International Journal of Pharmaceutics 391 (2010) 203–211

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

SNEDDS containing bioenhancers for improvement of dissolution and oral absorption of lacidipine. I: Development and optimization

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ARTICLE INFO

Article history:
Received 27 November 2009
Received in revised form 8 February 2010
Accepted 1 March 2010
Available online 7 March 2010

Keywords:
Lacidipine
Ternary phase diagram
Bioenhancers
SNEDDS
Dissolution
D-optimal mixture design

ABSTRACT

The aim of this study was to develop and optimize SNEDDS formulations containing surfactants reported to be bioenhancers for improvement of dissolution and oral absorption of lacidipine (LCDP). Preliminary screening was carried out to select proper components combination. D-optimal mixture experimental design was applied to optimize a SNEDDS that contains a minimum amount of surfactant, a maximum amount of lipid, and possesses enhanced emulsification and dissolution rates. Three formulation variables; the oil phase X_1 (a mixture of Labrafil®/Capmul®), the surfactant X_2 (a mixture of Cremophor®/Tween® 80) and the co-surfactant X_3, were included in the design. The systems were assessed for droplet size, light absorbance, optical clarity, drug release and emulsification efficiency. Following optimization, the values of formulation components (X_1, X_2, and X_3) were 34.20%, 40.41% and 25.39%, respectively. There is a good correlation between light absorbance and droplet size analysis of diluted SNEDDS (R^2 = 0.883). Transmission electron microscopy demonstrated spherical droplet morphology. The stability of the optimized formulation was retained after storage at 40 °C/75% RH for three months. The optimized formulation of LCDP showed a significant increase in dissolution rate compared to the drug suspension under the same conditions. Our results proposed that the optimized SNEDDS formulation, containing bioenhancing surfactants, could be promising to improve oral absorption of LCDP.

1. Introduction

Lacidipine (LCDP) is a calcium channel blocker developed for oral administration and widely used in therapy since the early 1990s. LCDP is used in the treatment of hypertension and atherosclerosis. It also possesses an antioxidant effect (Lee and Bryson, 1994; McCormack and Wagstaff, 2003). The active Trans form is used in therapy (De Filippis et al., 2002); unfortunately, lacidipine is a highly lipophilic drug of poor water solubility and undergoes extensive first-pass hepatic metabolism with a mean absolute bioavailability of ~10% (range 3–59%). It is completely metabolized in the liver by cytochrome P450 3A4 (CYP3A4) to pharmacologically inactive metabolites (Tang et al., 2008). In spite of the marked role of the drug in hypertension therapy, no reported trials were so far adopted to enhance its oral absorption.

Formulation design can be a useful approach to improve the absorption and thus the oral bioavailability of such drug candidates (Pouton, 2006; Balakrishnan et al., 2009). Nanoemulsions, including self-emulsifying (SEDDS) and self-nanoemulsifying drug delivery systems (SNEDDS) are among the methods used to improve the oral bioavailability of poorly soluble drugs (Kommuru et al., 2001; Hong et al., 2006). SNEDDS comprise isotropic mixtures of natural or synthetic oils with surfactants and co-surfactants. These systems spontaneously emulsify when exposed to GIT fluids to form oil in water nanoemulsion with nanometric droplet size, in the range of 20–200 nm (Mou et al., 2008; Porter et al., 2008). The small droplet size confirms the highly efficient absorption of these oil droplets due to the rapid drug dissolution and release (Rao and Shao, 2008). SNEDDS are characterized by high solvent capacity and excellent stability. In addition, they can improve oral bioavailability through enhancing permeation across the intestinal membrane, reduce or eliminate food effect, solubilization, droplet size reduction and improvement of drug dissolution (Rane and Anderson, 2008; Wasan et al., 2009; Wang et al., 2010). The drug can also be delivered by lymphatic transport through the intestine avoiding the hepatic first-pass metabolism (Porter and Charman, 2001). Furthermore, SNEDDS formulations, containing bioenhancers that contain certain types of surfactants such as Cremophor®, Tween 80® and Labrasol®, are also reported to further improve the bioavailability of absorbed compounds by facilitating transcellular and paracellular absorption. Bioenhancers act also as p-glycoprotein and/or CYP450 enzymes inhibitors decreasing intestinal efflux and drug biotransformation (Yu et al., 1999; Chen, 2008; Elnaggar et al., 2009).

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doi:10.1016/j.ijpharm.2010.03.008

Labrasol® enhances both membrane permeability and intestinal absorption of cephalexin, a widely used β-lactam antibiotic (Koga et al., 2002) and significantly improves the efficacy of Vancomycin, a water soluble glycopeptide antibiotic with poor absorption characteristics (Prasad et al., 2003).

Development of a pharmaceutical formulation is time consuming and labor intensive. This is especially true for the SNEDDS, because its composition usually is complex, involving multiple components. The application of a mixture experimental (D-optimal) design to pharmaceutical formulation development has been demonstrated to be an efficient and satisfactory method for optimization of the formulation and to acquire the necessary information to understand the relationship between controllable (independent) variables and performance or quality (dependent variables) in a formulation (Gao et al., 2004).

Literature lacks any data about the use of SNEDDS for improvement of the dissolution and oral absorption of LCDP. Thus, the aim of this study was the formulation and optimization of LCDP-loaded SNEDDS containing surfactants reported to be bioenhancers. Experimental mixture design and desirability function were applied to optimize SNEDDS that contain a minimum amount of surfactant, a maximum amount of lipid, and possess enhanced emulsification and dissolution rates. As part of the optimization process, the main effect, interaction effects and quadratic effects of amounts of lipid, surfactant and co-surfactant on drug release, droplet size and emulsification time were investigated. The optimized formulation exhibiting promising in vitro drug dissolution is anticipated to improve oral absorption of the drug. Development and in vivo evaluation of controlled release solid dosage forms of the optimized LCDP formulation for management of hypertension are presently investigated.

2. Materials and methods

2.1. Materials

Lacidipine was kindly supplied by EgyPharm (Egypt). Labrafil® M 1944 CS (Labrafil-M, oleoyl macrogolglycerides), Labrafil® M 2125 CS (Labrafil, linoleoyl macrogolglycerides), Transcutol® P (purified diethylene glycol monoethyl ether), Maisine® 35-1 (glyceryl monolinoleate), Labrasol® (PEG-8 glycol caprylate), Lauroglycol 90 (propylene glycol monolaurate) were kindly obtained from Gattefosse (France). Capmul® MCM C8 (glyceryl monocaprylate) was obtained from Abitec Corp. (Janesville, WI). Miglyol® 812 (caprylic/capric triglyceride) was obtained from Sasol (Witten, Germany). Cremophor® RH 40 (Polyoxyl 40 hydrogenated castor oil) from BASF (Germany). Tween® 80 (polysorbate 80) and HCl were purchased from Merck (Germany). All other chemicals and solvents were of analytical grade and used without further purification.

2.2. Solubility studies

The solubility of LCDP in various oils, surfactants, and co-surfactants was determined. 2 g of each of the selected vehicles were added to each vial containing known excess of LCDP (500 mg). After sealing, the mixtures were shaken at 30 ± 0.5 °C for 48 h in a thermostatically controlled shaking water bath (Model 1083, GLF Corp., Germany). After reaching equilibrium, the mixtures were centrifuged at 3000 rpm for 5 min, followed by filtration through a Millipore membrane filter (0.45 μm).

The filtrate was diluted with chloroform and quantified spectrophotometrically (UV-1601 PC, Shimadzu, Japan) for dissolved LCDP via a validated method at 360 nm using chloroform as a blank. Each experiment was carried out in triplicate.

2.3. Preliminary screening of various surfactants for their emulsifying ability

Emulsification ability of various surfactants was screened according to the method described by Date and Nagarsenker (2007). 300 mg of each surfactant (Labrasol®, Cremophor® and Tween® 80) was added to 300 mg of the oily phase (Labrafil®). The mixtures were gently heated at 50 °C for homogenizing the components. 50 mg of each mixture was accurately weighed and appropriately diluted with double distilled water to yield fine emulsion. The ease of formation of emulsions was monitored by noting the number of volumetric flask inversions required to give uniform emulsion. The resulting emulsions were observed visually for the relative turbidity, then allowed to stand for 2 h and their transmittance was assessed spectrophotometrically (UV-1601 PC, Shimadzu, Japan) at 638.2 nm using double distilled water as blank.

2.4. Construction of ternary phase diagrams

The existence of self-nanoemulsifying oil formulation fields that could self-emulsify under dilution and gentle agitation were identified from ternary phase diagrams of systems containing oil, surfactant and co-surfactant. A series of self-emulsifying systems were prepared in each of the four formulation systems with varying concentrations of oils; Miglyol® and Labrafil®, surfactants; Cremophor® and Tween® 80 and co-surfactants; Transcutol®. For any mixture, the total of surfactant, co-surfactant and oil concentrations always added to 100%.

2 g of each mixture was prepared by the addition of variable proportions of the oil, surfactant and co-surfactant into a 10-mL capped glass vial. The components were mixed by vortex mixer (Paramix II, Julabo, Germany) for 60 s. The efficiency of nanoemulsion formation was assessed by adding 100 mg of each mixture to 20 mL double distilled water, followed by gentle agitation using a magnetic stirrer. The lipid-based formulations were assessed visually according to the rate of emulsification and the final appearance of the emulsion (Date and Nagarsenker, 2007). Only clear or slight bluish dispersions of droplet size 200 nm or lower were considered in the nanoemulsion region of the diagram (Zhang et al., 2008).

2.5. Formulation optimization of LCDP-loaded SNEDDS

The mixture experimental study was designed based on a three component system: the oil phase X_1 (a mixture of Labrafil®/Capmul®, 2:1, w/w), the surfactant X_2 (a mixture of Cremophor®/Tween® 80, 1:1, w/w) and the co-surfactant X_3 (Transcutol®). The total concentration of the three components summed to 100%. The drug content is kept constant 4 mg/g of the prepared SNEDDS. Based on the previous results obtained from phase diagram, the range of each component was selected as follows: X_1 (10–50%), X_2 (30–60%) and X_3 (20–50%). The absorbance of diluted SNEDDS (Y_1), mean droplet size (Y_2) and cumulative amount of drug released after 15 min (Y_3) were used as the responses (dependent variables). The responses of all model formulations were treated by Design-Expert® software (version 7; Stat-Ease, Inc., Minneapolis, MN). Suitable models for mixture designs consisting of three components include linear, quadratic and special cubic models. The best fitting mathematical model was selected based on the comparisons of several statistical parameters including the standard deviation (SD), the multiple correlation coefficient (R^2), adjusted multiple correlation coefficient (adjusted R^2) and the predicted residual sum of square (PRESS), proved by Design-Expert software. Among them, PRESS indicates how well the model fits the data, and for the chosen model it should be small relative to the other models under consideration (Huang et al., 2004).

Table 1
The formulations of mixture design and their characterization results.

Mixture no.	Labrafil®/Capmul® (2:1, w/w) (X_1)	Cremophor®/Tween® 80 (1:1, w/w) (X_2)	Transcutol® (X_3)	Absorbance of diluted SNEDDS (Y_1)	Mean droplet size (nm) (Y_2)	(%) released after 15 min. (Y_3)	Emulsification time (s)
1	50	30	20	1.42	151.70	94.00	39
2	22	44	34	0.62	24.30	97.04	21
3	20	60	20	0.66	33.50	85.33	21
4	20	30	50	0.70	44.30	87.93	16
5	35	30	35	0.81	105.70	82.00	24
6	50	30	20	1.69	133.80	98.86	29
7	10	60	30	0.69	20.10	70.22	21
8	20	30	50	0.87	66.00	101.19	14
9	21	37	42	0.77	26.00	97.04	13
10	36	37	27	0.78	23.20	100.70	19
11	10	50	40	0.70	29.00	74.67	15
12	10	60	30	0.55	32.20	61.19	18
13	20	60	20	0.74	44.90	83.85	23
14	10	40	50	0.55	17.70	73.19	10
15	10	40	50	0.65	25.00	72.00	12
16	35	45		0.62	20.00	95.10	19

D-optimal design was selected since it minimizes the variance associated with the estimates of the coefficients in the model (Holm et al., 2006). The software selected a set of candidate points as a base design. These included factorial points (high and low level from the constraints on each factor, centers of edges, constraint plane centroids, axial check point, and an overall center point). The base design consisted of 16 runs (Table 1). The optimum formulation of this study is selected to have a droplet size as small as possible (<90 nm), absorbance of diluted SNEDDS ranging between 0.5 and 0.7 and a maximum cumulative amount released after 15 min (95–100%).

2.6. Characterization of the prepared LCDP-loaded SNEDDS

2.6.1. Spectroscopic characterization of optical clarity

The optical clarity of aqueous dispersions of SNEDD formulations was measured spectroscopically. The formulations were diluted to 25 times with double distilled water. The absorbance of each solution was measured at 400 nm, using double distilled water as a blank.

2.6.2. Droplet size measurement

100 mg of each formulation was introduced into 20 mL of purified water at 25 °C and the contents were gently stirred using a magnetic stirrer. The droplet size of the resultant emulsions was determined by photon correlation spectroscopy using a Zetasizer 3000 (Malvern Instruments, UK) able to measure sizes between 10 and 5000 nm. A laser beam at 632 nm wavelength was used and light scattering was monitored at 25 °C at a 90° angle.

2.6.3. In vitro release studies

500 mg of each of the prepared formulations containing 2 mg of LCDP were filled into size 4 soft gelatin capsules. Filled capsules were stored at room temperature for 24 h to allow complete solidification of the systems before use. The release of LCDP from the capsules filled with SNEDD formulations was performed using a USP dissolution tester, apparatus II (VK 700, Vankel, USA) at 37 ± 0.5 °C and at a rotating speed of 50 rpm in 100 mL of 0.1N HCl. At selected time intervals for a period of 60 min, aliquots each of 3 mL were withdrawn from the dissolution medium through a Millipore membrane filter (0.45 μm) and replaced with an equivalent amount of the fresh dissolution medium. Concentrations of LCDP were determined spectrophotometrically at 364.8 nm using the regression equation of a standard curve developed in the same medium. The dissolution experiments were carried out in triplicate.

Table 2
Solubility of lacidipine in various vehicles.

Type of vehicle	Solubility (mg/mL) ± SD, $n = 3$
Labrafil® M 1944 (Labrafil-M)	8.20 ± 0.24
Labrafil® M 212 CS (Labrafil)	9.51 ± 0.64
Miglyol®	12.77 ± 0.37
Maisine®	5.35 ± 0.15
Lauroglycol® 90	5.10 ± 0.04
Labrasol®	33.45 ± 0.48
Tween® 80	26.01 ± 0.36
Cremophor®	24.46 ± 0.89
Capmul®	12.96 ± 0.87
Transcutol®	72.90 ± 2.10

2.6.4. Determination of the emulsification time

In order to determine the emulsification time (the time needed to reach the emulsified and homogeneous mixture, upon dilution). 1 g of each formulation was added to 200 mL of 0.1N HCl at 37 °C with gentle agitation using magnetic stirrer. The formulations were assessed visually according to the rate of emulsification and the final appearance of the emulsion.

2.6.5. Transmission electron microscopy (TEM)

The morphology of the optimized LCDP-loaded SNEDDS was observed by TEM (Joel, JEM-100 CX electron microscope, Joel, Tokyo, Japan). After sample dilution with water (1:1000), a sample drop was placed on a copper grid. The excess was drawn off with a filter paper. Samples were subsequently stained with 1% phosphotungistic acid solution for 30 s.

2.6.6. Stability study of LCDP-loaded SNEDDS

The optimized LCDP-loaded SNEDDS was stored at 40 °C/75% RH for three months. Optical clarity, emulsification time and transmission electron microscopy (TEM) including globule size measurement were performed for the stored samples using the same procedures adopted for the fresh samples.

3. Results and discussion

3.1. Solubility studies

The self-emulsifying formulations consisting of oil, surfactants, co-surfactants, and drug should be a clear and monophasic liquid at ambient temperature when introduced to aqueous phase and should have good solvent properties to allow presentation of the

drug in solution (Kommuru et al., 2001). The solubility of LCDP in various vehicles is presented in Table 2. Amongst the various oily phases that were screened, Miglyol® and Labrafil® provided the highest solubility of LCDP so were chosen for further investigations. The three surfactants, namely; Cremophor®, Tween® 80 and Labrasol®, showed good solubilizing power for LCDP, therefore, the selection of the surfactants in the further studies was governed by their emulsification efficiency rather than their ability to solubilize the drug (Date and Nagarsenker, 2007). Transcutol, which is a solubilizer and absorption enhancer, was found to be a very efficient solubilizer for LCDP, and so was chosen as a co-surfactant in the development of SNEDD formulations aiming to improve the drug loading capabilities.

3.2. Screening of various surfactants for emulsifying ability

In this study, the three selected non-ionic surfactants (Tween® 80, Labrasol®, and Cremophor®) were reported to possess bioenhancing activity. The bioenhancing activity includes effects on tight junction allowing paracellular transport such as Labrasol® and inhibitory effects on P-gp and CYP450 enzymes such as Cremophor® and Tween® 80 (Buggins et al., 2007; Chen, 2008; Elnaggar et al., 2009). These findings were confirmed by Zhang et al. (2003) who demonstrated increased AUC and C_{max} for orally administered digoxin in rats when co-administered with Tween®. Increasing the dose of Tween® increased the extent of absorption; AUC and C_{max} were increased by 30% and 163% respectively after dosing in 1% Tween®, and by 61% and 161% after dosing in a 10% solution. Cremophor® was reported to have a role in improving bioavailability of some drugs formulated as self-emulsifying formulations, such as atorvastatin (Shen and Zhong, 2006) and utilized in one of the few marketed SMEDDS products; Neoral® (Elnaggar et al., 2009).

The results indicated that Cremophor® and Tween® 80 exhibited the highest emulsification efficiency (98.9% and 92.8% transmittance) requiring only one flask inversion for homogenous emulsion formation. Labrasol® exhibited the lowest emulsification efficiency as indicated by the lower transmittance value (57.8%). Although the HLB values of the used surfactants were close in the range of 13–16, the difference observed in their emulsifying ability could be attributed to the difference in their structure and chain length (Date and Nagarsenker, 2007). Cremophor® and Tween® 80 yielded clear nanoemulsions requiring short time for nanoemulsification. Thus, they were selected for further investigation.

3.3. Construction of ternary phase diagram

Based on the results of preliminary screenings, four phase diagram formulations were constructed namely; system I: Miglyol/Cremophor/Transcutol; system II: Miglyol/Tween 80/Transcutol; system III: Labrafil/Cremophor/Transcutol; system IV: Labrafil/Tween 80/Transcutol. The phase diagrams were depicted in Fig. 1. The shaded region indicates nanoemulsion region. Wider region indicates better self-nanoemulsifying ability (Elnaggar et al., 2009). In the current investigation, it could be seen that systems III and IV showed wider nanoemulsification regions compared to systems I and II indicating better self-nanoemulsification properties of the former systems. Also, systems III and IV, containing Labrafil®, yielded nanoemulsion containing as high as 30% oily phase composition. On the other hand, systems I and II, containing Miglyol, produced nanoemulsion till a maximum oil concentration of 20% only. Thus, Labrafil® was selected for formulation of LCDP-loaded SNEDDS using a mixture of Tween® 80 and Cremophor® (1:1) due to their bioenhancing activity.

3.4. Optimization of LCDP-loaded SNEDDS

The system components were selected based on the ability of the previously prepared ternary systems to form nanoemulsion containing the highest oil content. Capmul®, a medium chain monoglyceride, was added to the selected oil, Labrafil®, in order to increase the lipid content of the formulated nanoemulsion without losing its ability to form clear dispersion upon dilution. It was reported that medium chain monoglycerides (polar lipids) promote water penetration and self dispersibility of lipid formulations and have good solvent capacity for drugs (Pouton, 2007). Moreover, Capmul® is likely to increase the interfacial fluidity of surfactant boundaries in the micelles because of the entrapment of Capmul® in the high HLB surfactant enhancing the emulsification process upon dilution with aqueous medium (Taha et al., 2004).

In order to rapidly obtain the optimal LCDP-loaded SNEDDS, D-optimal mixture experimental design was applied in this study. The mixture of Labrafil®/Capmul® (2:1, w/w) (X_1), a mixture of Cremophor®/Tween® 80 (1:1, w/w) (X_2) and Transcutol® (X_3) were chosen as formulation variables and the absorbance of diluted SNEDDS (Y_1), mean droplet size (Y_2) and cumulative amount released after 15 min (Y_3) were selected as response variables. The responses of these formulations are summarized in Table 1. The independent and response variables were related using polynomial equation with statistical analysis through Design-Expert® software. As shown in Table 3, the approximation of response values of Y_1 and Y_2 based on the quadratic model was the most suitable because its PRESS was smallest. The values of the coefficients X_1, X_2 and X_3 are related to the effect of these variables on the response. A positive sign of coefficient indicates a synergistic effect while a negative term indicates an antagonistic effect upon the response (Huang et al., 2005). The larger coefficient means the independent variable has more potent influence on the response.

As shown in Table 1, Absorbance of the studied aqueous dispersion of SNEDDS varied between 0.55 and 1.69. As expected, compositions with lower absorbance showed lowest droplet size since aqueous dispersions with small absorbance are optically clear and oil droplets are thought to be in a state of finer dispersion (Subramanian et al., 2004). Based on the calculated model for the mean absorbance and droplet size, the contour plots are shown in Figs. 2 and 3, respectively. The oil mixture provides the largest contribution to the absorbance of the diluted SNEDDS and consequently the droplet size of the produced microemulsion. The two other formulation components have more limited effects on both responses. This result was similar to earlier studies (Liu et al., 2009) which reported that the droplet size of systems, containing Maisine® 35-1:Labrafac® CC (1:1, w/w) as oil mixture, increased from 24 to 110 nm when the oil concentration increased from 20 to 50%. As shown in Table 3, the coefficient of X_1X_2 for both responses was largest, showing the negative effect of combination of oil mixture and surfactant mixture on the droplet size of the diluted microemulsions and thus, their absorbance. As shown in Fig. 4, there is a good correlation between absorbance of diluted SNEDDS and droplet size analysis ($R^2 = 0.883$). Thus, spectrophotometric absorbance of diluted SNEDDS could be used as a simple technique for determining droplet size of SNEDDS rather than the expensive and sophisticated equipment used for measurement of droplet size.

From Table 1, it can be inferred that the three independent factors have a profound effect on drug release. In order to obtain a formulation having rapid drug release within 15 min, D-optimal design optimization was used to determine the levels of these factors. Special cubic model was the most appropriate mathematical model for Y_3 (Table 3).

As illustrated in Table 4, a P-value of ≤ 0.05 for any factor in analysis of variance (ANOVA) indicates significant effect of the cor-

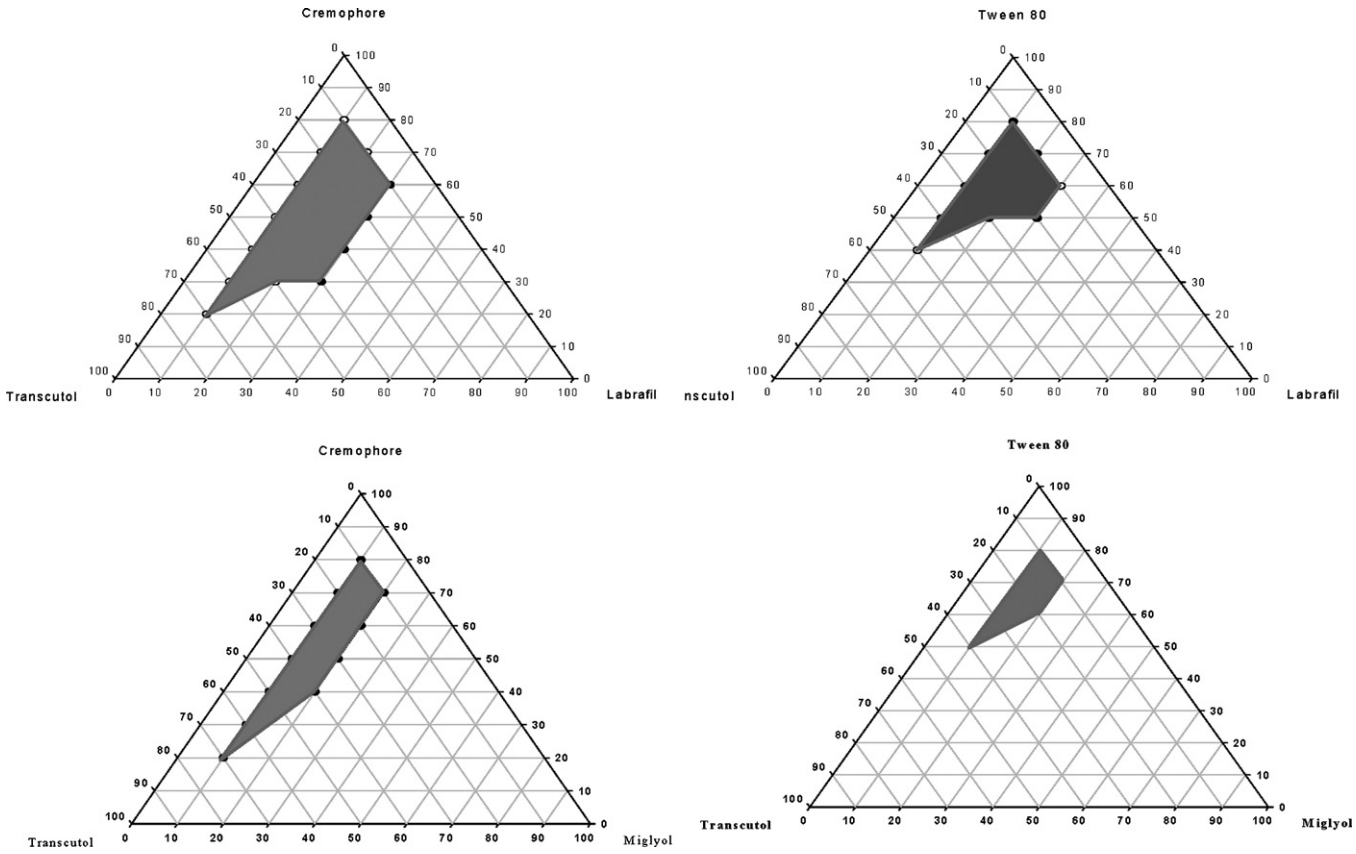

Fig. 1. Ternary phase diagrams of different selected systems dispersed in water at 25 °C. The shadow area represents o/w nanoemulsion region.

responding factors on the dissolution after 15 min (Y_3). It can be inferred that the interaction terms X_1X_2, X_1X_3 and X_2X_3 have a non-significant effect on the drug release from formulation dissolution for 15 min. The interaction term $X_1X_2X_3$ have a significant synergistic effect on drug release as indicated by the positive value of the coefficient $X_1X_2X_3$ (Table 3). Fig. 5 shows the contour diagrams illustrating the effect of varying ratios of (X_1), (X_2) and (X_3) on the release of drug from LCDP-loaded SNEDDS. It is obvious that there is an optimum ratio of all the mixture components for microemulsion formulation and drug release from emulsions as indicated by the central solid portion of the plot. The figure indicates that sufficient concentration of co-surfactant is needed for maximal effect of surfactant on emulsification of lipophilic substance.

Fig. 6 is a representative two component mixture plot, which elucidates the effects of varying ratio of oil mixture (X_1) and surfactant mixture (X_2) with a fixed amount of Transcutol (X_3) on response Y_3. It is clear that the emulsification of oil increases as the concentration of surfactant is increased. Maximum dissolution of the drug was found at oil concentration range from 20 to 30% with surfactant levels from 35 to 45%.

Table 3
Regression results of the measured responses.

Model	Coefficient	Y_1	Y_2	Y_3
	$b1(X_1)$	+0.073	+9.232	+5.340
	$b2(X_2)$	+0.027	+4.971	+2.341
	$b3(X_3)$	+0.028	+3.629	+4.618
	$b12(X_1X_2)$	−1.446E−003	−0.258	−0.153
	$b13(X_1X_3)$	−8.931E−004	−0.059	−0.275
	$b23(X_2X_3)$	−7.721E−004	−0.159	−0.135
	$b123$ ($X_1X_2X_3$)	–	–	+8.445E−003
Linear	SD	0.20	28.47	9.35
	R^2	0.6356	0.6131	0.5223
	Adjusted R^2	0.5795	0.5535	0.4488
	PRESS	0.87	15698.67	1681.41
Quadratic	SD	0.13	16.76	7.56
	R^2	0.8807	0.8968	0.7597
	Adjusted R^2	0.8210	0.8452	0.6395
	PRESS	0.49	6936.49	1630.13
Sp. cubic	SD	0.12	17.57	6.02
	R^2	0.9094	0.8980	0.8631
	Adjusted R^2	0.8490	0.8300	0.7718
	PRESS	0.41	10509.86	1260.91

Table 4
ANOVA of cumulative amount of drug released after 15 min (Y_3).

Source	Sum of squares	DF	Mean square	*F*-Value	*P*-Value	Significance
Model	2054.51	6	342.42	9.45	0.0018	Significant
Linear mixture	1243.39	2	621.70	17.16	0.0008	Significant
AB	15.23	1	15.23	0.42	0.5329	Not significant
AC	32.17	1	32.17	0.89	0.3706	Not significant
BC	108.33	1	108.33	2.99	0.1178	Not significant
ABC	246.12	1	246.12	6.79	0.0284	significant
Residual	326.00	9	36.22			
Lack of fit	183.65	4	45.91	1.61	0.3035	Not significant
Pure error	142.36	5	28.47			
Cor total	2380.51	15				

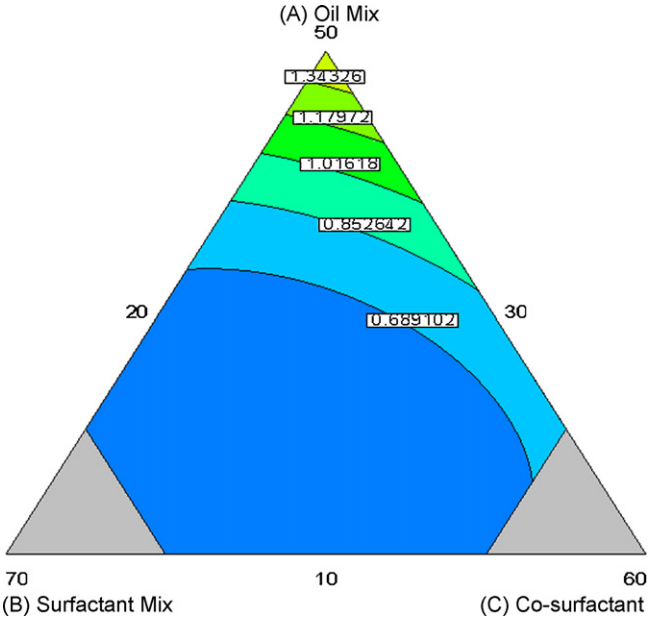

Fig. 2. 2D contour plots for the effect of variables on the absorbance of diluted LCDP-loaded SNEDDS (Y_1).

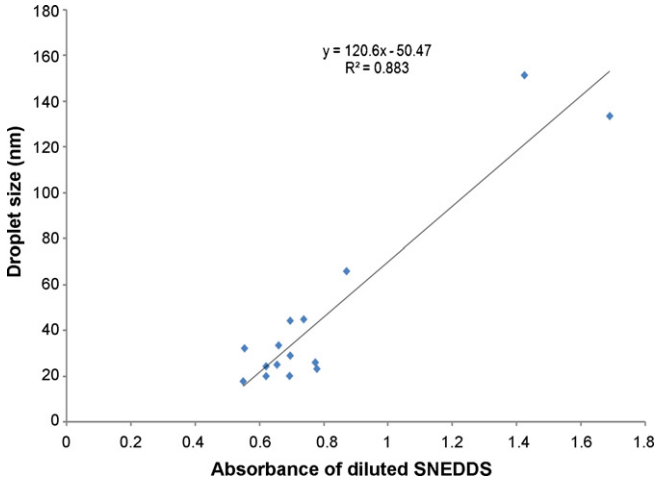

Fig. 4. Correlation between droplet size analysis and absorbance of diluted LCDP-loaded SNEDDS.

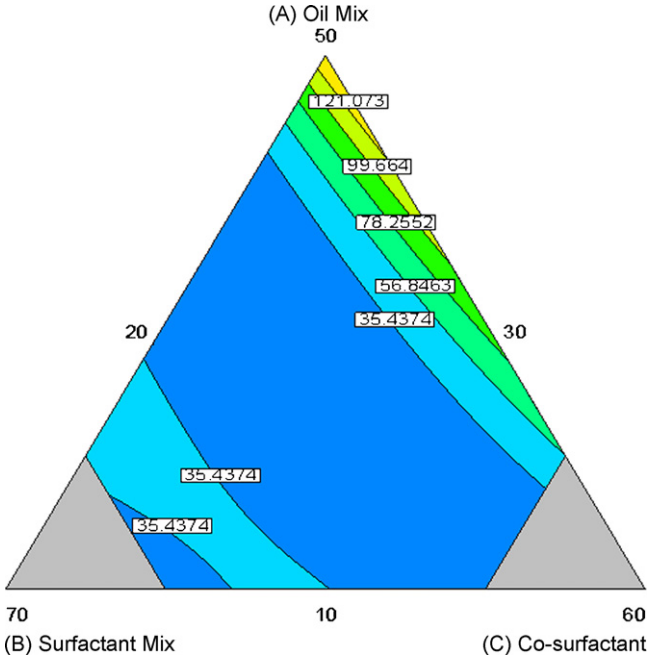

Fig. 3. 2D contour plots for the effect of variables on droplet size of LCDP-loaded SNEDDS (Y_2).

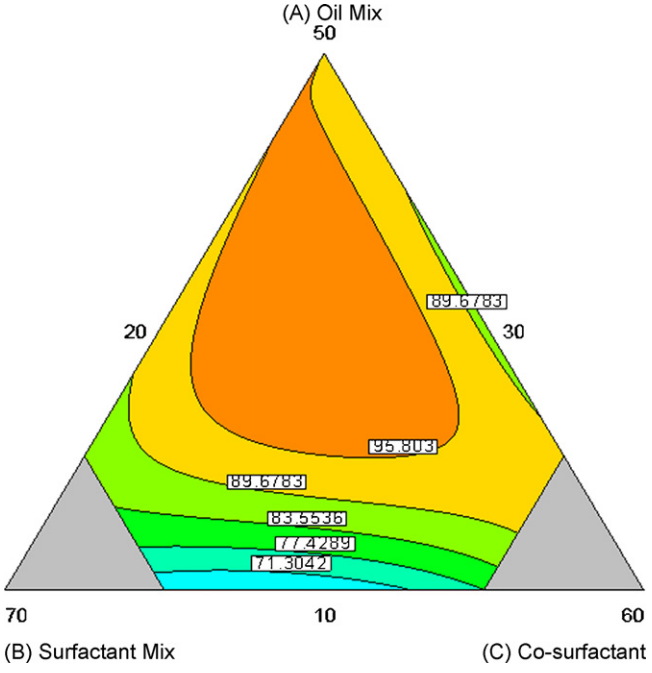

Fig. 5. 2D contour plots for the effect of variables on the cumulative amount of LCDP released after 15 min from SNEDDS (Y_3).

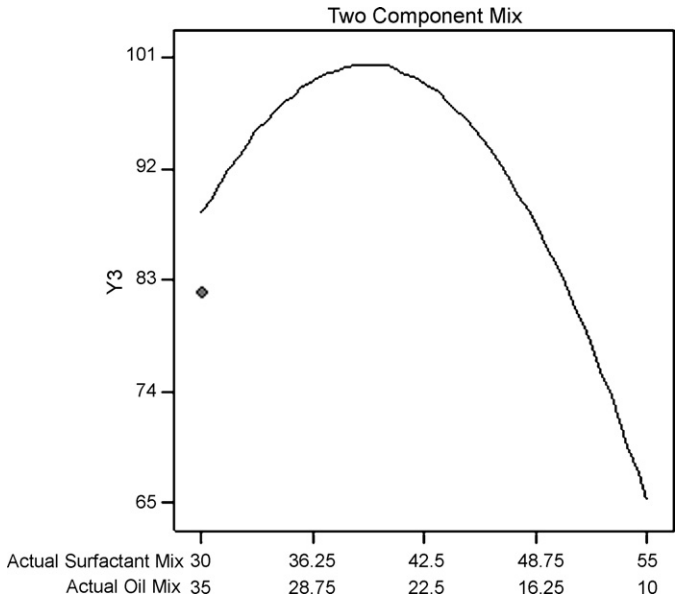

Fig. 6. Two component mixture plot for the effects of varying ratios of oil mixture (X_1) and surfactant mixture (X_2) with a fixed amount of Transcutol (X_3) on response Y_3.

The aim of the optimization of pharmaceutical formulations is generally to determine the levels of the variable from which a robust product with high quality characteristics may be produced. Some of the measured responses have to be minimized. In this case, these responses comprise the droplet size (<90 nm) and the absorbance (<0.8). Some responses, such as the dissolution of drug within 15 min, have to be maximized (>95%) in order to produce a product of desired characteristics. Under these conditions, these three responses were then combined to determine an all over optimum region. Fig. 7 shows an acceptable region met the requirement

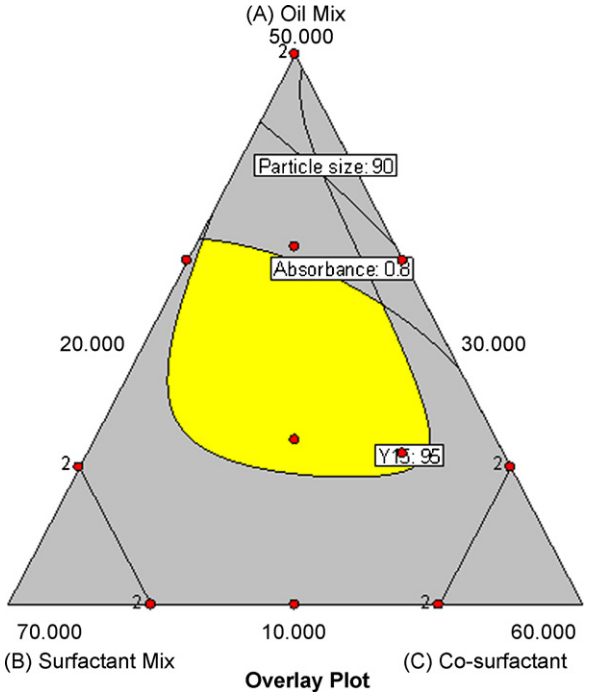

Fig. 7. Overlay plot for the effect of different variables on the three responses; absorbance (Y_1), droplet size (Y_2) and cumulative amount of LCDP released after 15 min from SNEDDS (Y_3).

Table 5
Predicted and observed responses of optimized composition of LCDP SNEDDS.

Variables	Values	Response	Observed values	Predicted values	Error %
X_1	34.20	Y_1	0.71	0.75	5.63
X_2	40.41	Y_2	34.30	35.60	3.79
X_3	25.39	Y_3	98.74	100.45	1.73

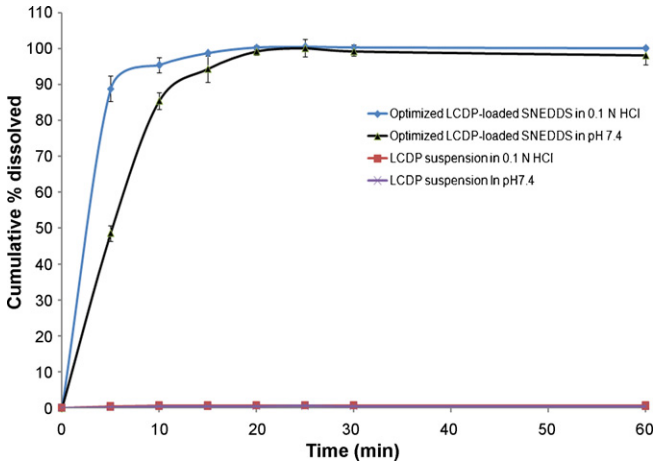

Fig. 8. In vitro dissolution profiles of LCDP from optimized LCDP-loaded SNEDDS formulation compared to that from aqueous drug suspension (mean % ± SD).

of these responses. According to the selection criteria, only those LCDP-loaded SNEDDS compositions with a maximum lipid content (>30%) were chosen for verification. A SNEDDS formulation satisfying these criteria was prepared and evaluated. The SNEDDS composition is reported in Table 5, along with the predicted and observed responses. An optimum response was found with Y_1, Y_2, and Y_3 of 0.75, 35.60 nm and 100.45% at X_1, X_2 and X_3 values of 34.20%, 40.41% and 25.39%, respectively. To verify these values, the optimum formulation was prepared according the above values of the factors and subjected to previous tests. The predicted and observed values of Y_1, Y_2, and Y_3 of the optimum formulation showed small percentage error, 5.63%, 3.76% and 1.73%, respectively. A good agreement is obtained between the model prediction and experimental observation. Thus, the validity of the model was established.

Fig. 8 demonstrates comparison of drug dissolution from LCDP-loaded SNEDDS formulation to that from aqueous drug suspension in 100 mL simulated gastric fluid (0.1N HCl), and simulated intestinal fluid (phosphate buffer pH 7.4). Both formulations contained the same drug amount (2 mg/0.5 g distilled water or optimized SNEDDS formulation). Dissolution profiles reveal that LCDP was completely dissolved from SNEDDS formulations after 15 and 20 min in simulated gastric fluid and simulated intestinal fluid, respectively. LCDP-loaded SNEDDS dispersions in both media showed no signs of precipitation, cloudiness or separation for 24 h. There was no drug dissolved from aqueous drug suspension after 60 min in both media. These results confirm the role of SNEDDS formulation to improve LCDP solubilization and in vitro dissolution.

3.5. Determination of the emulsification time

The rate of emulsification was taken as an important index for the assessment of the efficiency of self-emulsification (Lia et al., 2005; Wei et al., 2005; Balakrishnan et al., 2009) that is the SNEDDS should disperse completely and quickly when subjected to dilution under mild agitation.

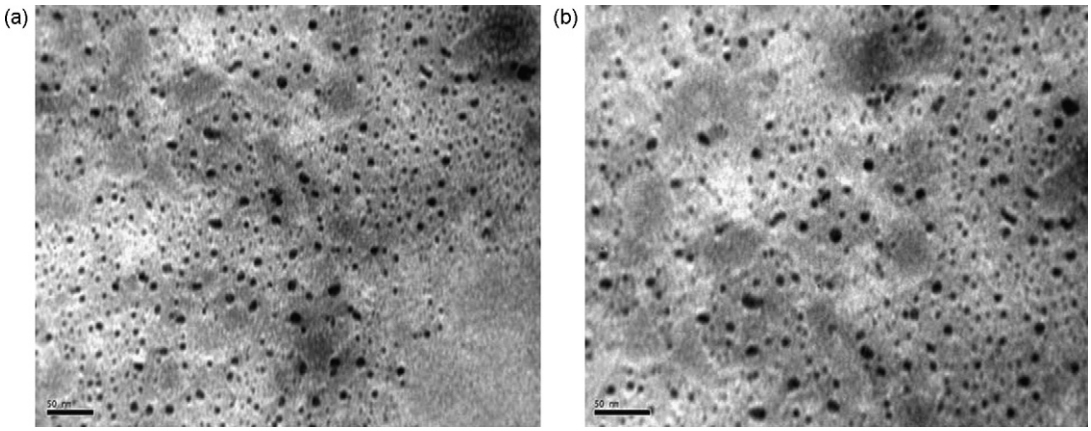

Fig. 9. TEM of optimized LCDP-loaded SNEDDS formulation (50,000×), fresh (a) and stored (b).

All the formulations exhibited a rapid rate of emulsification ranging from 10 to 39 s (Table 1). It is obvious that rapid emulsification is correlated with lower content of oil and higher content of co-surfactant which result in lower viscosity of the system (Lia et al., 2005). An attempt to find an adequate mathematical model between emulsification time and LCDP-loaded SNEDDS formulations failed. Gao et al. (2004) reported a failed attempt to find an adequate mathematical model between the droplet size and a SEDDS formulation consisting of glycerol dioleat, glycerol monooleate, Cremophor® EL, and PEG 400.

3.6. Stability of the optimized LCDP-loaded SNEDDS

The optimized LCDP-loaded SNEDDS was stable when stored at 40 °C/75% RH for three months where there was no obvious change in visual appearance. The emulsification times of the fresh and stored formulations were 14.5 ± 2.12 and 13 ± 2.82 (nm ± SD, $n = 3$), respectively. The morphology of the fresh and stored SNEDDs formulations was observed using TEM. The photographs depicted in Fig. 9 reveal that all droplets after dilution possessed nearly the same size and spherical shape.

4. Conclusion

In this study, SNEDD systems of LCDP were prepared and in vitro evaluated. All the formulations showed good release profiles and exhibited a rapid rate of emulsification. D-optimal mixture experimental design was applied in order to rapidly obtain the optimal LCDP-loaded SNEDDS formulation so to contain minimum amount of surfactant, maximum amount of lipid, highest emulsification and dissolution rates. The stability of the optimized formulation was retained after storage at 40 °C/75% RH for three months. The optimized SNEDDS formulation of LCDP showed a significant increase in dissolution rate compared to aqueous drug suspension in simulated gastric fluid and simulated intestinal fluid under the same conditions. The significant increase in drug dissolution and the inclusion of bioenhancing agents in the developed SNEDDS of LCDP propose that the prepared system could be promising to improve oral absorption of LCDP. Further studies for development and in vivo evaluation of controlled release solid dosage forms of the optimized LCDP formulation are presently investigated.

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