7.1 INTRODUCTION

SMEDDS formulation is composed of oil, surfactant and cosurfactant. The system has the ability of forming oil-in-water (o/w) microemulsion when dispersed by aqueous phase under gentle agitation. The agitation required for self-emulsification comes from the digestive motility provided by the movement of stomach and intestine in the gastrointestinal tract. SMEDDS present drugs in small droplet size and increase the dissolution and permeability (1). Moreover, drugs can be loaded in the inner-phase and delivered by lymphatic bypass mechanism, SMEDDS prevent drugs against hydrolysis by enzymes in the gastrointestinal tract and reduce the presystemic clearance and hepatic first-pass metabolism (2). The principle of self-emulsification is still the subject of speculation. Various models, and speculation about the absorption in the gastrointestinal tract, have been proposed to improve bioavailability of water-insoluble compounds caused by lipidic excipients, such as altering the gastrointestinal motility, increasing bile flow and mesenteric lymph flow, etc.

Factorial designs are used where the effects of different factors or conditions on choice for simultaneous determination of the effect of several factors and their interaction. Factorial design is used to study the effect of independent variables on the dependent variables of any formulation. Based on the principle of design of experiments, factorial design is employed to evaluate the effect of two independent factors (3). The regression equation for the response is calculated using the equation below:

$$Y = b_0 + b_1X_1 + b_2b_2 + b_3X_1^2 + b_4X_2^2 + b_5X_1X_2 \dots\dots\dots\dots\dots\dots\dots Equation\ 7.1$$

In this mathematical approach, each experimental response (Y) can be represented by a quadratic equation of the response surface. Y is the measured response and b is the estimated coefficient for the factor X. The coefficients corresponding to linear effects (X_1 and X_2), interaction (X_1X_2) and the quadratic effects (X_1^2 and X_2^2) (4).

Lurasidone hydrochloride (LH) is an atypical antipsychotic agent for the treatment of schizophrenia and bipolar disorder. LH is a BCS class II drug having absolute oral bioavailability of 9-19%. In addition to extensive first-pass metabolism (only 1% of unchanged lurasidone was recovered in urinary and biliary routes after intravenous administration of LH 19), incomplete absorption of LH due to poor water solubility and low dissolution is the major reason for its low oral bioavailability. Hence, LH loaded SMEDDS (LH-SMEDDS) were prepared and optimized using 3^2 factorial design to improve its solubility, dissolution and bioavailability (5).

7.2 MATERIALS

Lurasiodne hydrochloride (LH) was received as a gift sample from Alembic Pharmaceuticals Ltd., Vadodara, India. Peceol, Capryol 90, Lauroglycol 90, Labrasol, Capmul MCM, Labrafil M 1944 CS, Labrafil M 2125 CS, Transcutol HP, Lauroglycol FCC were obtained as gift samples from Gattefosse India Private Limited, Mumbai, India. Capmul MCM C8, Captex 500, Capmul PG8 were obtained from Abitec, USA. Cremophor EL, Cremophor RH40 were obtained as gift samples from BASF India Ltd., Mumbai, India. All other chemicals and reagents used were of analytical grade.

7.3 PREFORMULATION STUDIES

7.3.1 Solubility of LH in oils, surfactant and co-surfactants

Screening of oil was performed as described in section 5.3.1.

7.3.2 Screening of surfactants

Screening of surfactants was performed as described in section 5.3.2.

7.3.3 Screening of co-surfactants

Screening of surfactants was performed as described in section 5.3.3.

7.3.4 Construction of pseudoternary phase diagram

Pseudoternary phase diagrams were constructed as described in section 5.3.4.

7.4 FORMULATION DEVELOPMENT

LH was added in the oil in small increment with continuous stirring. The surfactant system was prepared by mixing separately the chosen surfactant and cosurfactant in their determined ratios. Oil phase containing LH was added in the surfactant system solution with continuous stirring till the homogenous mixture formed. LH-SMEDDS formulation was subjected to further characterization (6).

7.5 OPTIMIZATION USING 3^2 FACTORIAL DESIGN

A three level two factor full factorial design was employed for systemic study of joint influence of the effect of independent variables [concentration of oil (X_1) and surfactant to cosurfactant (K_m) ratio (X_2)] on critical dependent variables. Coded and actual values are shown in table 7.1. Globule size (Y_1), %transmittance (Y_2) and self-emulsification time (Y_3) were taken as dependent variables (7).

Table 7.1: The coded and actual values of independent variables of LH-SMEDDS

Factors	**Levels**		
	-1	**0**	**+1**
X_1: Oil Concentration (%)	10	20	30
X_2: Surfactant:cosurfactant ratio (km)	1	2	3
Dependent Variables	**Goal**		
Globule size (Y_1)	Minimize		
Percentage transmittance (Y_2)	Maximize		
Self-emulsification time (Y_3)	Minimize		

Contour and response surface and perturbation plots were generated to study response variations against independent variables using Design Expert 8.0.7.1 (Stat-Ease. Inc. Minneapolis, MN) software. Check point batch suggested by software was prepared and the percentage relative error of each response was calculated using following equation in order to judge validity of the model (7).

$$\%\ Relative\ Error = \frac{Predicted\ value - Experimental\ value}{Predicted\ value} * 100$$

7.5.1 Optimization using Desirability function

All three responses were simultaneously optimized using the general linear scale desirability function introduced by Derringer and Suich (8). The desirability function seeks out the most favourable and compromising point in the design space that fulfils the set goal for dependent variables such as minimum globule size and maximum transmittance (6).

For simultaneous optimization of globule size, percentage transmittance and self-emulsification time, desirability function (multi-response optimization technique) was applied and total desirability was calculated using Design Expert software (version 7.0.3, Suite, Minneapolis, MN). The desirability lies between 0 and 1 and it represents the closeness of a response to its ideal value (9).

7.5.2 Analysis of design space robustness

Overlay plot was generated using design expert to evaluate robustness of established design space with selecting response to higher and lower value of established design

space. The software suggested values for independent variables in and around established design space along with value of the desired responses (10).

7.5.3 Statistical Analysis

The results were presented as mean ± standard error of the mean. The experimental data were validated by ANOVA, regression coefficient, and p value less than 0.05 was considered as significant.

7.6 CHARACTERIZATION

Characterization of lurasidone HCl loaded SMEDDS were performed as per section 5.6.

7.7 IN VITRO DRUG RELEASE STUDY

In- vitro drug release of LH-SMEDDS was carried out same as procedure described in section 5.7 except LH-loaded SMEDDS and LH suspension equivalent to 20 mg was placed in the dialysis bag and phosphate buffer pH 6.8 containing 0.1% SLS. Drug concentration was determined using UV spectroscopy at 232 nm.

7.8 EX-VIVO PERMEATION STUDY

Ex vivo permeation study of LH-SMEDDS was carried out same as procedure described in section 5.8 except LH-loaded SMEDDS dispersion and LH suspension equivalent to 20 mg was taken for the study and phosphate buffer pH 7.4 containing 0.1% SLS. Drug concentration was determined using UV spectroscopy at 232 nm.

7.9 STABILITY STUDY

Stability study of LH loaded SMEDDS was carried out as per procedure described in section 5.9.

7.10 RESULTS AND DISCUSSION

7.10.1 PREFORMULATION STUDIES

7.10.1.1 Solubility of LH in oils, surfactant and co-surfactants

In this study, selection of oil was done on the basis of its capacity to solubilize maximum amount of drug. This might be attributed to the fact that in SMEDDS drug should be in its dissolved state, as this form have been reported to possess greater concentration of drug. The high concentration gradient provides driving force for the permeation of drug through GI tract. The maximum solubility of LH was obtained in Capmul MCM C8 (Figure 7.1) Capmul MCM C8 is also reported to have good emulsifying properties and has GRAS (generally regarded as safe) status. Thus, the same was chosen as the oily carrier phase for formulating the SMEDDS system (11).

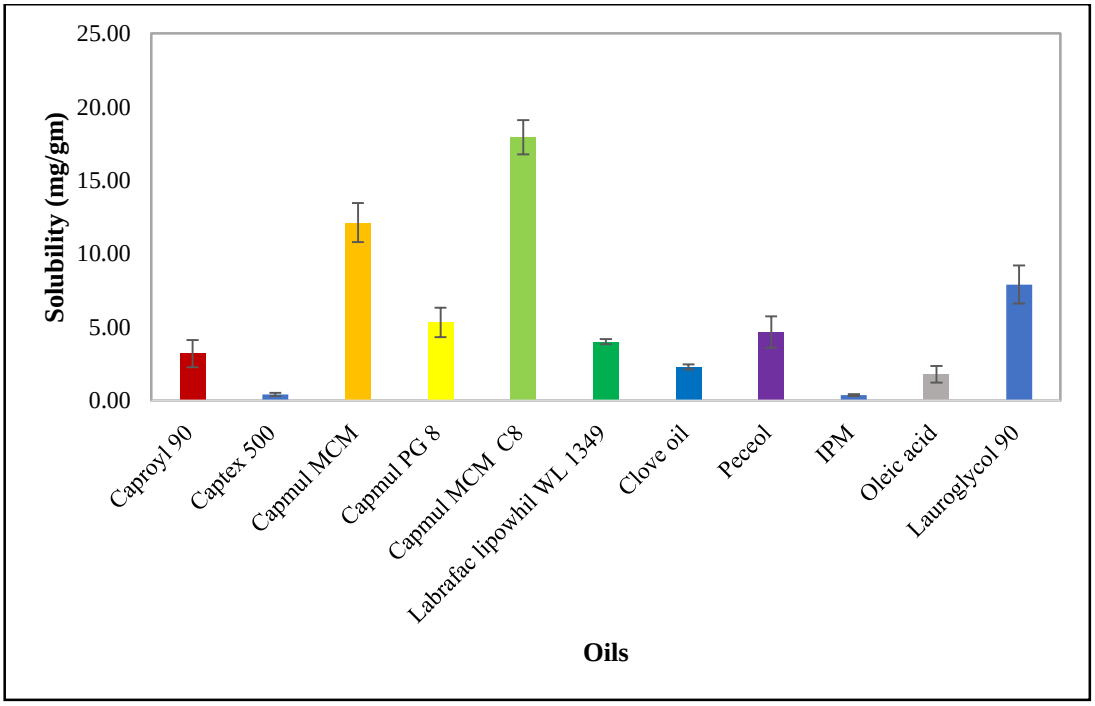


Figure 7.1: Solubility of lurasidone HCl in various oils

The solubility study in surfactants and co-surfactants was also done and it was observed that maximum solubility of LH was observed in Tween 20 and Propylene glycol as a surfactant and co-surfactant respectively (Figure 7.2 and 7.3). However, the selection of surfactant and cosurfactant for SMEDDS was not done on the basis of solubility studies since it was strongly believed that both of them play a crucial role in emulsification of oil phase. Good solubility of drug in surfactant and cosurfactant was considered as an additional advantage as this feature may prevent drug precipitation during storage (7).

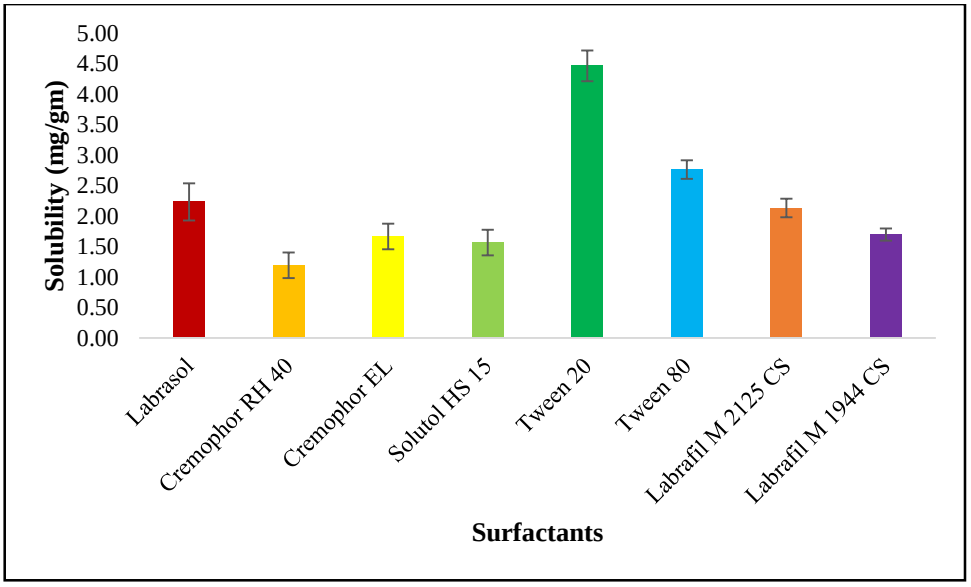


Figure 7.2: Solubility of lurasidone HCl in various surfactants

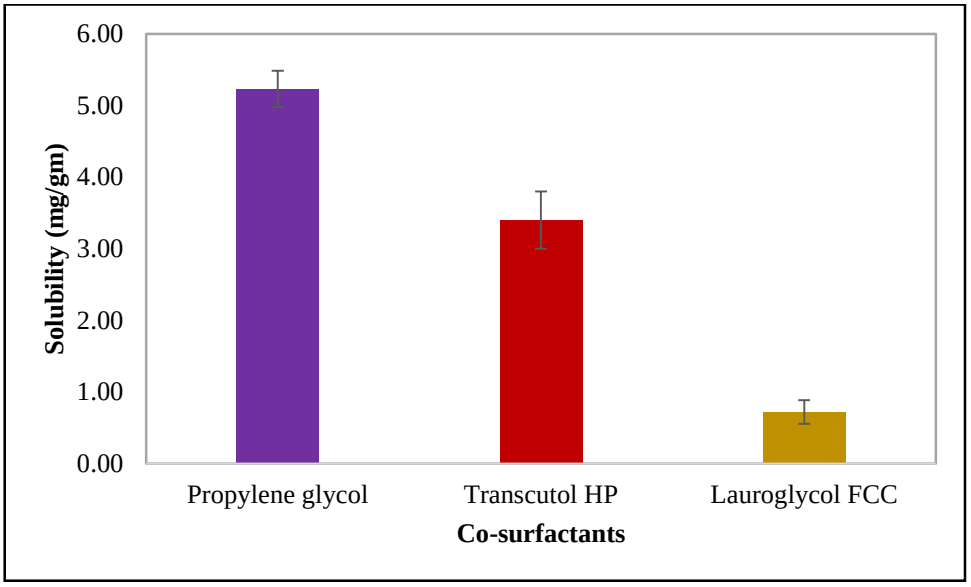


Figure 7.3: Solubility of lurasidone HCl in various co-surfactants

7.10.1.2 Screening of Surfactants

It is possible that the surfactant with good solubilizing properties for drugs may not have equally good affinity for the oil. Thus, the selection of the surfactant and co-surfactant was governed by the emulsification efficiency for oil rather than the ability to solubilize LH (12).

Nonionic surfactants are generally considered safer than the ionic surfactants and are usually accepted for oral ingestion (13). They are also reported to provide better stability to emulsion over a wider range of pH and ionic strength. In addition, they can produce reversible changes in intestinal mucosal permeability (11). The result revealed (Figure 7.4) that Cremophor EL (HLB 12-14) had highest ability to emulsify Capmul

MCM C8 as compared to other surfactants. Thus, Cremophor EL was selected as surfactant due to its both bioenhancing (12) and emulsifying property for formulation development.

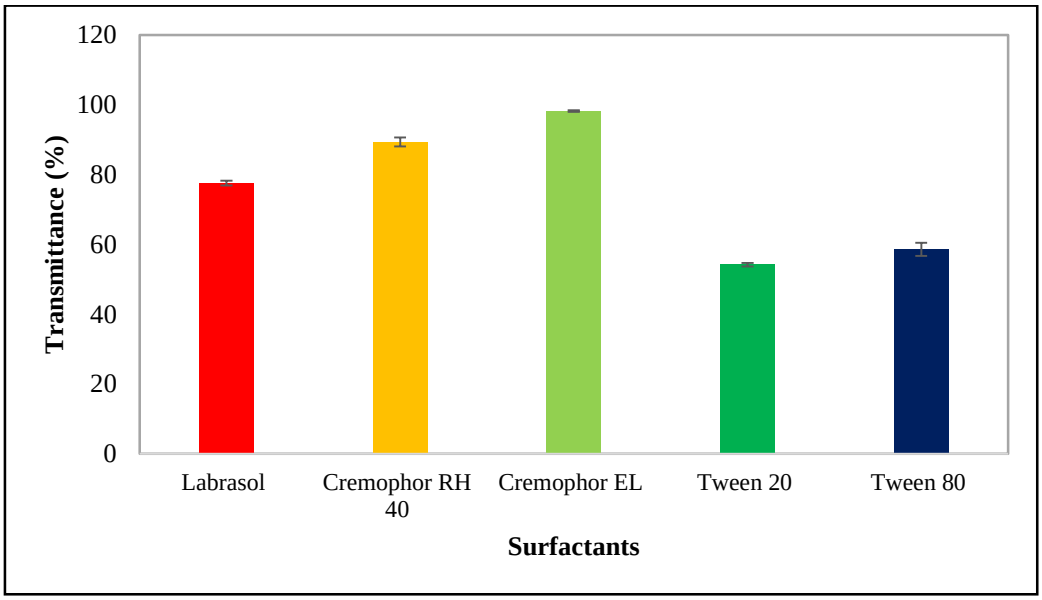


Figure 7.4: Emulsification study of lurasidone HCl in surfactants

7.10.1.3 Screening of co-surfactants

It was reported that co-surfactants improve the self-emulsification ability of surfactants by increasing the interfacial fluidity at the interface (11). The maximum transmittance was observed with Transcutol HP (Figure 7.5) which indicated its good emulsifying property as compared to other co-surfactants. So, Transcutol HP was selected for further investigation.

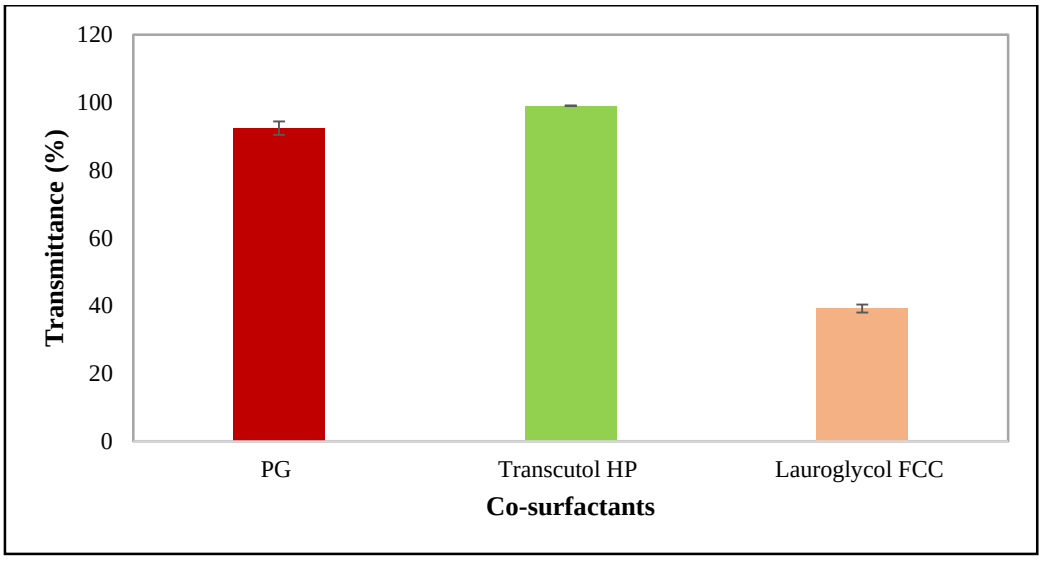
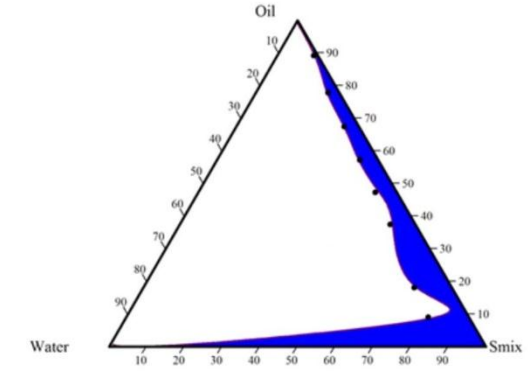


Figure 7.5:Emulsification study of lurasidone HCl in co-surfactants

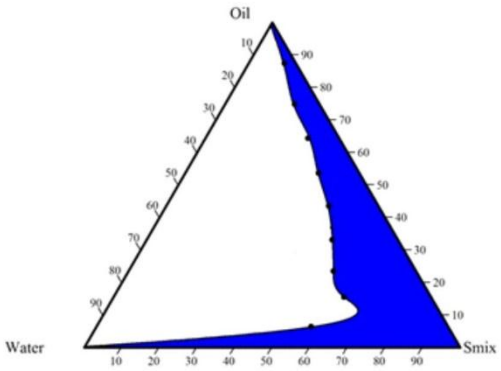
7.10.1.4 Construction of pseudo ternary phase diagrams

The relationship between the phase behavior and the composition of the SMEDDS mixtures was studied utilizing pseudo-ternary phase diagrams. Pseudo-ternary phase diagrams were constructed in the absence of LH to identify the self-emulsifying regions and to optimize the percentage of oil, surfactant, and co-surfactant for the SMEDDS formulations (12). The pseudo ternary phase diagrams in different weight ratios of surfactant to co-surfactant (S/Cos) were plotted between Capmul MCM C8, Cremophor RH 40 and Transcutol HP as oil, surfactant and co-surfactant phase respectively (Figure 7.6).

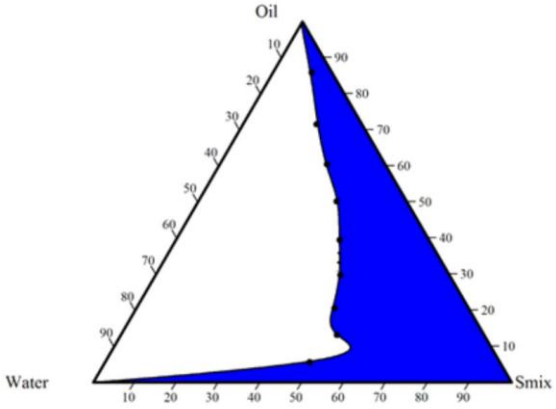
It was observed that at 1:0 ratio of S/Cos, smaller microemulsion region was observed as only surfactant was not be able to reduce o/w interfacial tension sufficiently. It was observed that as surfactant concentration increased from 1:1 to 3:1, microemulsion region was found to be increased. This might be due to increased adsorption of surfactant molecules at the oil–water interface leading to a decrease in the interfacial tension, which facilitates the formation of smaller droplets. Further increase in surfactant concentration from 3:1 to 4:1 didn't show any change in microemulsion region. This might be attributed to optimum emulsification has been achieved at 3:1 ratio. However, increase in co-surfactant concentration to 1:3 showed decrease in formation of microemulsion region which might be due to the fact that at high concentrations, cosurfactant not only stay into the interfacial film but also enter into the inner oil phase, leading to the expansion of interfacial film (1,14,15). Hence, 3:1 ratio was considered as optimum surfactant:co-surfactant ratio and used for further investigation.


Oil
Water
Smix

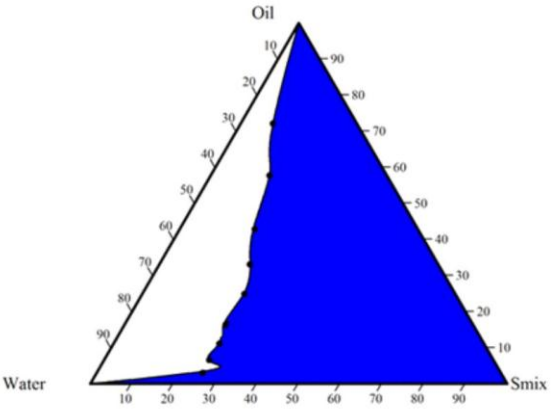
(a)


Oil
Water
Smix

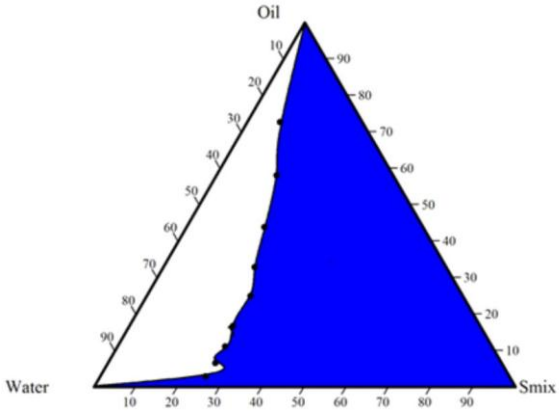
(b)


Oil
Water
Smix

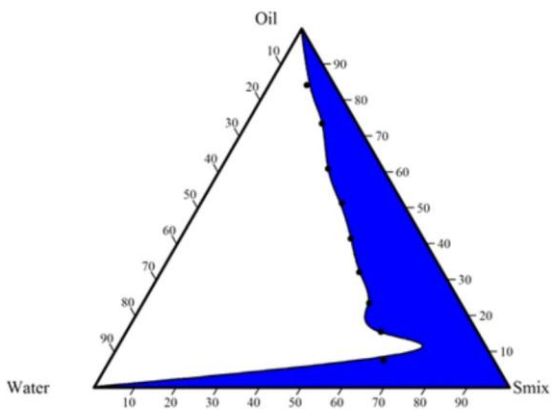
(c)


Oil
Water
Smix

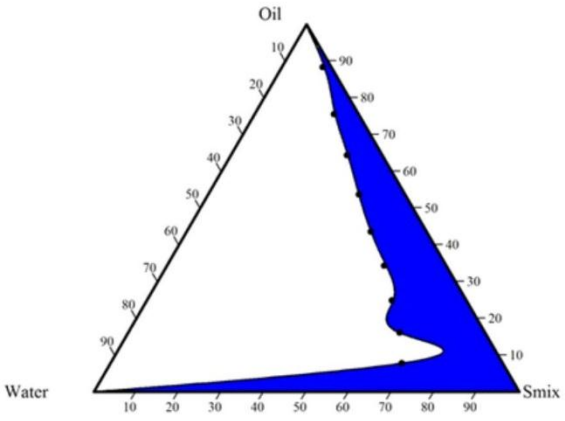
(d)


Oil
Water
Smix

(e)


Oil
Water
Smix

(f)



(g)

Figure 7.6:Ternary diagrams of Capmul MCM C8 with different ratio of Cremophor EL and Transcutol HP (a)1:0, (b)1:1, (c)2:1, (d)3:1, (e)4:2, (f)1:2 and (g)1:3 of LH-SMEDDS

7.10.2 OPTIMIZATION USING 3^2 FACTORIAL DESIGN

In order to ascertain the optimum formulation, it is necessary to evaluate the effect of formulation parameters and their interactions on the properties of the final product. Design of an immaculate SMEDDS requires rational blends of diversely behaving oils, surfactants and cosurfactants which cannot be achieved using a traditional One Variable at a Time (OVAT) approach. Systematic optimization of such isotropic delivery systems using Design of Experiment (DoE) on the other hand offers numerous advantages including high degree of precision and prognosis along with economic advantages. Optimization of LH-SMEDDS was carried out using 3^2 factorial design and the results of 3^2 factorial design are shown in table 7.2.

Table 7.2: 3^2 factorial design runs of LH-SMEDDS

Sr. No	X_1 (%)	X_2	Y_1 (nm)	Y_2 (%)	Y_3 (Sec)
1	20	2	111.7±3.4	84.6±1.2	81±3.5
2	30	3	103.7±5.8	88.4±0.5	55±5.0
3	20	3	97.9±6.2	91.8±1.1	61±3.4
4	30	2	120.7±8.4	85.2±1.4	93±6.0
5	10	3	49.2±4.5	99.7±0.4	35±4.5
6	30	1	133.9±3.6	88.8±0.7	112±5.0
7	10	2	66.8±5.7	98.5±0.8	55±3.7
8	20	1	121.1±2.9	90.3±1.1	94±3.2
9	10	1	80.56±4.1	99.8±1.6	45±5.9

7.10.2.1 Influence of independent variables on globule size

The globule size of the emulsion is crucial factor in SMEDDS formulation, as this determines the rate and extent of drug release as well as absorption. A second order polynomial regression equation was generated to determine influence of independent variables on globule size. The full model equation (Equation 7.2) was generated using software as shown below:

Full model equation:

$$Y_1 = 111.57 + 26.96X_1 - 14.13X_2 + 0.29X_1X_2 - 17.76X_1^2 - 2.01X_2^2 \ldots\ldots\ldots\ldots Equation\ 7.2$$

The regression coefficients having $p<0.05$ are highly significant. The terms having coefficients with $p>0.05$ are least contributing in the prediction of response (Table 7.3). The results showed that X_1, X_2 and X_1^2 were main contributing parameters to globule size ($p<0.05$) and X_1X_2 and X_2^2 were least contributing factors to globule size ($p>0.05$). Thus, neglecting non-significant ($p>0.05$) terms from the full model and applying regression between significant terms gives equation of reduced model (Equation 7.3).

Reduced model equation:

$$Y_1 = 110.23 + 26.96X_1 - 14.13X_2 - 17.76X_1^2 \ldots\ldots\ldots\ldots Equation\ 7.3$$

Table 7.3: Results of ANOVA for globule size of LH-SMEDDS

Source	Sum of Squares	df	Mean square	F Value	p-value prob>F
Model	6196.34	5	1239.27	193.74	0.0006
X_1	4359.97	1	4359.97	681.61	0.0001
X_2	1197.38	1	1197.38	187.19	0.0008
X_1X_2	0.34	1	0.34	0.053	0.8334
X_1^2	630.60	1	630.60	98.58	0.0022
X_2^2	8.05	1	8.05	1.26	0.3435
Residual	19.19	3	6.40		
Cor Total	6215.53	8			

It was observed from the ANOVA (Table 7.3) that both X_1 (p=0.0001) and X_2 (p=0.0008) had prominent effect on globule size. The magnitude of the co-efficients indicates its effect on response (Equation 7.3). A positive sign in front of the terms indicates synergistic effects while the negative sign indicates antagonistic effect of the factors. It was observed that X_1 (oil concentration) had positive effect on response i.e. as concentration of oil increased, globlue size increased. This might be attributed to higher oil concentration led to increased surface tension at oil water interface and surfactant concentration was unable to reduce interfacial tension sufficiently which led to aggregation of droplets and ultimately increased globule size. K_m (X_1) ratio had negative effect on the globule size i.e. as surfactant concentration increased, globule size decreased. This might be attributed to higher proportion of surfactant may provide closely packed interfacial surfactant film, thereby stabilizing the oil droplets.

The three-dimensional response surface plots and two-dimensional contour plots are graphical representations of the regression equation and express two independent variables at once against the response (Figure 7.7). Thus, the statistically significant relationship between the dependent and independent variables was further interpreted by using response surface analysis. These results were further confirmed by contour and response surface plot shown in figure 7.7. It also showed increase in globule size with increase in X_1 and decrease in X_2.

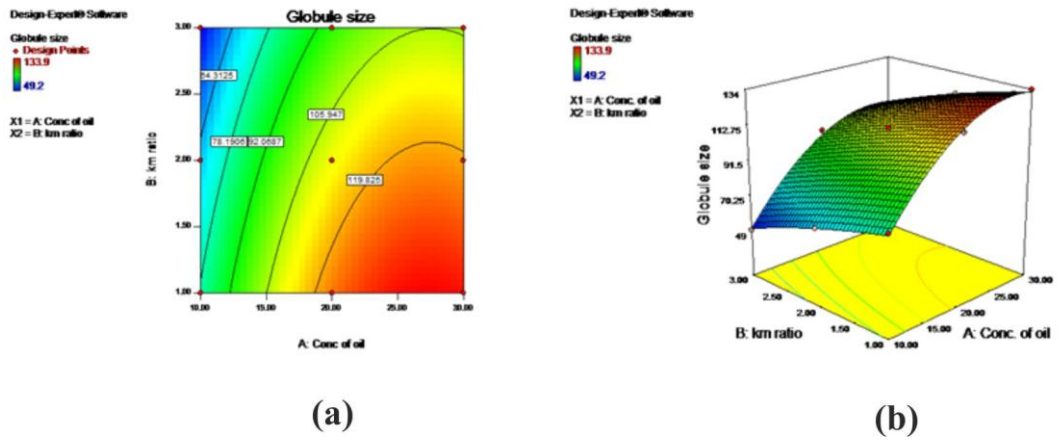


Figure 7.7: (a) Contour and (b) Response surface plot showing effect of independent variables on globule size of LH-SMEDDS

7.10.2.2 Influence of independent variables on percentage transmittance

A second order polynomial regression equation was generated to determine influence of independent variables on percentage transmittance. The full model equation generated using software is shown in equation 7.4:

Full model equation:

$$Y_2 = 86.43 - 5.93X_1 + 0.17X_2 - 0.075X_1X_2 + 4.50X_1^2 + 3.70X_2^2 \ldots\ldots\ldots\ldots Equation\ 7.4$$

The results showed (Table 7.4) that X_1 and X_1^2 were main contributing parameters to percentage transmittance ($p<0.05$) while X_2, X_1X_2 and X_2^2 were least contributing factors to percentage transmittance ($p>0.05$). So, neglecting non-significant terms led to generation of reduced model equation (Equation 7.5):

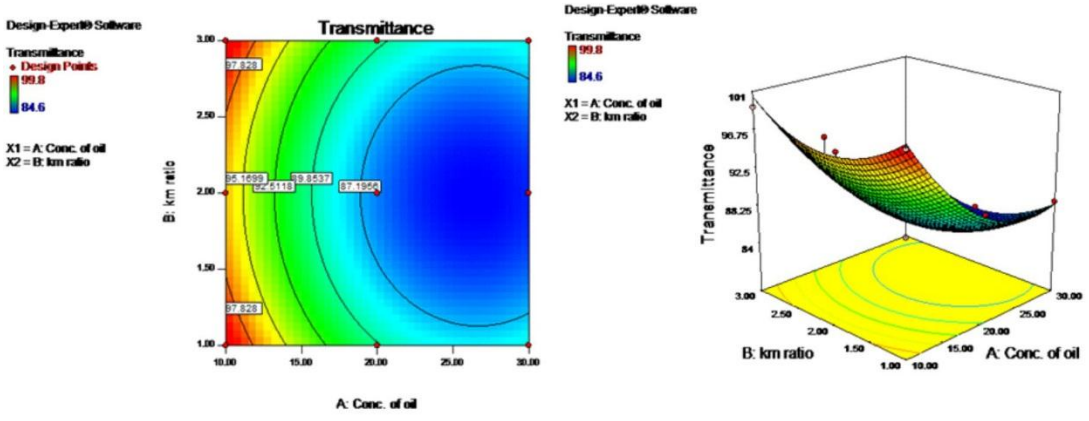
Reduced model equation:

$$Y_2 = 88.90 - 5.93X_1 + 4.50X_1^2 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots Equation\ 7.5$$

Table 7.4: Results of ANOVA for percentage transmittance of LH-SMEDDS

Source	Sum of Squares	df	Mean square	F Value	p-value prob>F
Model	272.28	5	54.46	14.80	0.0215
X_1	206.51	1	206.51	56.11	0.0042
X_2	0.060	1	0.060	0.016	0.8384
X_1X_2	2.500E-003	1	2.500E-003	6.792E-004	0.9401
X_1^2	39.31	1	39.31	10.68	0.0405
X_2^2	26.40	1	26.40	7.17	0.0652
Residual	11.04	3	3.68		
Cor Total	6215.53	8			

It was observed that X_1 had prominent and negative influence on percentage transmittance indicating increase in X_1 led to decrease in percentage transmittance. This effect is similar to the efffect on globule size but in opposite direction. This may be attributed to increased interfacial tension between oil and aqueous phase due to insufficient concentration of surfactant system (16). It was observed from response surface plot (Figure 7.8) that as the X_1 increased from 10 to 30 %, the percentage transmittance was decreased from 100 to 80%. As X_2 (K_m ratio) increased from 1 to 3, the percentage transmittance was increased to 100%. However, maximum transmittance was obtained at 10% oil concentration and K_m ratio 3 indicating that the system was optically clear which a prerequisite for microemulsions.



(a) **(b)**

Figure 7.8: (a) Contour and (b) Response surface plot showing effect of independent variables on %transmittance of LH-SMEDDS

7.10.2.3 Influence of independent variables on self-emulsification time

A second order polynomial regression equation was generated to determine influence of independent variables on self-emulsification time. The full model equation generated using software is shown below (Equation 7.6):

$$Y_3 = 84.89 + 20.83X_1 - 16.67X_2 - 11.75X_1X_2 - 12.83X_1^2 - 9.33X_2^2 \ldots\ldots\ldots\ldots Equation\ 7.6$$

The results showed that (Table 7.5) X_1 ,X_2 , X_1X_2 , X_1^2 , and X_2^2 all had significant effect on self-emulsification time ($p<0.05$).

Table 7.5: Results of ANOVA for self-emulsification time of LH-SMEDDS

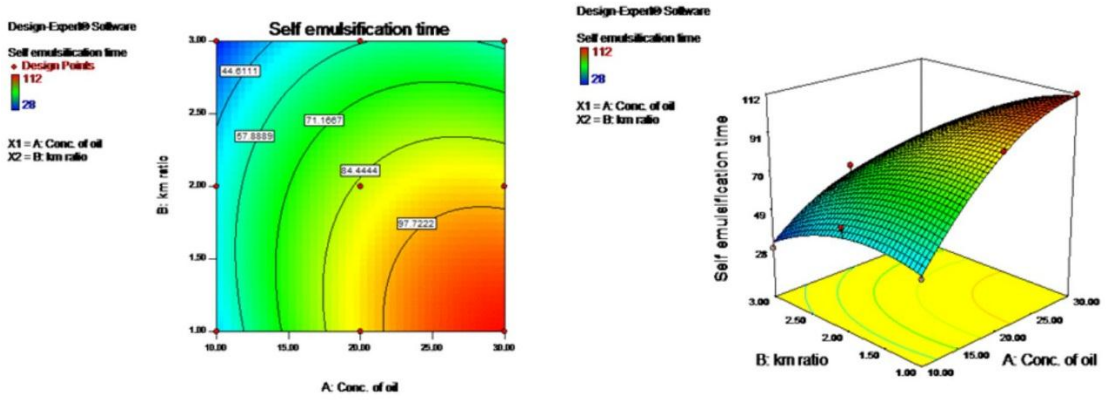
Source	Sum of Squares	df	Mean square	F Value	p-value prob>F
Model	5326.69	5	1065.34	72.32	0.0025
X_1	2604.17	1	2604.17	176.78	0.0009
X_2	1666.67	1	1666.67	113.14	0.0018
X_1X_2	552.25	1	552.25	37.49	0.0088
X_1^2	329.39	1	329.39	22.36	0.0179
X_2^2	174.22	1	174.22	11.83	0.0413
Residual	44.19	3	14.73		
Cor Total	5370.89	8			

X_1 had agonistic effect on self-emulsification time i.e. increase in oil concentration increased emulsification time required for SMEDDS. This behavior might be due to the fact that at higher concentrations of the oily phase and with a low amount of surfactant mixture, the proportion of the surfactant mixture that facilitates water penetration decreases and the mixture becomes more lipophilic causing increased difficulty of emulsification (17).

X_2 had antagonistic effect on self-emulsification time i.e. increase in K_m decreased self-emulsification time. This could be due to at low K_m, there was a lower amount of surfactant present which was unable to emulsify the amount of oil present whereas at high levels of K_m the surfactant amount is sufficient enough to emulsify oil (7). Another reason may be due to higher surfactant concentration leads to penetration of aqueous phase into the oil phase causing massive interfacial disruption and ejection of droplets into the bulk aqueous phase (16).

This discrepancy might be due to the differences in the hydrophilic lipophilic balance (HLB) of Capmul MCM C8 (X_1) and Cremophor EL (X_2) mixtures. Bachynsky et al (18) showed that the HLB of the surfactant mixtures has a significant effect on the performance of the self emulsifying system. However, optimum surfactant mixture should be obtained at an appropriate combination with the oily phase (17).

The effect of independent variables on self-emlusification time was further elucidated using contour and response surface plot. It was observed that (Figure 7.9) as oil concentration increased from 10 to 30%, there was significant increase in self-emulsification time whereas as K_m ratio increased from 1 to 3, there was decrease in self-emulsification time due to increase in spontaneity of microemulsion formation at higher surfactant concentration. It was confirmed that lowest emulsification time was obtained at 10% oil concentration and K_m ratio 3.



(a) **(b)**

Figure 7.9: (a) Contour and (b) Response surface plot showing effect of independent variables on self-emulsification time of LH-SMEDDS

7.10.2.4 Statistical analysis of designed experiment

The adequacy of the cubic model was verified by ANOVA and multiple correlation coefficient (R^2) tests provided by Design- Expert software. The results of ANOVA showed that p value for response globule size, % transmittance and self-emulsification time was found to be 0.0006, 0.0215 and 0.0025 respectively, for quadratic model. Thus, it can be concluded that all the responses fitted the quadratic model well ($p<0.05$) Furthermore, the multiple regression analysis for the quadratic model is shown as R^2 value, which signifies the measure of the amount of variation around the mean explained by the model. In our study, the R^2 values for the responses globule size,

%transmittance and self-emulsification time were 0.9969, 0.9610 and 0.9862 respectively (Table 7.6).

Adjusted R2 on the other hand, provides an estimate of amount of variation about the mean explained by the model, adjusted for specific number of parameters. Predicted R2 represents the amount of variation in new data explained or predicted by the mathematical model. The closeness of predicted R^2 with R^2 and adjusted R^2 indicated goodness of fit to the data.

The term "Adequate precision" represents signal to noise ratio, and a value of more than 4 is desirable. The high value of adequate precision depicts adequate model discrimination, in other words, adequacy of the signal. The results indicated that all the responses were affected by variations in the studied oil concentration and K_m ratio.

Table 7.6: Regression analysis for all responses of LH-SMEDDS

	Globule size	**%transmittance**	**Self-emulsification time**
R^2	0.9969	0.9650	0.9918
Adjusted R^2	0.9918	0.9067	0.9781
Predicted R^2	0.9624	0.6977	0.9298
Adeq precision	39.789	10.539	23.932

7.10.2.5 Validation of design

Criteria for selection of optimized batch were arbitrarily selected as minimum globule size, maximum transmittance and minimum emulsification time. The experimental value (Table 7.7) was found to be in close agreement with predicted value for all responses with lower percent prediction error which suggested suitability of design applied**.**

Table 7.7: Predicted and observed responses for check point batch of LH-SMEDDS

Response	**Predicted value**	**Experimental value**	**Percentage prediction error**
Y_1 (nm)	50.43	49.2±1.60	2.44
Y_2(%)	100.49	99.7±0.30	1.10
Y_3 (Sec)	36.97	35.0±2.0	5.33

The concept behind a desirability function using multi-response optimization process is to transform multi-response into single response using mathematical calculation (9). The desirability for the selected quadratic model was found to be 0.987 (Figure 7.10) indicating accurate and reliable approaches in the optimization process.

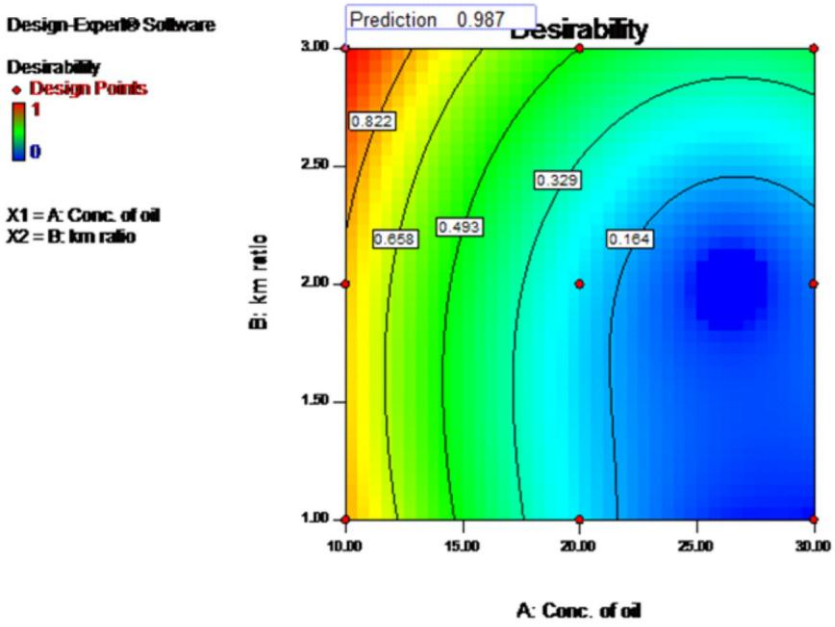


Figure 7.10: Desirability plot of optimized batch of LH-SMEDDS

7.10.2.6 Analysis of Design Space robustness

The design space was generated using the aforementioned criteria by a graphical-optimization technique. Figure 7.11 portrays the optimal design space region within the requisite knowledge space and location of the optimum formulation within the desired limits. It was observed that value of independent variables outside the design space showed variation in response (Table 7.8). So, it proved that the design space was sensitive to variation in independent variables. The yellow area showed desired response proving the robustness of design space.

Table 7.8: Evaluation of sensitivity of obtained design space of LH-SMEDDS

X_1	X_2	Y_1 (nm)		Y_2 (%)		Y_3 (sec)	
		Predicted	**Observed**	**Predicted**	**Observed**	**Predicted**	**Observed**
10.00	3	50.43	49.2±2.9	100.81	99.7±1.30	36.97	35.0±2.0
16.55	1.63	105.12	108.7±6.8	89.45	87.4±1.50	79.54	82.0±3.0

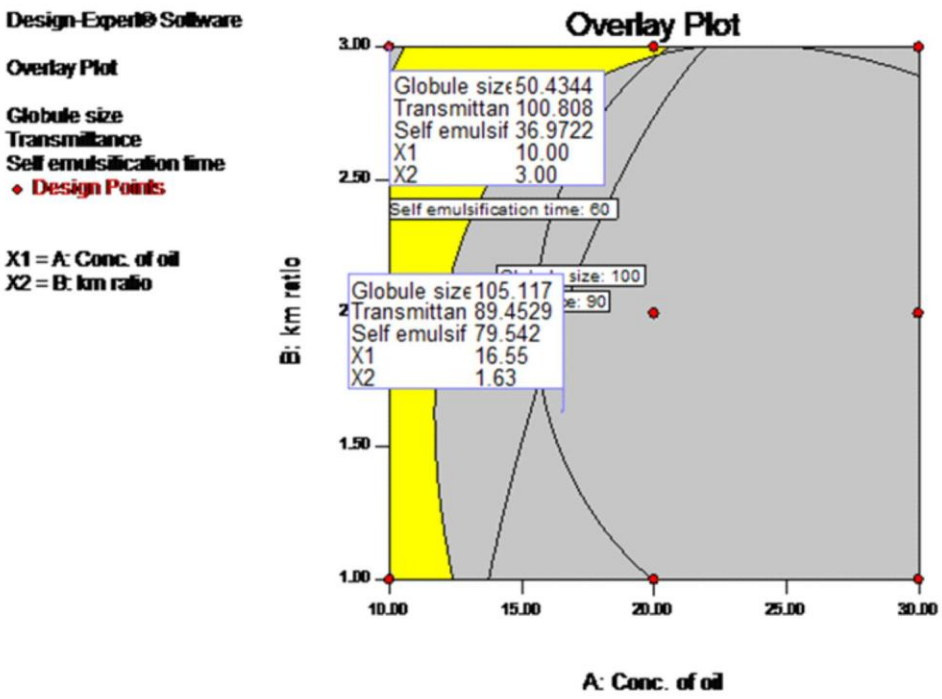


Figure 7.11: Overlay plot depicting the design space for optimized batch of LH-SMEDDS

7.11 CHARACTERIZATION

7.11.1 Globule size determination

The globule size of the optimized batch was found to be 49.22±1.60 nm (Figure 7.12). The smaller is the droplet size, the larger is the surface area provided for drug absorption (19). The size of the globule is also found to be in the nanometer indicating the possibility of enhanced permeation through the biological membrane. The PDI of the optimized batch of LH loaded SNEDDS was found to be 0.177±0.012 which illustrated narrow size distribution of globules.

Figure 7.12: Globule size of optimized batch of LH-SMEDDS

7.11.2 Zeta potential determination

The zeta potential of optimized formulation was found to be -10.3±2.3 mV (Figure 7.13). This might be due to the presence of free fatty acids in the oil phase of SMEDDS since both surfactant and cosurfactant used were non-ionic in nature. This negative zeta potential value indicates greater electrostatic repulsive forces between the globules which prevents the coalescence of globules and therefore, gives indication of physical stability of SMEDDS.

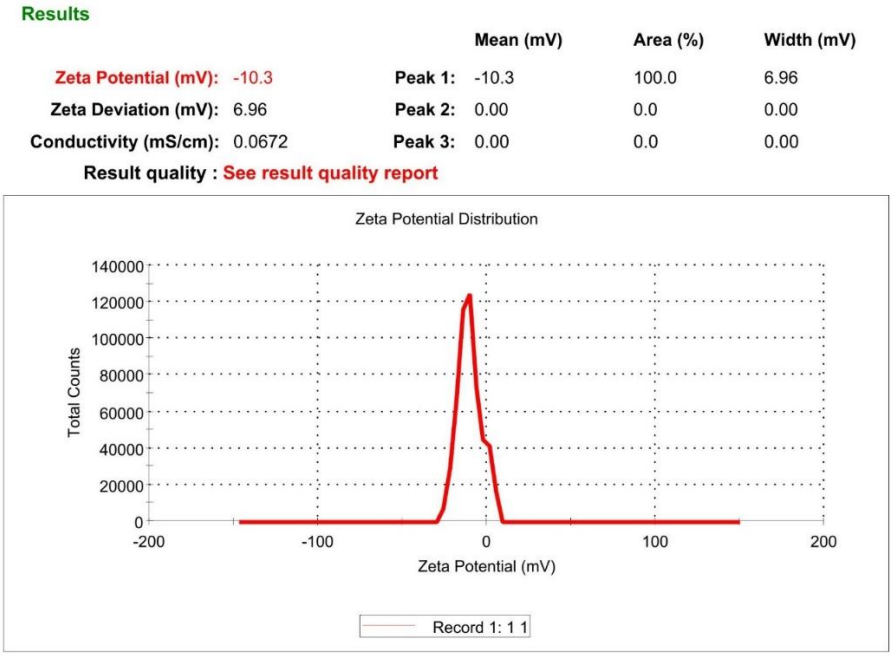


Figure 7.13: Zeta potential of optimized batch of LH-SMEDDS

7.11.3 Robustness to dilution

The optimized SMEDDS formulation was exposed to different pH media such as distilled water, simulated gastric fluid (pH 1.2) and simulated intestinal fluid (pH 6.8) to mimic the in vivo conditions. The globule size at all dilutions was found to be less than 55 nm (Table 7.9). The study revealed no precipitation or phase separation indicating all the formulations were found to be robust towards dilution in different pH conditions. Moreover, the formulations were robust over wide degree of dilutions without any signs of drug precipitation and phase separation.

Table 7.9: Robustness to dilution with different media of LH-SMEDDS

Media	50 times (nm)	100 times (nm)	250 times (nm)	500 times (nm)	1000 (nm)
Distilled water	49.2±1.3	47.1±1.8	48.4±2.3	45.6±1.2	44.4±2.8
pH 1.2	50.5±2.8	48.5±1.4	49.1±1.9	47.3±3.7	47.6±2.5
pH 6.8	50.2±1.6	49.3±2.2	46.5±2.0	47.5±2.5	46.8±2.2

7.11.4 Determination of self-emulsification time

The rate of emulsification is an important parameter for the assessment of spontaneity of emulsification for the systems. The SMEDDS should disperse completely and quickly when subjected to aqueous dilution under mild agitation of GI tract. The emulsification time of the optimized batch of LH-SMEDDS was found to be 35.0±2.0 sec, which portrayed spontaneity of system.

7.11.5 Cloud Point determination

Cloud point is a critical parameter especially for non-ionic surfactants containing SMEDDS in terms of their stability. When the temperature of the system is higher than its cloud point, an irreversible phase separation occurs. Hence, the cloud point should be > 37 °C, in order to avoid phase separation of formulations in GI tract. The cloud point of the optimized batch of IRB-loaded SNEDDS was found to be 74.5 °C which confirmed stability of system at physiological temperature in vivo.

The reason for higher cloud point temperature may be attributed to solubility of drug in oil and surfactant system, optimized ratio of S/ CoS and/or surfactants with higher HLB values. This infers good thermal stability of optimized SMEDDS. Above 74.5 °C phase separation and precipitation was observed, this is due to dehydration of POE (poly oxy ethylene) moiety of Cremophor EL and alkyl chains of Transcutol HP (16,20,21).

7.11.6 Drug content determination

The drug content in the optimized formulation was around 99.5±2.00% indicating high entrapment in the oil phase.

7.11.7 Viscosity determination

The viscosity of optimized batch was found to be 44.23±2.30 cP.

7.11.8 % Transmittance

% Transmittance of the optimized batch was found to be 99.7±1.30 % indicating the system was optically clear which is a prerequisite for microemulsions.

7.11.9 Thermodynamic stability

The SMEDDS formulation undergoes spontaneous in situ solubilization in the GI lumen to form a microemulsion system. As such, formulation should possess considerable stability in order to prevent precipitation, creaming or cracking. Hence, thermodynamic stability study was designed to identify and avoid the metastable systems. This study revealed that optimized formulation could withstand wide range of temperature changes (heating cooling cycle) and centrifugal stress (centrifugation study) without any phase separation and drug precipitation (22).

7.11.10 Fourier Transform Infrared (FTIR) spectroscopy

The IR spectra of LH, Capmul MCM C8, physical mixture of drug and excipients and drug loaded SMEDDS are shown in figure 7.14. The FTIR spectra of LH showed characteristic peaks at 2936 cm^{-1}, 2258.0 cm^{-1} and 1687.5 cm^{-1} indicative of C-H stretching, N-H stretching and C=O stretching respectively. The physical mixture of excipients showed characteristic peaks of all functional groups of drug and excipients. No additional peak was observed indicating compatibility between drug and excipients. FTIR spectra of LH-SMEDDS showed absence of characteristic peaks of LH indicating that drug was completely solubilized in oil phase of SMEDDS.

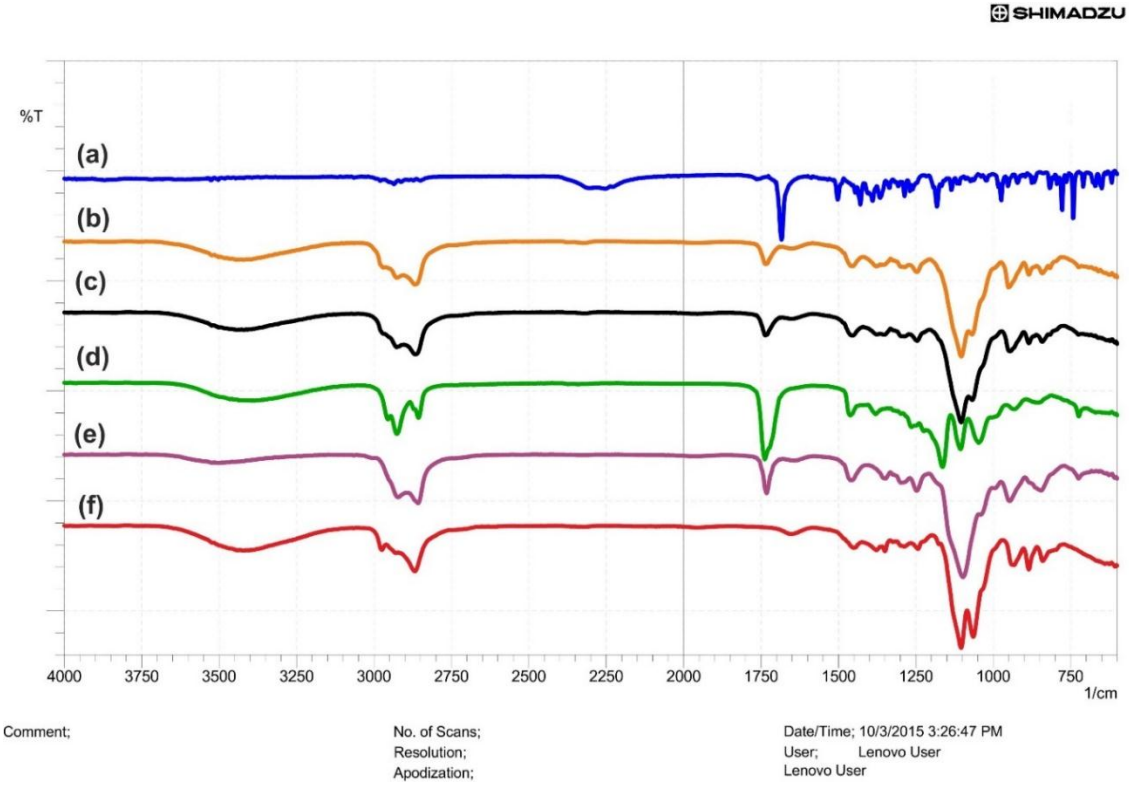


Figure 7.14: FTIR spectrum of (a) LH (b) Capmul MCM C8 (c) Cremophor EL (d) Transcutol HP (e) Physical mixture of LH and excipients (f) LH-SMEDDS

7.11.11 Transmission Electron Microscopy (TEM)

TEM image of the optimized SMEDDS (Figure 7.15) after dilution appeared as dark, spherical globules. The droplets were also found to be of uniform size distribution. The size was found to be in the range of 30-50 nm which is in accordance with DLS results.

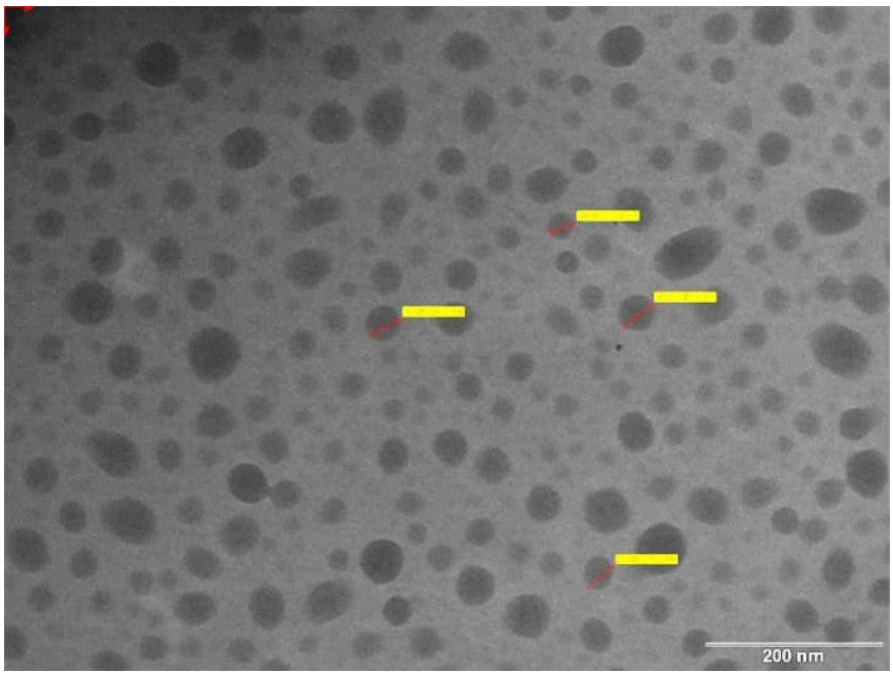


Figure 7.15:TEM of optimized batch of LH-SMEDDS

7.12 IN VITRO DRUG RELEASE STUDY

The in vitro drug release profile of LH-SMEDDS and LH suspension is shown figure 7.16. In case of LH suspension, a fast release (91.67±3.78%) of LH was observed in 2 h during acidic condition. Subsequent release of the drug in pH 6.8 was relatively slower and total 94.6±3.11% of drug was released at the end of 8 hr.

In contrast, LH-SMEDDS showed only 18.44±3.12% of LH was released in acidic medium whereas more than 98.2±4.19% of LH was released at the end of 8 h in PBS. Such slow release of LH from SMEDDS formulation indicates good affinity of LH for the oil phase (Capmul MCM C8). More amount of drug would be reaching to intestine inside the small microemulsion globules which in turn will help in enhancing oral bioavailability via lymphatic uptake.

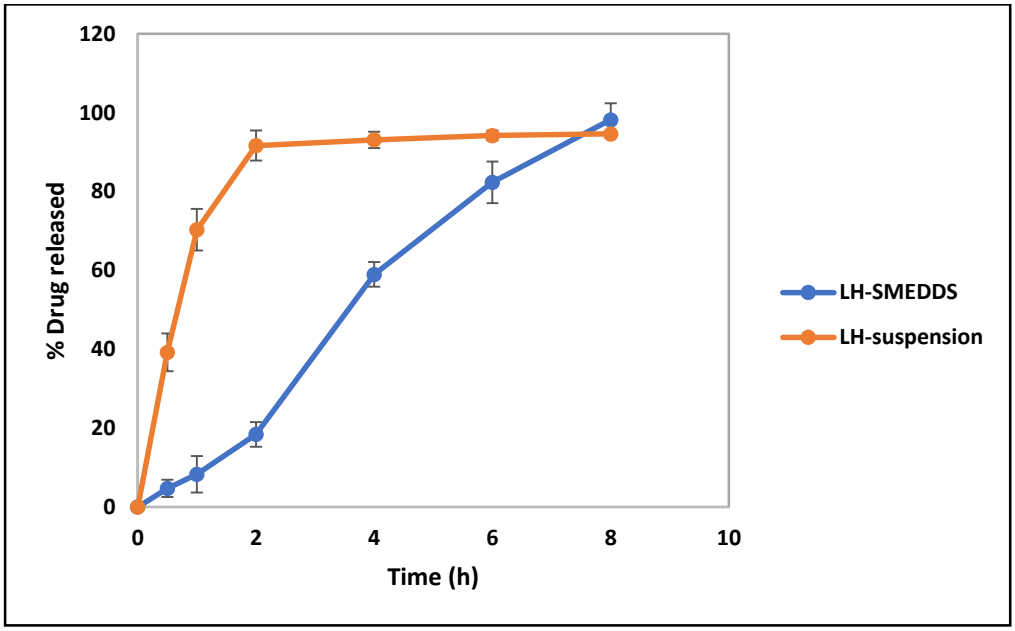


Figure 7.16: In-vitro drug release profile of LH-SMEDDS and LH suspension

7.13 EX VIVO PERMEATION STUDY

The ex vivo drug permeation study of LH suspension and LH-SMEDDS was shown in figure 7.17. The release profile of drug suspension indicated most of the drug was permeated through stomach (~83%) and drug available for lymphatic uptake was very low. Subsequent release of the drug from intestine was relatively slower and total 85.74±4.34% of drug was released at the end of 8 hr.

In case of LH-SMEDDS, it showed only 15% of drug diffusion through stomach and ~85% drug was diffused through intestinal membrane (Figure 7.15). Hence, it can be

said that significant amount of drug will be carried to the intestine. Moreover, it can be assumed that the characteristics of microemulsion, such as nano-sized droplet size which eventually provide larger surface area and the interaction between the surfactants (Cremophor EL, Transcutol HP) and intestinal membrane, may result in enhanced permeation of LH. Hence, it can be noted that permeation of the drug from the intestine was enhanced with SMEDDS, which fulfilled our objective of increasing intestinal permeability for enhancing the bioavailability of LH (23).

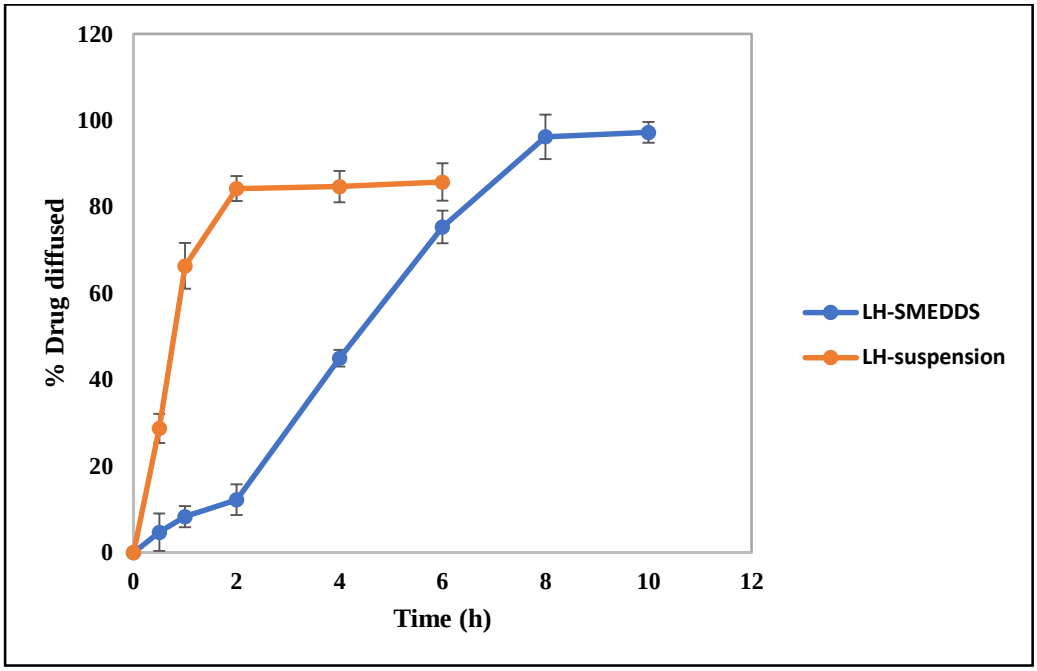


Figure 7.17 : Ex vivo permeation study of LH-SMEDDS and LH suspension

7.14 STABILITY STUDY

The stability of LH loaded SMEDDS was monitored for 3 months at RT in terms of globule size, zeta potential, drug content and self-emulsification time. No significant change in the globule size, zeta potential, drug content and self-emulsification time was observed during 3 months of storage at room temperature (Table 7.10).

Table 7.10: Characteristics of LH-SMEDDS after 3-months stability study

Time (months)	**Room temperature (30 °C/60±5%RH)**			
	Globule size (nm)	**Zeta potential (mV)**	**Drug content (%)**	**Self-emulsification time (sec)**
Initial	49.2±1.6	-10.3±2.3	99.5±2.0	35.0± 2.0
1 month	50.3±2.3	-10.1±2.8	99.3±3.2	35.0± 2.5
2 months	51.3±3.1	-9.7±3.1	98.5 ±4.1	36.0± 3.5
3 months	53.4±4.6	-8.9±1.9	97.7±3.2	37.0± 2.0

7.15 REFERENCES

1. Shen H, Zhong M. Preparation and evaluation of self-microemulsifying drug delivery systems (SMEDDS) containing atorvastatin. J Pharm Pharmacol. 2006:58;1183-1191.
2. Westesen K. Novel lipid-based colloidal dispersions as potential drug administration systems – expectations and reality. Colloid Polym Sci. 2000;278:608–618
3. Sahu BP, Das MK. Optimization of felodipine nanosuspensions using Full Factorial Design. Int J of PharmTech Res. 2013;5(2):553-561
4. Sawant KK, Patel MH, Patel K. Cefdinir nanosuspension for improved oral bioavailability by media milling technique: formulation, characterization and in vitro-in vivo evaluations. Drug Dev Ind Pharm. 2016;42:758-768.
5. Qian S, Heng W, Wei Y, Zhang J, Gao Y. Coamorphous Lurasidone Hydrochloride-Saccharin with Charge assisted Hydrogen Bonding Interaction Shows Improved Physical Stability and Enhanced Dissolution with pH-independent Solubility Behavior. Cryst Growth Des. 2015;15(6):2920-2928.
6. Cho HJ, Lee DW, Marasini N, Poudel BK, Kim JH, Ramasamy T, Yoo BK, Choi H, Yong CS, Kim JO. Optimization of self-microemulsifying drug delivery system for telmisartan using Box–Behnken design and desirability function. J Pharm Pharmacol. 2013;65:1440-1450.
7. Patel J, Dhingani A, Garala K, Raval M, Sheth N. Quality by design approach for oral bioavailability enhancement of Irbesartan by self-nanoemulsifying tablets. Drug Deliv. 2014;21(6):412-35.
8. Derringer G, Suich R. Simultaneous optimization of several response variables. J Qual Technol. 1980;12:214–219.
9. Kumar A, Sawant KK. Application of multiple regression analysis in optimization of anastrozole-loaded PLGA nanoparticles. J Microencapsul. 2014;31(2):105-114.
10. Patel M, Sawant K. A Quality by Design Concept on Lipid Based Nanoformulation Containing Antipsychotic Drug: Screening Design and Optimization using Response Surface Methodology. J Nanomed Nanotechnol. 2017;8(3): 1-11.

11. Shweta Gupta S, Chavhan S, Sawant KK. Self-nanoemulsifying drug delivery system for adefovir dipivoxil: Design, characterization, in vitro and ex vivo evaluation. Colloids Surf A. 2011;392:145– 155.
12. Khan AW, Kotta S, Ansari SH, Sharma RK, Ali J. Self-nanoemulsifying drug delivery system (SNEDDS) of the poorly water-soluble grapefruit flavonoid Naringenin: design, characterization, in vitro and in vivo evaluation. Drug Deliv. 2015;22(4):552–561.
13. Nazzal S, Smalyukh II, Lavrentovich OD, Khan MA. Preparation and in vitro characterization of a eutectic based semisolid self-nanoemulsified drug delivery system (SNEDDS) of ubiquinone: mechanism and progress of emulsion formation, Int J Pharm. 2002;235:247–265.
14. Poudel BK, Marasini N, Tran TH, Choi H, Yong CS, Kim JO. Formulation, Characterization and Optimization of Valsartan Self- Microemulsifying Drug Delivery System Using Statistical Design of Experiment. Chem Pharm Bull. 2012;60(11):1409–1418.
15. Wei Y, Ye X, Shang X, Peng X, Bao Q, Liu M, Guo M, Li F. Enhanced oral bioavailability of silybin by a supersaturatable self-emulsifying drug delivery system (S-SEDDS). Colloids Surfaces A. 2012;396:22– 28.
16. Kallakunta VR, Bandari S, Jukanti R, Veerareddy PR. Oral selfemulsifying powder of lercanidipine hydrochloride: formulation and evaluation. Powder Technol. 2012;221:375–82.
17. Zidan AS, Sammour OA, Hammad MA, Megrab NA, Habib MJ, Khan MA. Quality by design: Understanding the formulation variables of a cyclosporine A self-nanoemulsified drug delivery systems by Box–Behnken design and desirability function. Int J Pharm. 2007;332:55–63.
18. Bachynsky MO, Shah NH, Patel CI, Malick, AW. 1997. Factors affecting the efficiency of a self-emulsifying oral delivery system. Drug Dev Ind Pharm. 1997;23:809–816.
19. Dixit AR, Rajput S, Patel SG. Preparation and Bioavailability Assessment of SMEDDS Containing Valsartan. AAPS PharmSciTech. 2010;11(1):314-321.
20. Ping Z, Ying L, Nianping F, Jie X. Preparation and evaluation of self-microemulsifying drug delivery system of oridonin. Int J Pharm. 2008;355:269–276.

21. Elnaggar YSR, El-Massik MA, Abdallah OY. Self-nanoemulsifying drug delivery systems of tamoxifen citrate: design and optimization. Int J Pharm. 2009;380:133–141.
22. Singh AK, Chaurasiya A, Singh M, Upadhyay SC, Mukherjee R, Khar RK. Exemestane Loaded Self-Microemulsifying Drug Delivery System (SMEDDS): Development and Optimization. AAPS PharmSciTech. 2008;9(2):906-916.