of liquid crystals, at a magnification of 40× (Kossena *et al.*, 2004). Liquid crystalline (LC) phases were further identified as hexagonal or lamellar based on the observed birefringence patterns. Within the current study, liquid SNEF samples were examined to investigate the influence of CN loading on birefringence patterns in anhydrous samples, as well as 30% and 50% water dilution samples.

Determination of CN by using UPLC assay

CN was accurately quantified by using a recently reported UPLC reversed-phase method (Abdel-Hamid *et al.*, 2012) with minor modifications. The mobile phase composition was altered to 0.5% trifluroacetic acid:acentonitrile (55:45) and the run time was increased to 1.5 min to allow for higher resolution between the intact drug and degradation product peaks. Separation was achieved using an Acquity® UPLC BEH C18 (2.1 × 50 mm, 1.7 µm) column along with an Acquity guard filter, maintained at 50 ºC, and the flow rate was maintained at 0.5 ml/min. The UV detector was set at 251 nm and the injection volume was 1.0 µl.

Statistical analysis

The statistical significance of the results was analyzed using SPSS 22® software. One-way analysis of variance (ANOVA) followed by post hoc tests (LSD) were applied to compare solubility results (Atef, Belmonte, 2008; Shahba, Mohsin, Alanazi, 2012a). A value of $p < 0.05$ was considered significant.

RESULTS AND DISCUSSION

Equilibrium solubility studies

CN solubility was previously screened in diverse formulation compositions (Shahba, Mohsin, Alanazi, 2012a). In the current study, we aimed to maximize CN solubility and to develop a formulation design framework for weakly basic lipophilic drugs. Being a weak base, it was crucial to investigate the influence of formulation acidification on CN solubility. Formulation acidification was achieved using either an external acidifier (represented by HCl) or an internal acidifier (represented by free fatty acid present in the formulation).

Influence of external acidification on CN solubility

The influence of formulation acidification on CN solubility was investigated by acidifying SNEF by using 37% HCl solution as the external acidifier. HCl solution was added to the formulation at 1%, 2%, and 5% w/w. Preliminary data revealed that 2% HCl acidification showed the highest CN solubilization. This finding might be explained by the high formulation water content that led to lower CN solubilization in case of 5% acidification.

The study involved investigating the acidification of two formulations: medium chain triglyceride (MCT) based-formulation and long chain fatty acid (LCFA) based-formulation. In case of MCT formulations, 2% acidification resulted in a 100% solubility increase relative to the non-acidified counterpart. However, the 2% acidified LCFA formulation showed a 30% increase relative to the non-acidified counterpart (Figure 1). The solubility increase upon acidification complies with the fact that CN is a weak base and its solubility increases with decreasing pH (Gu *et al.*, 2005). Similar results have been obtained with other weakly basic drugs, such as albendazole, which was shown to present increased solubility with increased concentrations of acidified PEG_{400} in the formulation (Mukherjee, Plakogiannis, 2010). The solubility increase upon formulation

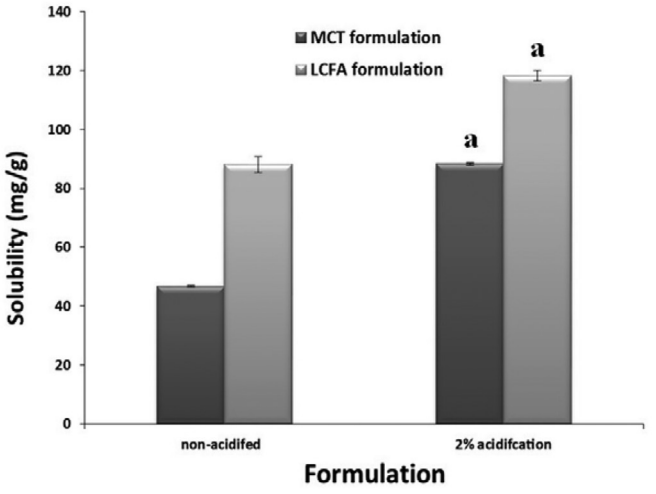

FIGURE 1 - Influence of external formulation acidification on CN solubility. MCT: medium chain triglycerides, LCFA: long chain fatty acid. Non-acidified MCT formulation was represented by M812/I308/T85 (25/25/50); 2% acidified MCT formulation by M812/I308/T85/HCl (24/24/50/2); non-acidified LCFA formulation by OL/I308/Cr-El (25/25/50); 2% acidified LCFA formulation by OL/I308/Cr-El/HCl (24.5/24.5/49/2). **a** denotes significant difference ($p < 0.05$) from the corresponding non-acidified formulation. Data are expressed as mean ± S.D., n=3–6.

acidification may be due to drug super-saturation or the transient formation of hydrochloride salts.

Influence of internal acidification (free fatty acids versus ester form)

CN shows significant solubility increase upon increasing the acidity of aqueous systems (Gu *et al.*, 2005). Interestingly, this phenomenon was also predominant in lipid-based systems (Figures 2 and 3).

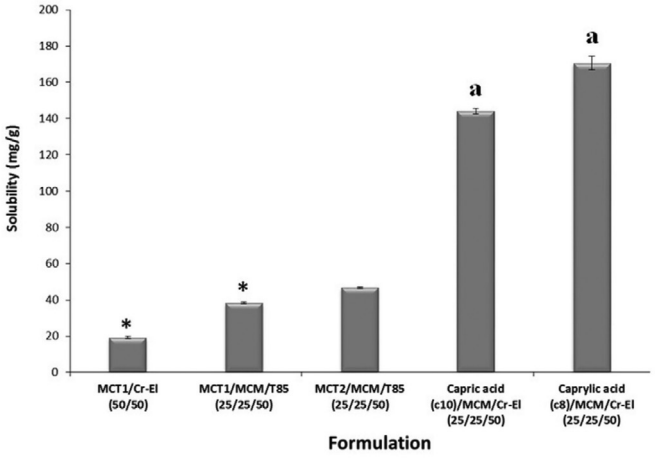

FIGURE 2 - Influence of internal formulation acidification on CN solubility in capric/caprylic acid-based formulations. MCT1 denotes medium chain capric/caprylic triglycerides (M810), MCT2 denotes medium chain capric/caprylic triglycerides (M812), and MCM denotes medium chain capric/caprylic mono-glycerides (I308). *: Data adapted from Shahba, Mohsin, Alanazi, 2012a; **a**: significant difference ($p < 0.05$) from other formulations. Data are expressed as mean ± S.D., n = 3.

Capric and caprylic acid (free fatty acids) formulations showed significantly ($p<0.05$) higher CN solubility relative to other capric/caprylic ester formulations (Figure 2). It seems that free fatty acids significantly increase CN solubility by acting as lipophilic solubilizers, as well as internal acidifiers.

Similarly, a COFA (high % of free lauric acid)-based formulation showed significantly ($p<0.05$) higher CN solubility relative to other lauric acid monoglycerides (esters) formulations (Figure 3).

Influence of fatty acid chain length

A more focused study was conducted to evaluate the influence of changing the fatty acid type within the formulation (Figure 4). CN solubility was significantly ($p<0.05$) increased upon decreasing fatty acid chain length. This finding may appear inconsistent with the finding of a previous study (Shahba, Mohsin, Alanazi, 2012a) that showed higher CN solubility in long chain lipid formulations. However, thorough data review revealed that the previously compared formulations were uneven since the previous study (Shahba, Mohsin, Alanazi, 2012a) compared long chain free fatty acid with medium chain ester formulations. For a fair comparison, formulators should consider whether the evaluated fatty acid is in the free or ester form.

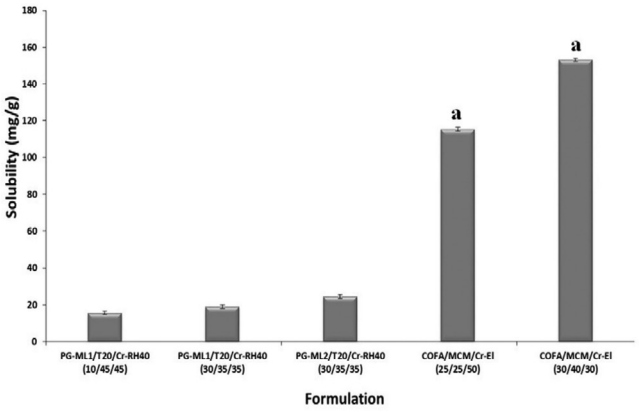

FIGURE 3 - Influence of internal formulation acidification on CN solubility in lauric acid-based formulations. PG-ML1 denotes propylene glycol monolaurate (Lauroglycol 90), PG-ML2 denotes propylene glycol monolaurate (Capmul PG-12). T20: Tween 20; Cr-RH40: Cremophor RH40; COFA: coconut oil free fatty acids (high % of lauric acid); MCM: medium chain capric/caprylic mono-glycerides (I308). **a**: significant difference ($p < 0.05$) from other formulations. Data are expressed as mean ± S.D., n = 3.

The phenomenon found in Figure 4 was correlated with the fact that the acid value of fatty acids increases as the fatty acid chain length decreases. Finally, a direct linear relationship was established between the reported acid value of the fatty acids in the formulation and CN solubility (Figure 5). CN solubility significantly ($p<0.05$) increased as the acid value increased. This point provides a suggested platform for the formulation design of weakly basic lipophilic drugs.

Self-emulsification assessment

Influence of external acidification

Medium chain glyceride formulations (acidified by HCl) showed satisfactory self-emulsification behavior upon water and acidic dilution at pH 1.2. However, all formulations (except for the 5% acidified formulation) presented indispersible flakes upon shifting from pH 1.2 to pH 6.8 (Table I). This may be due to the interaction of the hydrochloride salt present in the formulation with the buffer salts of the alkaline media.

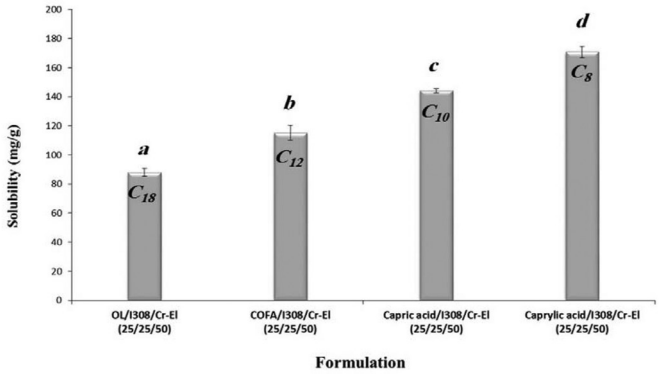

FIGURE 4 - Influence of fatty acid chain length on CN solubility. OL denotes oleic acid (c18) and COFA denotes coconut oil free fatty acids (high % of lauric acid, C12). Different letters above the bars indicate significant difference ($p<0.05$) between the solubility values. Data are expressed as mean ± S.D., n = 3–6.

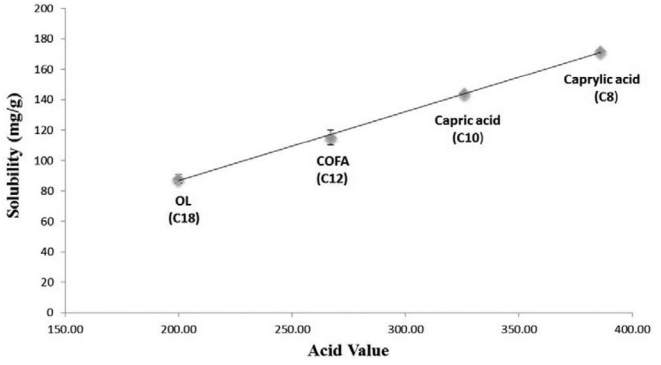

FIGURE 5 - Influence of the acid value of the oil portion on CN solubility in the whole formulation. The oil portion represented 25% of the formulation while the remaining excipients were kept constant as 25% I308 and 50% Cr-El. OL denotes oleic acid (c18) and COFA denotes coconut oil free fatty acids (high % of lauric acid, C12). Data are expressed as mean ± S.D., n = 3-6.

Influence of internal acidification

Incorporation of free fatty acids in SNEFs offers dual advantages of serving as lipophilic portions as well as internal acidifiers within the formulation. Different fatty acids were assessed in order to explore their influence on formulation self-emulsifying efficiency.

Although some formulations containing COFA showed efficient self-emulsification upon water and acidic dilution, they presented indispersible flakes upon shifting to pH 6.8 (Table II). A follow-up study was conducted to assess whether buffer salts interact with formulation components and produce these flakes. To distinguish the influence of the buffer salts, both formulation components and solution pH were maintained nearly equal while various buffer salts were investigated. COFA/I308/HCO-60 (25/25/50) was selected as a control formulation and various buffer salts (with solution pH = 6.8 ± 1) were assessed (Table III). The model formulation presented poor homogeneity/dispersibility with all the investigated buffer salts. These results suggest that the poor self-emulsification of the COFA formulation (at pH 6.8) might be related to the fatty acid itself rather than any interaction with the buffer salts.

Most capric acid (c10) and caprylic acid (c8) formulations showed excellent self-emulsification behavior upon acidic dilution (Table IV). However, they presented poor homogeneity/dispersibility upon shifting to pH 6.8. On the other hand, OL formulations exhibited superior performance compared with other fatty acids (Table V). Most of the tested formulations showed excellent self-emulsification in water, at pH 1.2, and even at pH 6.8. This observation is in agreement with recent studies (Larsen *et al.*, 2012; Patel, Sarma, Vavia, 2013) where OL self-emulsifying formulations have exhibited excellent self-emulsification in all tested media. This promising OL characteristic might be explained by the low acid value of OL compared with other fatty acids (Figure 5) leading to a decreased precipitation tendency at pH 6.8. Another possible reason may be that OL is thought to behave as a co-surfactant at neutral pH (Larsen *et al.*, 2012).

The aforementioned discussion highlights the importance of conducting the self-emulsification assessment in three different media (distilled water, pH 1.2, and pH 6.8), especially for weakly basic drugs. The usual practice of conducting the self-emulsification assessment in water only could lead to inaccurate excipient selection, resulting in wasted time and unsuccessful formulation optimization.

Experimentally designed phase diagrams

For each phase diagram, dots represented the investigated points in the design. Dots marked with a "2" or a "3" indicate points that were duplicated or triplicated, respectively. The analysis of response variables were carried out by using Design Expert® software. Data transformation was performed if necessary. Model fitting was carried out to select a model with insignificant lack-of-fit, high adjusted and predicted R-squared values, small standard deviation, small predicted residual sum of squares (press), and no aliasing (Mukherjee, Plakogiannis, 2010).

COFA/I308/Cr-El system

The D-optimal mixture design was applied to examine the influence of varying formulation components

TABLE I - Influence of HCl acidification on self-emulsification efficiency in medium chain glyceride formulations

Formulation (%W/W)	Excipient miscibility	Aqueous media	Homogeneity/ dispersibility	Spontaneity	Overall performance
M812/I308/T85 (25/25/50)	miscible	D.W	good	< 1 min	√
		pH 1.2	good	< 1 min	√
		shift to pH 6.8	poor	-	×
M812/I308/T85/HCl (24.5/24.5/50/1)	miscible	D.W	good	< 1 min	√
		pH 1.2	good	< 1 min	√
		shift to pH 6.8	poor	-	×
M812/I308/T85/HCl (24/24/50/2)	miscible	D.W	good	< 1 min	√
		pH 1.2	good	< 1 min	√
		shift to pH 6.8	poor	-	×
M812/I308/T85/HCl (22.5/22.5/50/5)	miscible	D.W	moderate	< 1 min	√
		pH 1.2	good	< 1 min	√
		shift to pH 6.8	good	-	√

(√): good self-emulsifying efficiency; (×): poor self-emulsifying efficiency; M812: miglyol 812; I308: Imwitor 308; T85: tween 85.

TABLE II - Influence of internal acidification by COFA on formulation self-emulsifying efficiency

Formulation (%W/W)	Excipient miscibility	Aqueous media	Homogeneity/ dispersibility	Spontaneity (min)	Overall performance
COFA/I308/Cr-El (25/25/50)	miscible	pH 1.2	poor	< 1	×
		shift to pH 6.8	poor	-	×
COFA/I308/Cr-El (10/20/70)	miscible	pH 1.2	good	< 1	√
		shift to pH 6.8	poor	-	×
COFA/S218/Cr-El (25/25/50)	miscible	pH 1.2	good	< 1	√
		shift to pH 6.8	poor	-	×
COFA/I308/T80 (25/25/50)	miscible	pH 1.2	good	< 1	√
		shift to pH 6.8	poor	-	×
COFA/I308/T20 (25/25/50)	miscible	pH 1.2	good	< 1	√
		shift to pH 6.8	poor	-	×
COFA/I308/PG/Cr-El (25/15/10/50)	miscible	pH 1.2	good	< 1	√
		shift to pH 6.8	poor	-	×
COFA/I308/M812/Cr-El (25/12.5/12.5/50)	miscible	pH 1.2	good	< 1	√
		shift to pH 6.8	poor	-	×
COFA/T85/Cr-El (33.3/33.3/33.3)	miscible	pH 1.2	good	< 1	√
		shift to pH 6.8	poor	-	×
COFA/I308/HCO-60 (25/25/50)	miscible	pH 1.2	good	< 1	√
		shift to pH 6.8	poor	-	×

(√): good self-emulsifying efficiency; (×): poor self-emulsifying efficiency; COFA: coconut oil fatty acids; I308: Imwitor 308; S218: sefsol 218; Cr-El: cremophor El; M812: miglyol 812; T20: Tween 20; PG: propylene glycol; T80: Tween 80.

on formulation OD_{600} after aqueous dilution at five different stages (Figures 6 and 7). The first and second responses were conducted using CN-free formulation (Figure 6). There was no substantial difference between OD_{600} at 5 min and 1 h after aqueous dilution of CN-free formulations (Figure 6 [A, B]). Both phase diagrams showed small

TABLE III - Influence of varying buffer solutions on formulation self-emulsifying efficiency

Formulation (%W/W)	Buffer solution	pH	Homogeneity/ dispersibility	Spontaneity (min)	Overall performance
COFA/I308/HCO-60 (25/25/50)	K_2HPO_4,KH_2PO_4, NaOH	7.5	poor	< 1	×
COFA/I308/HCO-60 (25/25/50)	Na_2HPO_4, KH_2PO_4	6.8	poor	< 1	×
COFA/I308/HCO-60 (25/25/50)	Na-acetate, 0.1N HCl	5.9	poor	< 1	×
COFA/I308/HCO-60 (25/25/50)	tri-Na citrate, 0.1N HCl	6.65	poor	< 1	×
COFA/I308/HCO-60 (25/25/50)	K_2HPO_4, 0.1 N HCl	6.75	poor	< 1	×
COFA/I308/HCO-60 (25/25/50)	Na-acetate, NaOH	6.7	poor	< 1	×

(√): good self-emulsifying efficiency; (×): poor self-emulsifying efficiency; COFA: coconut oil fatty acids; I308: Imwitor 308.

TABLE IV - Influence of internal acidification by capric (c10) and caprylic (c8) acid on formulation self-emulsifying efficiency

Formulation (%W/W)	Excipient miscibility	Aqueous media	Homogeneity/ dispersibility	Spontaneity (min)	Overall performance
Capric acid (c10)/I308/Cr-El (25/25/50)	miscible	pH 1.2	good	< 1	√
		shift to pH 6.8	poor	-	×
Capric acid (c10)/I308/T20 (25/25/50)	miscible	pH 1.2	good	< 1	√
		shift to pH 6.8	poor	-	×
Capric acid (c10)/I308/Cr-RH40 (25/25/50)	miscible	pH 1.2	good	< 1	√
		shift to pH 6.8	poor	-	×
Capric acid (c10)/I308/T85 (25/25/50)	miscible	pH 1.2	good	1–5	√
		shift to pH 6.8	poor	-	×
Capric acid (c10)/I308/HCO-30 (25/25/50)	miscible	pH 1.2	good	< 1	√
		shift to pH 6.8	poor	-	×
Capric acid (c10)/I988/Cr-El (25/25/50)	miscible	pH 1.2	good	< 1	√
		shift to pH 6.8	poor	-	×
Caprylic acid (c8)/I308/Cr-El (25/25/50)	miscible	pH 1.2	good	< 1	√
		shift to pH 6.8	poor	-	×

(√): good self-emulsifying efficiency; (×): poor self-emulsifying efficiency; I308: Imwitor 308; I988: Imwitor 988; Cr-El: cremophor El; Cr-Rh40: cremophor RH40; T20: Tween 20; T85: Tween 85.

bluish and large turbid and milky areas. On the other hand, CN-equilibrated formulations showed significant decrease in OD_{600} compared to their CN-free counterparts (Figure 6 [A, B], Figure 7 [A, B]). At 5 min, the phase diagram showed large transparent and bluish areas with no existence of turbid or milky areas (Figure 7A). At subsequent (1 h and 2 h) samples, OD_{600} showed a gradual shift upwards and a turbid area started to appear at the expense of the transparent area (Figure 7 [B,C]). It is worth noticing that all formulations showed sharply lower OD_{600} values after equilibration with CN. This is an unusual phenomenon because CN is a PWSD and its incorporation was expected to increase formulation turbidity upon aqueous dilution. For further investigation

TABLE V - Influence of internal acidification by OL on formulation self-emulsifying efficiency

Formulation (%W/W)	Aqueous media	Excipient miscibility	Homogeneity/ dispersibility	Spontaneity (min)	Overall performance
OL/I308/Cr-El (25/25/50)	pH 1.2	miscible	good	< 1	√
	shift to pH 6.8		good	-	√
OL/I308/Cr-El (30/30/40)	pH 1.2	miscible	good	< 1	√
	shift to pH 6.8		good	-	√
OL/I308/Cr-El (30/40/30)	pH 1.2	miscible	poor	< 1	×
	shift to pH 6.8		poor	-	×
OL/I308/HCO-60 (30/40/30)	pH 1.2	miscible	good	< 1	√
	shift to pH 6.8		good	-	√
OL/I308/HCO-60 (25/25/50)	pH 1.2	immiscible	good	< 1	×
	shift to pH 6.8		good	-	×

(√): good self-emulsifying efficiency; (×): poor self-emulsifying efficiency; OL: oleic acid, I308; Imwitor 308, Cr-El; cremophor El

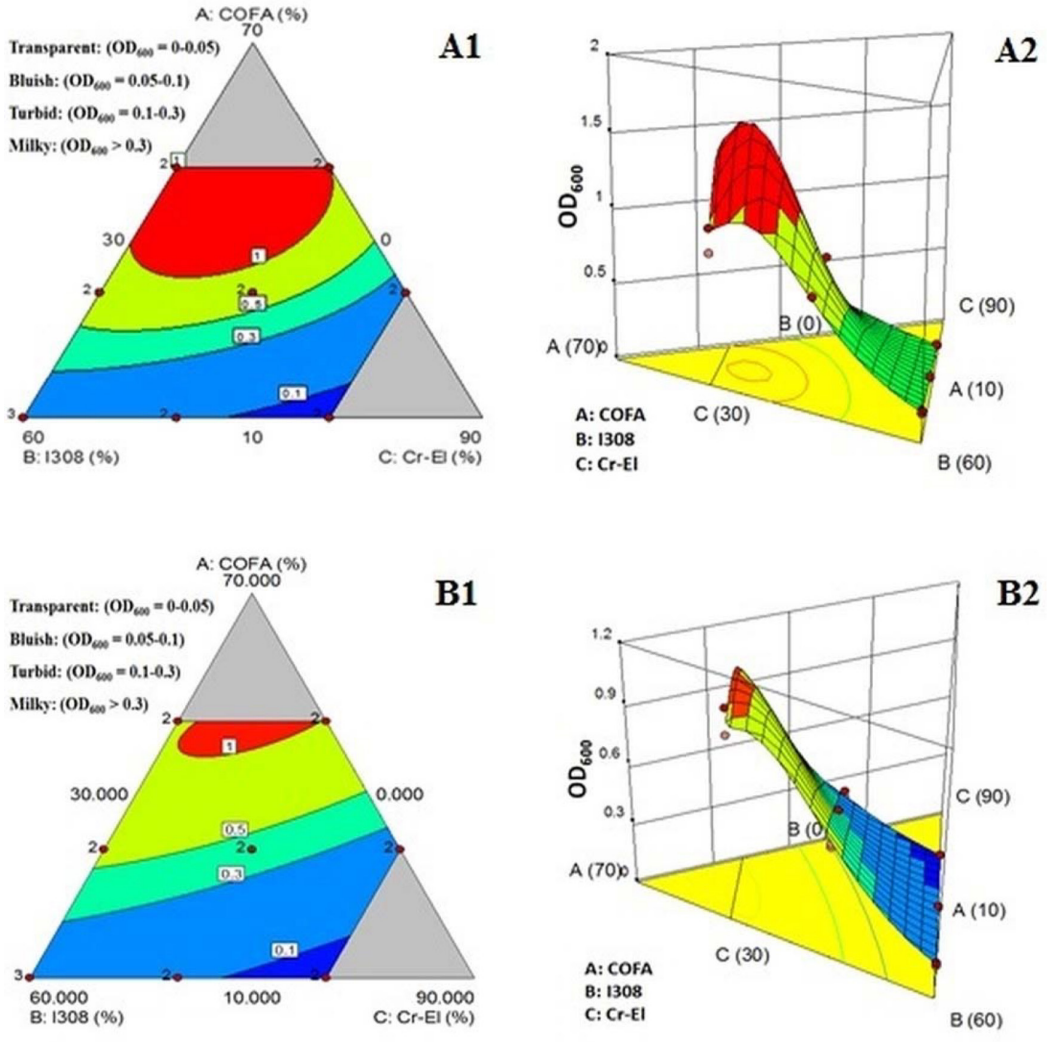

FIGURE 6 - Mixture design for the COFA/I308/Cr-El system showing: (A_1) phase diagram; (A_2): 3D plot of OD_{600} after 5 min of aqueous dilution of CN-free formulation; (B_1) phase diagram; (B_2): 3D plot of OD_{600} after 1 h of aqueous dilution of CN-free formulation. COFA denotes coconut oil fatty acids, I308: Imwitor 308, and Cr-El: cremophor El.

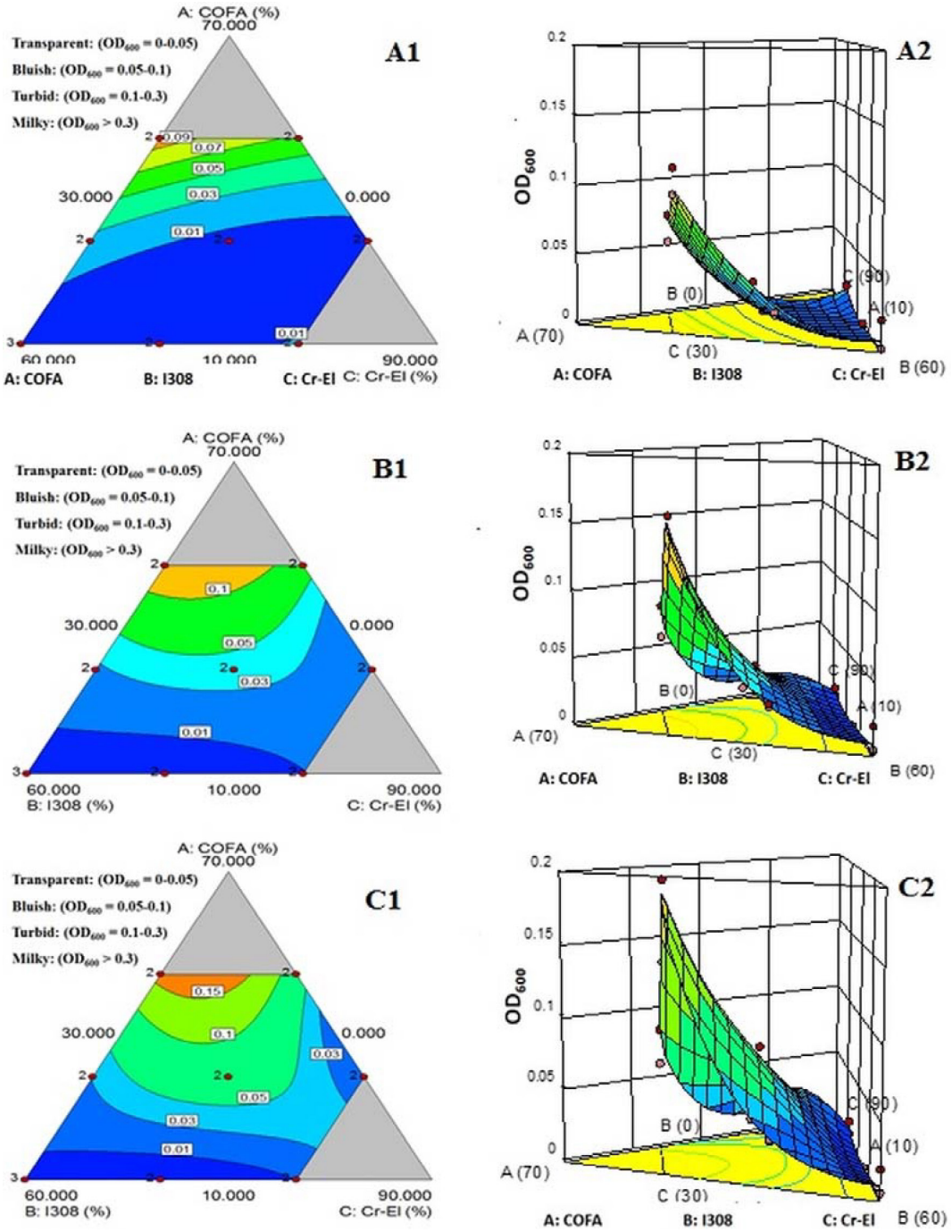

FIGURE 7 - Mixture design for COFA/I308/Cr-El system showing: (A_1) phase diagram; (A_2) 3D plot of OD_{600} after 5 min of aqueous dilution of CN-equilibrated formulation; (B_1) phase diagram; (B_2) 3D plot of OD_{600} after 1 h of aqueous dilution of CN-equilibrated formulation; (C_1) phase diagram; (C_2) 3D plot of OD_{600} after 2 h of aqueous dilution of CN-equilibrated formulation. COFA denotes coconut oil fatty acids, I308: Imwitor 308, and Cr-El: cremophor El.

of this phenomenon, another system was investigated using OL, rather than COFA, as the oil component.

OL/I308/Cr-El system

D-optimal mixture design was applied to examine the influence of varying formulation components on droplet size and equilibrium solubility. The droplet size of the diluted CN-free formulations showed high variability, ranging from less than 100 nm up to ≈ 1250 nm (Figure 8A). Formulations with droplet size above 100 nm are expected to show a turbid or milky appearance (Shahba, Mohsin, Alanazi,

2012b). However, formulation phase behavior completely changed upon CN incorporation (Figure 8B). Diluted CN-equilibrated formulations showed less variability and significantly smaller droplet sizes, with a maximum of 87 nm. This observation closely matches the results obtained in the previous COFA/I308/Cr-El phase diagram, where the incorporation of CN led to smaller droplet sizes, decreased turbidity, and increased SNEF efficiency. Many researchers have examined the influence of PWSD incorporation into SNEFs on droplet size. Some articles showed droplet size increases upon drug incorporation (Kommuru *et al.*, 2001; Kang *et al.*, 2004), while a few articles showed no droplet size difference upon drug incorporation (Nielsen *et al.*, 2007). However, one recent publication showed significant droplet size decrease upon drug incorporation (Patel, Sarma, Vavia, 2013). This last publication may be in agreement with the current work because it has also investigated a very similar formulation composition (OL/medium chain monoglycerides/Cr-El) with a weakly basic drug (lumefantrine). According to that recent publication (Patel, Sarma, Vavia, 2013), this phenomenon might be due to the interaction between the drug amine group and the OL carboxylic group, which leads to the formation of an ion-pairing complex. The formed complex presented more efficient self-emulsification and lower droplet sizes than the drug-free formulation. The observed phenomenon could be extrapolated for other weakly basic lipophilic drugs.

The third response involved the equilibrium CN solubility in anhydrous SNEFs (Figure 8C). Maximum CN solubility was achieved using higher OL and lower Cr-El proportions. This result matches the solubility data, which showed a significant ($p < 0.05$) rise in CN solubility upon increasing the free fatty acid proportion in the formulation (Figure 3).

Experimental model validation

To achieve the optimum CN-SNEF, it was desirable to select the formulation presenting lower droplet size and higher CN solubility. The formulation [OL/I308/Cr-El (25/25/50)] was selected as a confirmation point to validate the predictions of the experimental model. The design was utilized to predict the three design responses for this formulation. Experiments were then conducted to obtain the actual response values. All the actual mean values were close to the predicted mean, and fall between the 95% lower and higher prediction intervals (Table VI). These results confirm the model accuracy in predicting different design responses. The selected formulation showed ultra-fine (43 nm) droplet size (in case of CN loading), high (88 mg/g) CN solubility, and therefore has great potential to present an efficient CN-SNEF. These results match the recently reported data regarding CN-SNEFs (Shahba, Mohsin, Alanazi, 2012a).

Cross polarizing light microscopy

The optimal liquid SNEF (OL/I308/Cr-El [25/25/50]) was examined to investigate the influence of CN loading on the birefringence pattern in anhydrous samples, as well as 30% and 50% water dilutions. Anhydrous liquid SNEF showed a transparent isotropic oily phase in both CN-free and CN-equilibrated samples. After hydration, CN-free liquid SNEF showed a two-phase emulsion mixture (without LC) at both 30% and 50% water ratios (Figure 9A, B). However, hydrated samples of CN-loaded SNEF became more transparent and presented a birefringent LC phase at 50% water ratio (Figure 9C, D). On the basis of the observed pattern, this sample was identified as lamellar LC phase (Mohsin, Long, Pouton, 2009; Mohsin, Pouton, 2012). The presence of a lamellar LC phase could lead to the enhancement of the formulation self-emulsification efficiency. A very recent article (Lee *et al.*, 2016) showed pronounced enhancement of bioavailability upon using lamellar LC nanoparticles. It is worth noticing that the LC phase was only observed in the CN-loaded formulation. This is in close correlation with the phenomenon observed in experimentally designed phase diagrams, which showed enhanced SNEF efficiency upon CN incorporation into the formulation.

CONCLUSION

Pre-competitive research should be promoted to build-up advanced formulation design frameworks for different PSWD categories. In the current study, we investigated the weakly basic lipophilic drug category by using CN as a model. The formulation acidity and the pH of the dispersion media were found to exert strong influences on drug solubility and formulation efficiency, respectively. Formulation acidification produced significant drug solubility increase. It was critical to conduct the self-emulsification assessment in media with different pH rather than water alone. This step is of particular importance for weakly basic drugs. OL was found to be a vital component in the development of SNEFs for weakly basic lipophilic drugs. It offers several advantages, serving as a lipophilic solubilizer, an internal acidifier, along with maintaining excellent self-nanoemulsification efficiency. As shown in the current study, experimentally designed phase diagrams could be easily utilized for fast, accurate, and reliable optimization

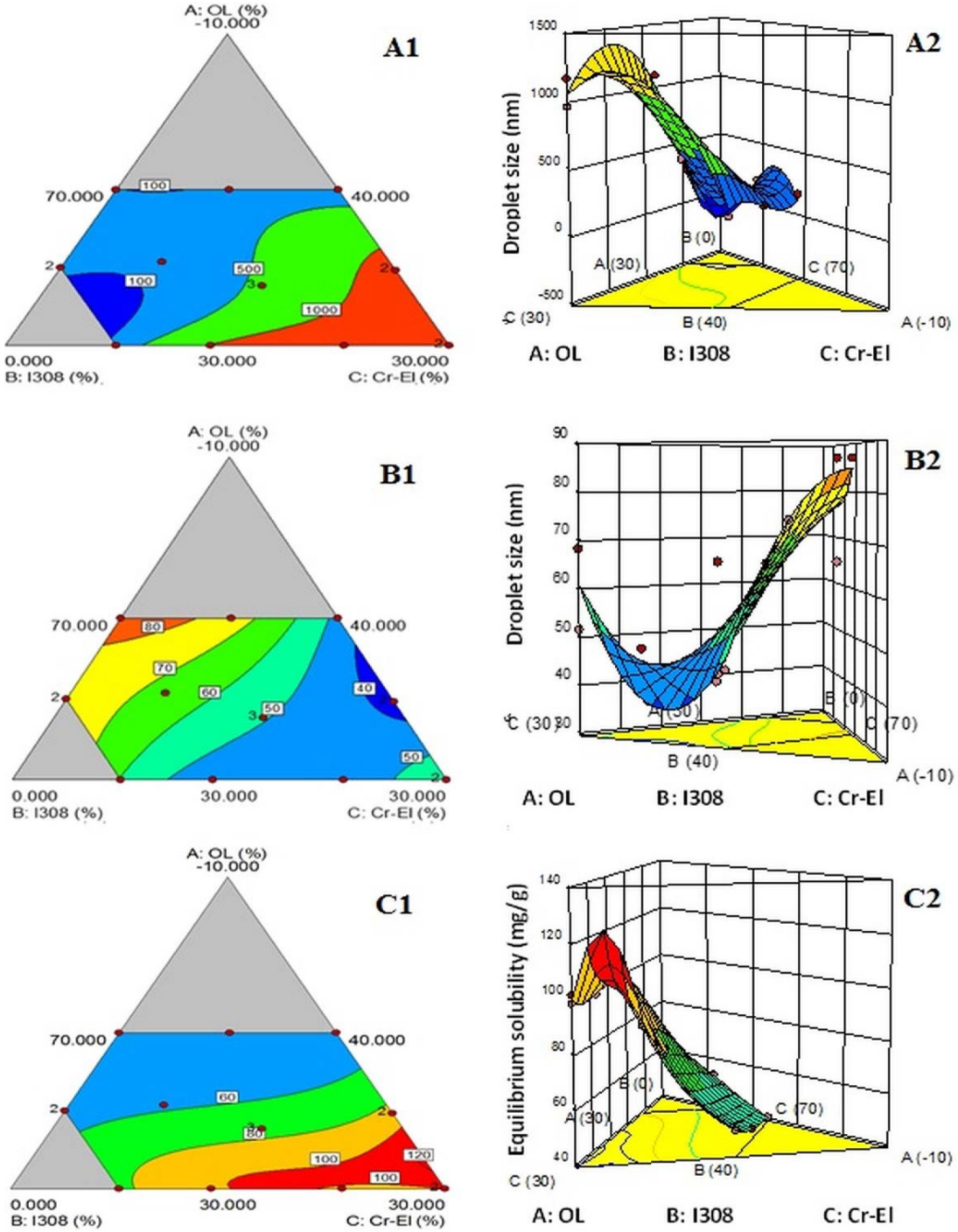

FIGURE 8 - Mixture design for OL/I308/Cr-El system showing: (A_1) phase diagram; (A_2) 3D plot of droplet size of diluted CN-free formulation (nm); (B_1) phase diagram; (B_2) 3D plot of droplet size of diluted CN-equilibrated formulation (nm); (C_1) phase diagram; (C_2) 3D plot of formulation equilibrium solubility (mg/g). OL denotes oleic acid, I308: Imwitor 308, and Cr-El: cremophor El.

of SNEFs. Future work may involve *in vitro* dissolution and lipolysis studies in formulation optimization by experimental design. The adopted optimization approach explored critical characterization issues, the role of formulation acidification, SNEF optimization by experimental design, and can be generalized for the whole category of weakly basic lipophilic drugs.

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TABLE VI - Validation of the experimental design model using the formulation [OL/I308/Cr-EL (25/25/50)]

Response	S.D.	n	Predicted mean	Actual data mean	95% PI low	95% PI high
R1. Droplet size after 1 h of aqueous dilution of drug-free formulation (nm)	105.8	3	652	677	448	857
R2. Droplet size after 1 h of aqueous dilution of drug-equilibrated formulation (nm)	9.6	3	48.1	43.0	32.0	64.2
R3. Equilibrium solubility (mg/g)	0.04	6	86.6	88.0	83.0	90.7

n: number of replicates; **S.D.**: standard deviation; **PI:** prediction interval

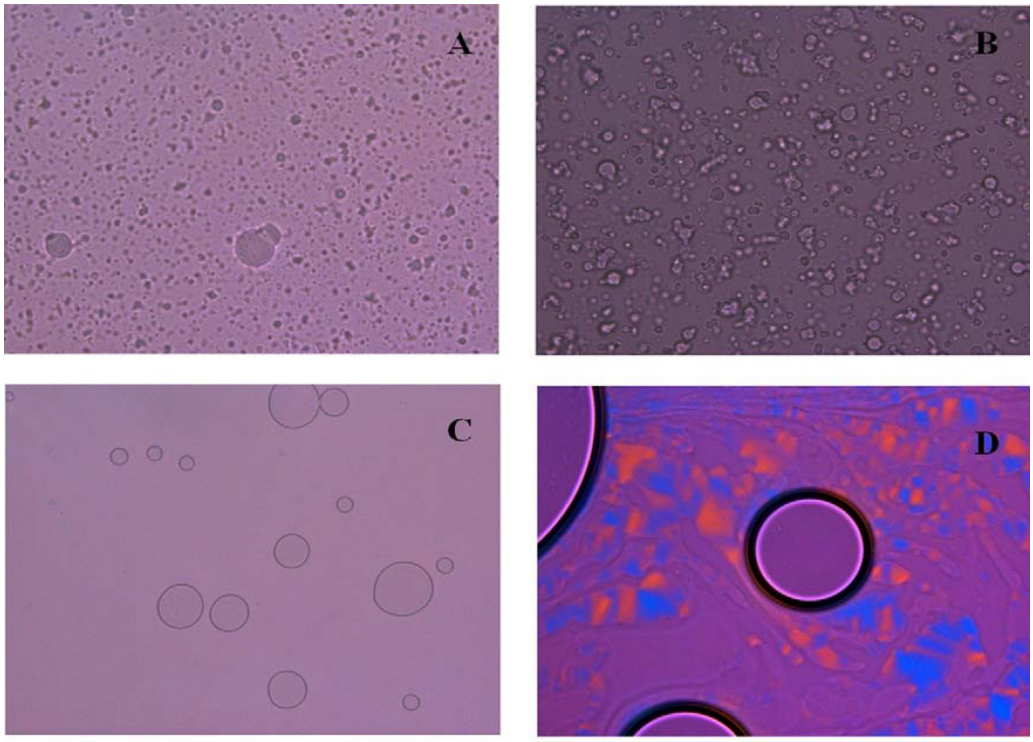

FIGURE 9 - Polarizing microscope images of liquid SNEF showing A: CN-free SNEF/water (70/30, w/w), B: CN-free SNEF/water (50/50, w/w), C: CN-loaded SNEF/water (70/30, w/w), and D: CN-loaded SNEF/water (50/50, w/w).

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