Formulation Development (SMEDDS)

Part A: Experimental

Chapter 4

4.1 Materials and Equipments

4.1.1 Materials

Dabigatran Etexilate (DE) and Nisoldipine (NISO) were received as gift samples from Alembic Research Centre, Vadodara, India. Peceol, Labrasol, Lauroglycol 90, Capryol 90, Transcutol HP, Labrafac PG and Labrafac Lipophile WL1349 were obtained as gift samples from Gattefosse India Private Limited, Mumbai, India. Capmul MCM C8, Capmul MCM, Captex 500 and Capmul PG8 were obtained from Abitec, USA. Cremophor EL and Cremophor RH40 were obtained as gift samples from BASF India Ltd., Mumbai, India. PEG 200 was obtained as gift sample from Croda Chemicals Pvt. Ltd., Mumbai, India. All other chemicals and reagents used were of analytical grade.

4.1.2 Equipments

Following is the list of equipments and instruments used for the preparation of SMEDDS of DE and NISO.

Table 4.1.1: List of Equipments and Instruments

Name of equipment/ Instrument	Model	Make
Magnetic stirrer- with and without hot plate	1MLH	Remi Motors, India
Electronic weighing balance	ELB300	Shimadzu, Japan
Zeta sizer	Nano ZS	Malvern Instruments, UK
UV-visible double beam spectrophotometer	UV-1800	Shimadzu, Japan
Centrifuge	CPR-30	Remi, India
USP dissolution apparatus	DS8000	LabIndia, India
Melting point apparatus	VPM-PM	Veego, India
Microscope	DS-Fi2	Nikon Digital, Japan
Differential scanning calorimeter	DSC-60	Shimadzu, Japan
Fourier Transform Infrared spectrophotometer	IR Affinity-1	Shimadzu, Japan
Stability chamber	Tanco-PLT 258	S.R Lab Instruments, India

Special precautions were taken to protect NISO from light at all the stages of formulation development (use of dark area) and its analysis (use of amber coloured glasswares).

4.2 Preformulation

4.2.1 Authentication of drugs: Dabigatran etexilate (DE) and Nisoldipine (NISO)

4.2.1.1 UV Visible spectroscopy

Solutions containing 10 µg/mL of DE and NISO were prepared in 0.01N HCl and 0.1N HCl + 0.5% SLS respectively and scanned over the wavelength range of 200-400 nm against their respective blanks [0.01N HCl and 0.1N HCl + 0.5% SLS] using double beam UV-Visible spectrophotometer (UV-1800, Shimadzu Corporation, Japan). The UV absorption spectra for both the drugs were recorded.

4.2.1.2 Fourier Transform Infrared spectroscopy (FTIR)

Drugs (DE and NISO) were triturated individually in mortar and pestle to remove lumps, if any. A small amount of fine powder of each drug was kept in sample holder and the spectra were recorded by scanning in the wavelength region of 4000-600 cm^{-1} using FTIR spectrophotometer (IR Affinity-1, Shimadzu, Japan). The IR spectrum of individual drugs were compared with that of their respective reported spectra.

4.2.1.3 Differential scanning calorimetry (DSC)

Differential scanning calorimeter (Shimadzu, Japan) equipped with an intra-cooler and a refrigerated cooling system was used to analyze the thermal behaviour of DE and NISO in the range of 40 to 300ºC at a heating rate of 10 °C/min with Nitrogen supplied at 50 mL/min and 100 mL/min through cooling unit. Indium standard was used to calibrate the DSC temperature.

4.2.1.4 Melting point determination

Melting point of both the drugs was determined using capillary method by melting point apparatus (Veego-VPM-PM).

4.2.2 Drug-Excipients compatibility study

A drug-excipient compatibility study was carried out with potential formulation excipients to determine drug- excipients interaction. The drug-excipients compatibility study was carried out by visual observation and by using FTIR spectroscopy. The excipients viz. Capmul MCM C8, Cremophor EL and Transcutol HP were mixed with DE individually, while Peceol, Cremophor EL and Transcutol HP were mixed with NISO individually. Placebo mix and individual excipients with drug in 1:1 ratio were

kept under compatibility study for 4 weeks at 40ºC±2ºC / 75%±5% RH in sealed glass vials for DE and amber coloured sealed glass vials for NISO and were visually observed for 1, 2 and 4 weeks. Final (4 weeks) samples were analyzed by FTIR spectrophotometry.

4.3. Fabrication of SMEDDS

4.3.1. Selection of oil phase by solubility study

Solubility study of DE and NISO was carried out in wide variety of oils. An excess amount of individual drug (DE and NISO) was added to 1 mL of oil, vortexed (Spinix MC-01 Vortex Shaker, India) for 5 min and the resulting mixture was kept in sealed vials in shaker incubator (Scigenics, Orbitek, India) at 25°C temperature and 50 rpm speed. After 48h, the vials were centrifuged (Remi Centrifuge, India) at 6000 rpm for 10 min [1]. The supernatant was removed, filtered through 0.45 µm membrane filter and DE [2] and NISO [3] concentrations were analyzed spectrophotometrically at 315 nm and 236 nm respectively after appropriate dilution with methanol.

4.3.2. Selection of surfactant and cosurfactant by emulsification and solubility test

Surfactant and cosurfactant were selected on the basis of their ability to emulsify the oil phase. Selection was based on % transmittance and ease of emulsification. Ease of emulsification was judged by the number of flask inversions required to yield homogenous emulsion [4]. For % transmittance, 100 µL of surfactant was added to 100 µL of oil phase followed by heating the mixture at 45°C. 100 µL of mixture was then diluted with 50 mL distilled water in a glass stopper conical flask. The % transmittance was evaluated at 650 nm by using UV-visible spectrophotometer (UV-1800, Shimadzu, Japan) keeping distilled water as a blank. The microemulsion was further observed visually for any turbidity or phase separation. The surfactant and cosurfactant with the best emulsification capacity for the oil phase and highest % transmittance were used for development of DE and NISO SMEDDS [5]. Solubility test was performed similar to as explained in section 4.3.1.

4.3.3. Establishment of pseudo ternary phase diagram

Pseudo ternary phase diagrams were developed using the aqueous titration method [6]. Ternary diagrams of S_{mix}, oil and water were plotted, each of them representing an apex of the triangle. Ternary mixtures with varying ratios of surfactant, co-surfactant and oil were prepared for both the drugs with their respective oil surfactant and cosurfactant. For

any mixture, the sum total of surfactant, co-surfactant and oil concentrations was always equal to 100%. From solubility studies, Capmul MCM C8 and Peceol were selected as oil phase for DE and NISO respectively; while from emulsification test, Cremophor EL was selected as surfactant and Transcutol HP as cosurfactant for both the drugs. The surfactant was mixed with co-surfactant in ratios of 3:1, 2:1, and 1:1 respectively. Aliquots of s_{mix} were then mixed with oil in ratios of 0.5:4.5, 1:4, 1.5:3.5, 2:3, 2.5:2.5, 3:2, 3.5:1.5, 4:1, 4.5:0.5 in different vials and then titrated with distilled water at room temperature. The samples were then equilibrated for 30sec and visually observed after each addition. Only uniform, transparent, optically clear mixtures were considered as microemulsions. Pseudo ternary phase diagrams were then constructed using CHEMIX software [7].

4.3.4. Preparation of DE and NISO SMEDDS

Based on screening studies, drug loaded batches of DE SMEDDS and NISO SMEDDS were prepared.

For DE SMEDDS, 20mg of DE was dissolved in Capmul MCM C8 under continuous stirring followed by sonication for 3 min. S_{mix} of Cremophor EL and Transcutol HP was added to the drug mixture and stirred for 10 min using a magnetic stirrer (Remi, India). Clear, transparent SMEDDS formulation thus prepared was further characterized.

For NISO SMEDDS, 10 mg of NISO was dissolved in Peceol by vortex mixing followed by sonication for 1 min. Remaining procedure was same as that for DE SMEDDS.

4.3.5. Optimization of DE SMEDDS using D-optimal design and NISO SMEDDS using 3 level factorial design

Various formulation and process variables should be optimized simultaneously during developing pharmaceutical formulations. In conventional method of optimization, combined effects of the independent variables are not considered. The difficulties in optimizing a pharmaceutical formulation are due to the difficulty in understanding the real relationship between dependent and independent responses [8]. The D-optimal mixture design, a subtype of mixture design, is most popular response surface methodologies for optimizing the formulation variables which was used for optimizing DE SMEDDS. A three level factorial design was applied to optimize the formulation variables of NISO SMEDDS with basic need of understanding the interactions between independent variables. The Design Expert® software (Version 8.0.3, Suite, Minneapolis, USA) was used to generate both the designs.

Optimization of DE SMEDDS was carried out using D- Optimal Design to study the influence of effect of independent variables [concentration of oil (X1), concentration of surfactant (X2) and concentration of cosurfactant (X3)] on critical dependent variables [Globule Size (Y1) and % Transmittance (Y2)]. The different levels of the formulation variables were selected based on preliminary experiments to get desirable constraints for the two responses Globule size (GS) and % Transmittance (T) to judge the formation of a self-micro emulsifying system. The design layout is as shown in Table 4.3.1.

Table 4.3.1: Layout of D-optimal design for DE SMEDDS

	Levels	
Variables (Factors)	−1	1
X1: concentration of oil (%)	10	20
X2: concentration of surfactant (%)	40	60
X3: concentration of co-surfactant (%)	20	40
Responses	**Constraints**	
Y1= Globule size (nm)	Minimize	
Y2 =Transmittance (%)	Maximize	

The D-optimal design comprised of 16 runs and 3-factors at 2-levels with five centre point trials for reproducibility. The design allowed the fitting of cubic model on two responses for process optimization in preparation of DE SMEDDS with minimum GS and maximum %T. Contour plots and response surface plots were also generated.

Check Point Analysis/Desirability function for D-optimal design

After the fitting of mathematical model, the desirability function was used for optimization. The desirability function consolidates all the responses into one variable and leaves the possibility to anticipate the ideal levels for the independent variable. It is used to convert the multi responses problems into single response problems [9]. The established contour plots and response surface plots were confirmed by performing check point analysis. Difference in the predicted and actual values of experimentally obtained responses (Y1 and Y2) were checked using student's 't' test along with

desirability function. The criteria of desired GS and %T was set to predict optimum conditions [10].

A three level factorial design was performed for optimization of NISO SMEDDS formulation. 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 was employed to evaluate the effect of two independent factors [11] viz. concentration of oil (X_1) and mass ratio of surfactant to co-surfactant (Km) (X_2) on dependent factors as Globule size (Y1) and % transmittance (Y2). The design layout is as shown in Table 4.3.2.

Table 4.3.2: Layout of three level factorial design for NISO- SMEDDS

	Levels		
Variables (Factors)	−1	0	1
X1: concentration of oil (%)	10	20	30
X2: surfactant to cosurfactant (Km) ratio	1	2	3
Responses	**Constraints**		
Y1= Globule size (nm)	Minimize		
Y2 =Transmittance (%)	Maximize		

The three level factorial design comprised of 13 runs, 2-factors at 3-levels with 5 centre points. Contour plots and response surface plots were also generated.

Check Point Analysis/Desirability function for factorial design

A check point analysis was performed to confirm the usefulness of the established contour and response surface plots. The desirability function looks for the most favourable point in the design space that fulfils the set goal for dependent variables such as minimum globule size and maximum transmittance. For synchronized optimization of responses Y_1 (GS) and Y_2 (% T), the desirability function (multi-response optimization technique) was applied. The desirability between 0 and 1 represents the closeness of a response to its ideal value [10].

Statistical analysis

The experimental data were validated by ANOVA, regression coefficient, lack of fit test and $p < 0.05$ was considered as significant.

4.4. Characterization of DE SMEDDS and NISO SMEDDS

4.4.1. Robustness to dilution test

Robustness to dilution was studied by diluting the optimized SMEDDS formulations to 50, 100, 250 and 1000 times with distilled water, 0.01 N HCl and Phosphate buffer pH 6.8 for DE SMEDDS and with distilled water, 0.1 N HCl with 0.5% sodium lauryl sulphate (SLS) and Phosphate buffer pH 6.8 with 0.5% SLS for NISO SMEDDS. The diluted SMEDDS formulations for both the drugs were stored for 12h at room temperature and observed for any signs of phase separation or drug precipitation. Globule size and % Transmittance of all the samples were measured for both the formulations. [12].

4.4.2. Thermodynamic stability studies

Thermodynamic stability studies were performed to assess the stability of microemulsion formed after diluting the DE and NISO SMEDDS formulations at following accelerated stress conditions [13].

Heating cooling cycle

Six cycles between refrigerated temperatures 2-8˚C and 45˚C with storage at each temperature for 48h were studied. Formulation which passed this cycle was then subjected to centrifugation test.

Centrifugation test

The formulation was centrifuged at 3500 rpm for 30 min using cooling centrifuge (Remi equipments, India) and observed for phase separation visually. Uniform formulation was then taken further for freeze thaw stress test.

Freeze thaw cycle

Three freeze thaw cycles between -21˚C and +25˚C with storage at each temperature for 48h were performed. Phase separation, cracking, creaming and turbidity were observed visually.

4.4.3. Globule size, poly dispersibility index (PDI) and zeta-potential

The globule size, PDI and zeta potential of the diluted (1:250) DE and NISO SMEDDS formulations were determined using dynamic light scattering (Malvern, Nano ZS, UK). All studies were performed in triplicate [12].

4.4.4. % Transmittance

The optimized DE and NISO SMEDDS formulations were freshly diluted individually to 50, 100, 250, 1000 times with distilled water and dilutions were kept undisturbed for 10 min. Turbidity was then observed visually [13]. Thereafter, % transmittance was measured at 650 nm using UV–Visible spectrophotometer against distilled water as the blank.

4.4.5. Cloud point measurement

The optimized DE and NISO SMEDDS formulations were diluted with distilled water (250 times), placed in a temperature regulated water bath (IKA, HB 10 digital, USA) and temperature was increased gradually. The cloud point was determined visually, as the temperature at which there was a sudden appearance of cloudiness and by determining % transmittance at 650 nm by using UV–Vis spectrophotometer [14]. All studies were repeated in triplicates.

4.4.6. Viscosity

The viscosity of the optimized batch of DE and NISO SMEDDS was determined employing Brookfield viscometer (DV-III+ Rheometer, Brookfield, USA) using cone and plate at 10 rpm speed and 25°C temperature in triplicate.

4.4.7. Self emulsification time and precipitation assessment

The emulsification time of the optimized batch of SMEDDS was assessed by USP type II (paddle type) dissolution apparatus (DS 8000, LabIndia Instruments, India). Formulations equivalent to 20mg of DE and 10 mg of NISO were added to 250mL of 0.01N HCl and 0.1N HCl with 0.5% SLS respectively at 37.5°C. Mild agitation was provided by the paddle rotating at 50 rpm. The time required for complete dispersion of the formulation in aqueous phase to form microemulsion was recorded as self-emulsification time for both the formulations. Precipitation was evaluated by visual inspection of the resultant emulsion after 24h storage at 37°C. The formulations were then categorized as clear (transparent or transparent with bluish tinge) or non-clear (turbid), stable (no precipitation at the end of 24h) or unstable (showing precipitation within 24h) [15, 16]. All the studies were performed in triplicate.

4.4.8. Morphological examination using Transmission Electron Microscopy (TEM)

The morphology of the oil globules of DE and NISO SMEDDS were visualized using TEM. A drop of microemulsion formed after dilution (100-fold dilution in distilled

water) of the optimized SMEDDS of both the drugs were individually placed on a piece of parafilm. A carbon coated grid (3mm, 300#) was placed on the drop and left for 1 min. Excess fluid was removed by using filter paper. Negative staining was then performed by placing the grid on a drop of 2% phosphotungsten acid (PTA) for 1 min. The grid was examined under a transmission electron microscope (Tecnai 20, 200KV, Phillips, Netherland) [12].

4.4.9. Drug content

The optimized batches of DE SMEDDS and NISO SMEDDS, equivalent to 20mg of DE and 10 mg of NISO were dispersed in10mL of methanol, stirred sufficiently to dissolve the drug and centrifuged at 3000 rpm for 10min [5]. The supernatants were duly diluted and analyzed spectrophotometrically at 315nm for DE and at 236 nm for NISO.

4.4.10. Fourier transform infrared (FTIR) spectroscopy

The FTIR spectra of final optimized formulation was compared with individual spectra of both the pure drugs and placebo mix of excipients used in fabrication of DE and NISO SMEDDS.

4.4.11. Drug release studies

4.4.11.1. *In vitro* dissolution study

The dissolution study was performed using USP type II (paddle) dissolution apparatus (DS 8000, Labindia Instruments, India), using 900mL of 0.01N HCl and pH 6.8 phosphate buffer for DE SMEDDS and 0.1NHCl with 0.5% SLS and pH 6.8 phosphate buffer with 0.5% SLS for NISO SMEDDS at 37± 0.5°C and 50 rpm. The optimized SMEDDS formulation and drug suspension each equivalent to 20mg of DE and 10 mg of NISO were filled individually into hard gelatin capsules shells (Size: 00). A sample aliquot of 3mL was removed at each time interval followed by replacement with an equivalent amount of fresh dissolution medium in order to maintain the sink condition and analyzed spectrophotometrically at 325nm and 316nm for 0.01N HCL and pH 6.8 phosphate buffer respectively for DE SMEDDS [17] and at 238nm and 236nm for 0.1N HCl with 0.5% SLS and pH 6.8 phosphate buffer with 0.5% SLS respectively for NISO SMEDDS. All the experiments were performed in triplicate.

4.4.11.2. *In vitro* diffusion study

The diffusion study was performed using activated dialysis membrane with a molecular weight cut-off of 12000 Daltons and pore size 2.4 nm (Hi-media, India). The optimized DE and NISO SMEDDS formulations and drug suspensions (1mL) equivalent to 20mg of DE and 10 mg of NISO respectively were filled in the dialysis membrane bag which was sealed with the help of clips and immersed in 250mL of 0.01N HCl and pH 6.8 phosphate buffer for DE and in 250 mL of 0.1N HCl with 0.5% SLS and pH 6.8 phosphate buffer with 0.5% SLS for NISO under continuous stirring at 37± 0.5°C. A sample aliquot of 3mL was removed at each time interval followed by replacement with an equivalent amount of fresh diffusion medium in order to maintain the sink condition [15] and analyzed spectrophotometrically at 325nm and 316nm for 0.01N HCL and 6.8 phosphate buffer respectively for DE SMEDDS [18] and at 238nm and 236nm for 0.1N HCl with 0.5% SLS and pH 6.8 phosphate buffer with 0.5% SLS respectively for NISO SMEDDS [19, 20]. All the experiments were performed in triplicate. *In vitro* diffusion study data was further fitted to various release models viz zero order (Eq. 4.4.1), first order (Eq. 4.4.2), Hixon Crowell (Eq.4.4.3), Korsemeyer Peppas (Eq.4.4.4) and Higuchi model (Eq. 4.4.5) to identify the mechanism and kinetics of drug release from optimized DE and NISO SMEDDS formulations. Regression coefficient (r^2) was calculated to identify the best-fit model [10].

Zero Order equation

$$Q_t = Q_0 + K_0 t \qquad \text{(Eq. 4.4.1)}$$

First Order equation

$$\ln Q_t = \ln Q_0 + K_1 t \qquad \text{(Eq. 4.4.2)}$$

Hixon Crowell equation

$$W_o^{1/3} - W_t^{1/3} = K_s t \qquad \text{(Eq.4.4.3)}$$

Korsemeyer Peppas equation

$$\frac{M_t}{M_\infty} = a t^n \qquad \text{(Eq.4.4.4)}$$

Higuchi model

$$Q_t = K_H \sqrt{t} \qquad \text{(Eq. 4.4.5)}$$

Where, Q_t is the amount of drug released in time t, Q_0 is the initial amount of drug in the solution, W_o is the initial amount of drug in the pharmaceutical dosage form, W_t is the remaining amount of drug in the pharmaceutical dosage form at time t and K_s is a constant incorporating the surface–volume relation, M_t /M_∞ is the fractional release of drug, a is a constant incorporating structural and geometric characteristics of the drug dosage form, n is the release exponent, indicative of the drug release mechanism, K_0 is the zero order release constant, K_1 is the first order release constant and K_H is the Higuchi dissolution constant [21].

4.4.11.3. *Ex vivo* release study

All experiments and protocols described in this study were approved by the Institutional Animal Ethics Committee (IAEC) of Faculty of Pharmacy, The M. S. University of Baroda, Gujarat, India., and were conducted as per the norms of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India (Protocol No. MSU/IEAC/2016-17/1642) . Male Sprague-Dawley rats (250-300 g) were humanitarianly sacrificed and the stomach and small intestine were isolated and washed thoroughly with PBS to remove the mucous and lumen contents. The optimized DE SMEDDS formulation equivalent to 20mg of DE and optimized NISO SMEDDS formulation equivalent to 10mg of NISO were individually filled in both the stomach and intestine tissues. Both the ends of the tissue were tied properly to avoid any leakage and were placed in an organ bath with continuous aeration at 37 ± 0.5°C. The receptor compartment (organ tube) was filled with 50mL of phosphate buffer saline pH 7.4 for DE SMEDDS and phosphate buffer saline pH 7.4 with 0.5% SLS for NISO. At predetermined time intervals, samples were withdrawn from the receptor compartments and filtered through 0.22µ membrane filter. Fresh buffer was used to replenish the receptor compartments. Similarly, equivalent amount of DE and NISO suspensions were also studied [5]. Samples were analyzed spectrophotometrically at 316nm for the content of DE [2] and at 236nm for the content of NISO [19, 20]. All the experiments were performed in triplicate.

4.5. Stability study

The optimized batches of DE SMEDDS and NISO SMEDDS were subjected to accelerated and long term stability testing according to the ICH guidelines for zones III and IV (ICH Q1A (R2), 2003) at 40 ± 2^0C/75 ± 5% RH (for 0,1, 2, 3 and 6 months) and 25 ± 2^0C/60 ± 5% RH (for 0, 1, 3 and 6 months) conditions [22]. The optimized formulation of DE SMEDDS was kept in sealed glass vials while NISO SMEDDS optimized formulation was kept in sealed amber coloured glass vial [23]. The samples were withdrawn periodically and evaluated for different physicochemical parameters like globule size, drug content and % transmittance.

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Formulation Development (SMEDDS)

Part B: Result & Discussion

Chapter 4

4.6. Preformulation

4.6.1.Authentication of drugs: Dabigatran etexilate (DE) and Nisoldipine (NISO)

4.6.1.1. UV spectroscopy

The solutions containing 10µg/mL DE and NISO in 0.01N HCl and 0.1N HCl + 0.5% SLS respectively were scanned and wavelength maxima (λ_{max}) was found to be 325 nm and 238 nm respectively (Figure. 4.6.1 and 4.6.2). Spectra for both the drugs were found to be similar to their respective reported spectra [1, 2].

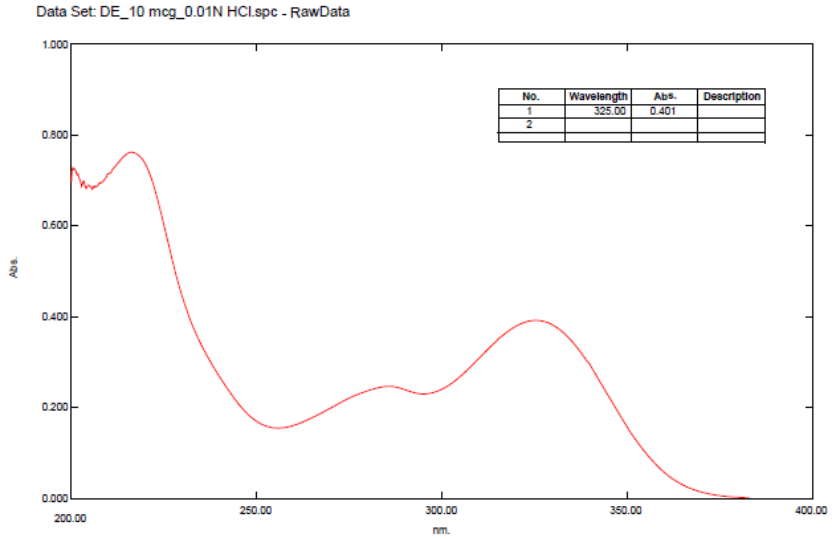


Figure 4.6.1: UV spectrum of DE in 0.01N HCl

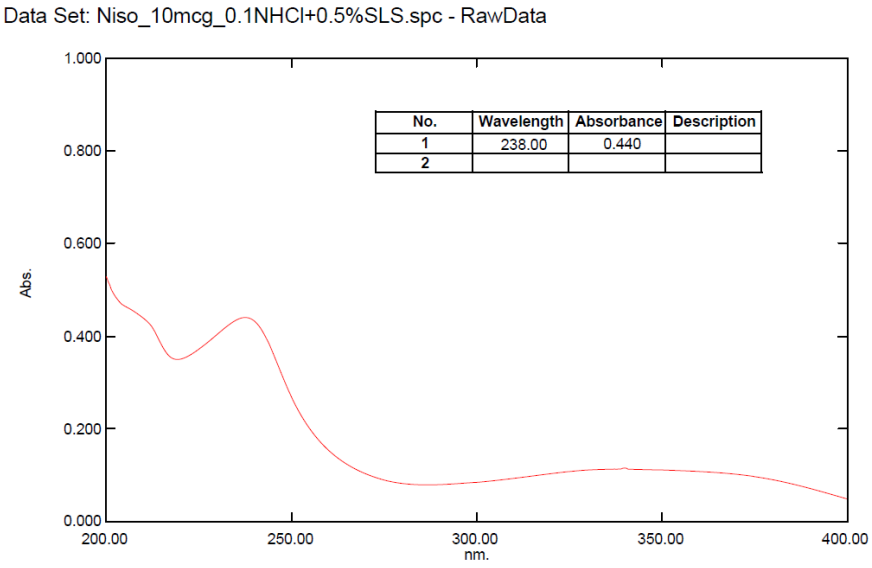


Figure 4.6.2: UV spectrum of NISO in 0.1N HCl with 0.5% SLS

4.6.1.2 Fourier transform infrared (FTIR) spectroscopy

The IR spectra of both the drugs were recorded (Figure 4.6.3 and 4.6.4) and the functional groups were interpreted as per the reported chemical structure of DE and NISO.

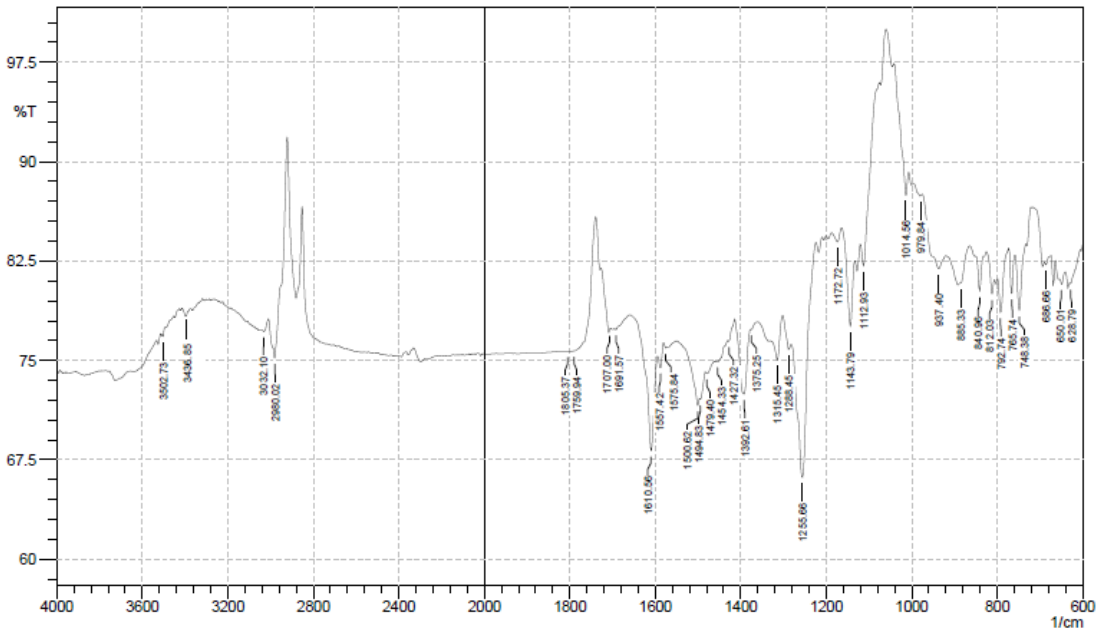


Figure 4.6.3: FTIR spectrum of DE

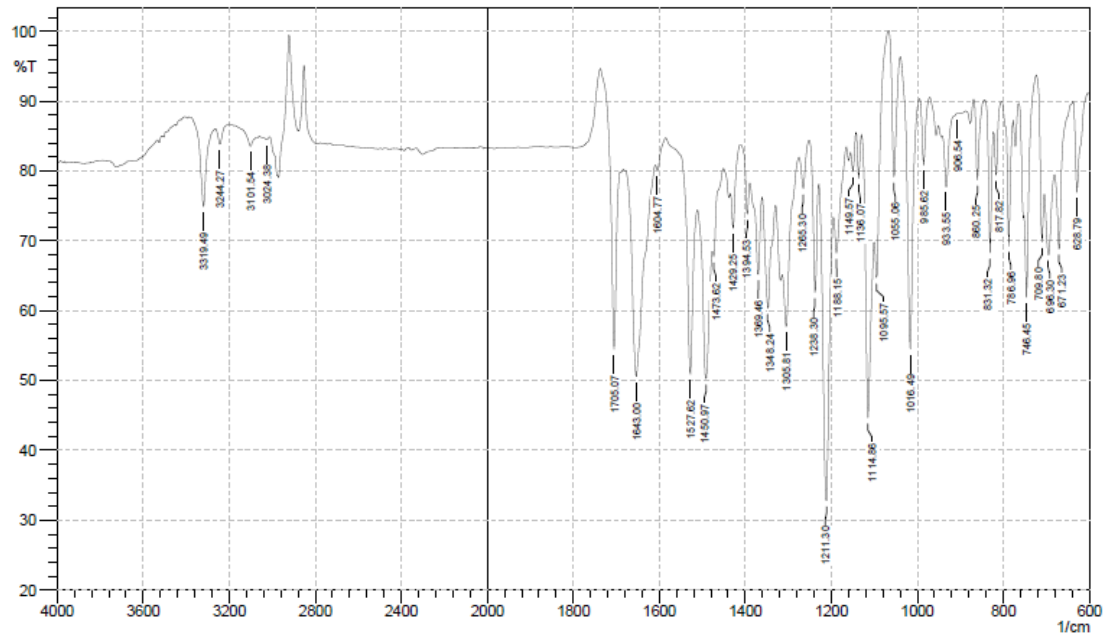


Figure 4.6.4: FTIR spectrum of NISO

The observed and reference values of stretching and bending vibrations in the IR spectra of DE and NISO along with their corresponding functional groups at their respective wave numbers are recorded in Table 4.6.1 and 4.6.2 respectively.

Table 4.6.1: Functional groups along with their wave numbers for DE

Functional Groups	Wave number (cm^{-1})	
	Observed	**Reference**
-NH_2 (Primary amine)		
-NH (Stretching)	3436	3500-3100
-NH (Bending)	1557	1640-1550
-OCO- (Ester)		
C=O (stretching)	1759	1750-1730
-NH (Stretching)	3436	3500-3100
-CO (Bending)	1143	1300-1000
-NH- (Secondary Amine)		
-NH (Stretching)	3436	3500-3100
-CN (Bending)	1255	1350-1000
-NHCO- (Amide)		
C=O (stretching)	1691	1680-1630
-NH (Stretching)	3436	3500-3100
Aromatic ring		
Stretching	3032	3150-3050
Bending	1610	1600-1475
Alkanes (-CH_3)		
-CH (Stretching)	2980	3000-2850
-CH (Bending)	1392	1450-1375

Table 4.6.2 Functional groups along with their wave numbers for NISO

Functional Groups	Wave number (cm^{-1})	
	Observed	**Reference**
-NH- (Secondary Amine)		
-NH (Stretching)	3319	3500-3100
-NH (Bending)	1643	1640-1550
-CN (Bending)	1348	1350-1000
-OCO- (Ester)		
C=O (stretching)	1705	1750-1730
-NH (Stretching)	3101	3500-3100
-CO (Bending)	1305	1310-1000
Aromatic ring		
Stretching	3244	3150-3050
Bending	1604	1600-1475
Alkanes ($-CH_3$)		
-CH (Stretching)	3024	3000-2850
-CH (Bending)	1450	1450-1375

From these results it can be concluded that samples of both the drugs used were authentic.

4.6.1.3 Differential scanning Calorimetry (DSC)

In DSC thermograms, the endothermic melting transition of DE and NISO was observed at 127.43°C and 149.13°C respectively (Figure 4.6.5 and 4.6.6) These melting points were corresponding to their respective reported melting points [3, 4].

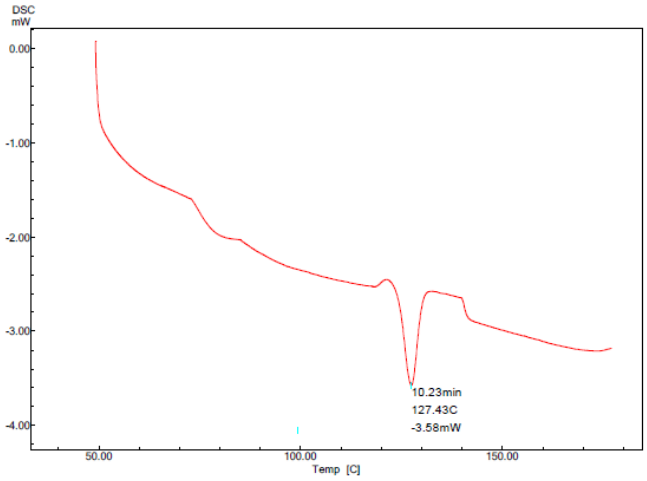


Figure 4.6.5: DSC thermogram of DE

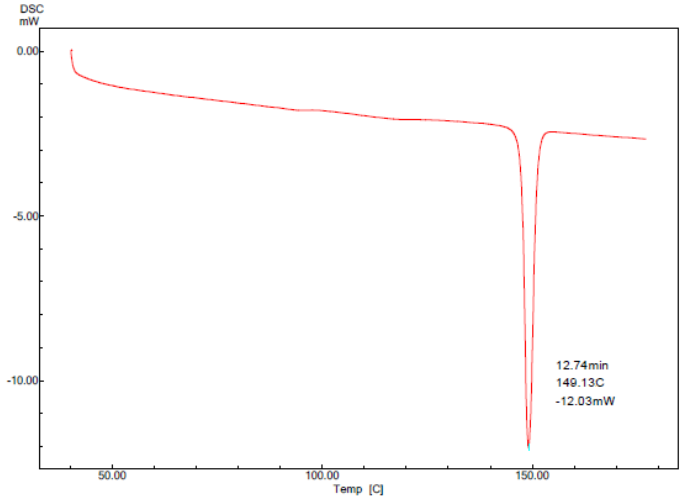


Figure 4.6.6: DSC thermogram of NISO

4.6.1.4 Melting point determination

The melting points of DE and NISO were observed to be in the range of 127-128°C (Reported melting point:128°C to 129°C) and 150°C to 151°C (Reported melting point-150°C to 155°C) respectively. The observed values were almost similar to the reported values, hence confirmed the authenticity of both the drugs.

4.6.2 Drug-Excipients compatibility study

4.6.2.1 Physical observations

No physical change was observed for the placebo mix of DE with excipients (mentioned in Table 4.6.3) and NISO with excipients (mentioned in Table 4.6.4) which were kept for compatibility study. Therefore it was concluded that both placebo mix and individual excipients were physically compatible with their respective drugs.

Table 4.6.3: Physical compatibility study of DE with excipients

Sr. No.	Physical mixture	Visual observation			
		Initial	**1 week**	**2 weeks**	**4 weeks**
1	DE + Capmul MCM C8	Clear liquid	No change	No change	No change
2	DE + Cremophor EL	Clear liquid	No change	No change	No change
3	DE + Transcutol HP	Clear liquid	No change	No change	No change
4	DE +Peceol	Clear liquid	No change	No change	No change
5	DE +Labrasol	Clear liquid	No change	No change	Very slight Hazy
6	DE + PEG 200	Clear liquid	No change	No change	No change
7	DE+ Capmul MCM C8 + Cremophor EL+ Transcutol HP	Clear liquid	No change	No change	No change

Table 4.6.4: Physical compatibility study of NISO with excipients

Sr. No.	Physical mixture	Visual observation			
		Initial	After 1 weeks	After 2 weeks	After 4 weeks
1	NISO+Peceol	Clear Yellow liquid	No change	No change	No change
2	NISO+ Cremophor EL	Clear Yellow liquid	No change	No change	No change
3	NISO+ Transcutol HP	Clear Yellow liquid	No change	No change	No change
4	NISO+ Capmul MCM C8	Clear Yellow liquid	No change	No change	No change
5	NISO +Labrasol	Clear Yellow liquid	No change	No change	No change
6	NISO+ PEG 200	Clear Yellow liquid	No change	No change	No change
7	NISO+ Peceol + Cremophor EL+ Transcutol HP	Clear Yellow liquid	No change	No change	No change

4.6.2.2 Fourier Transform Infrared spectroscopy

Individual IR spectra of DE, placebo mix and DE SMEDDS are shown in Figures 4.6.3, 4.6.7, and 4.6.8 respectively. Similarly, IR spectra of NISO, placebo mix and NISO SMEDDS are shown in Figures 4.6.4, 4.6.9, and 4.6.10 respectively. It was observed that the principal peaks of DE and NISO as shown in Table 4.6.1 and 4.6.2 respectively, were retained as such in the spectra of DE SMEDDS (Figure 4.6.8) and NISO SMEDDS (Figure 4.6.10) respectively, thereby indicating the absence of any significant interaction or incompatibility between the drug and excipients used in the formulations.

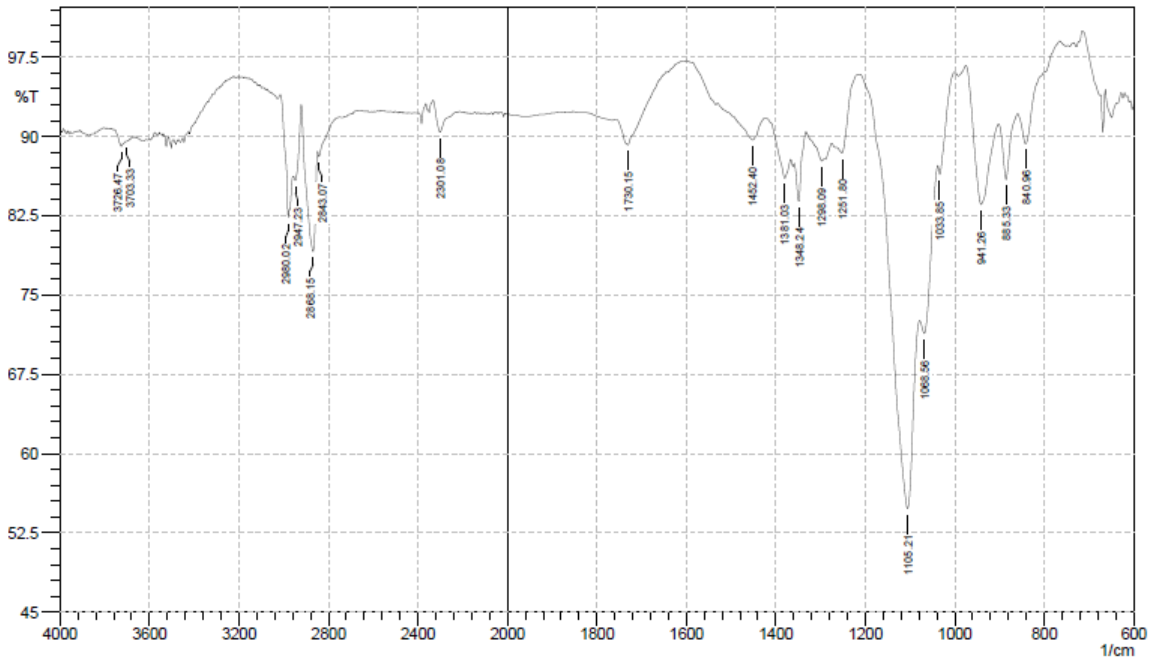


Figure 4.6.7: FTIR spectrum of placebo mix for DE SMEDDS

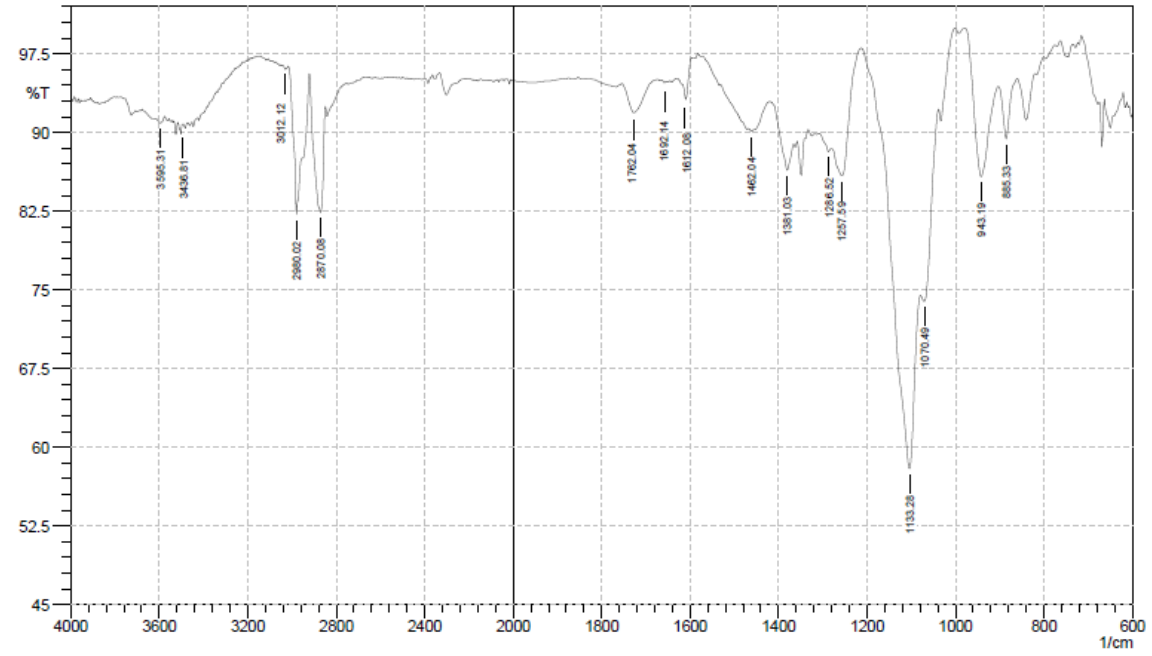


Figure 4.6.8: FTIR spectrum of DE SMEDDS

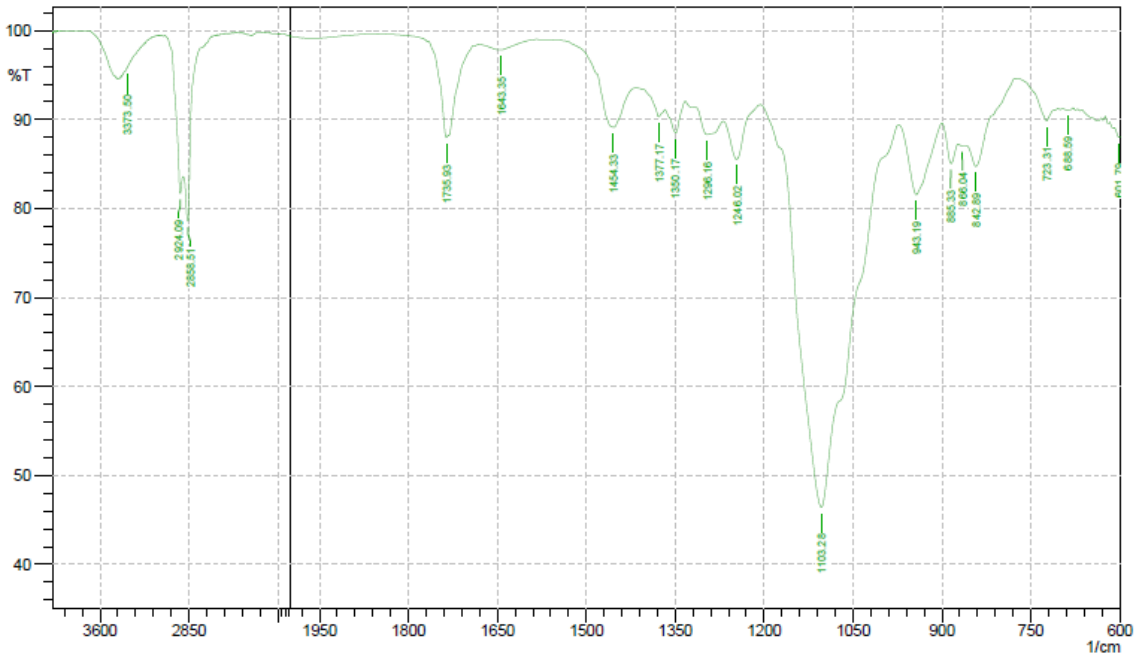


Figure 4.6.9: FTIR spectrum of placebo mix for NISO SMEDDS

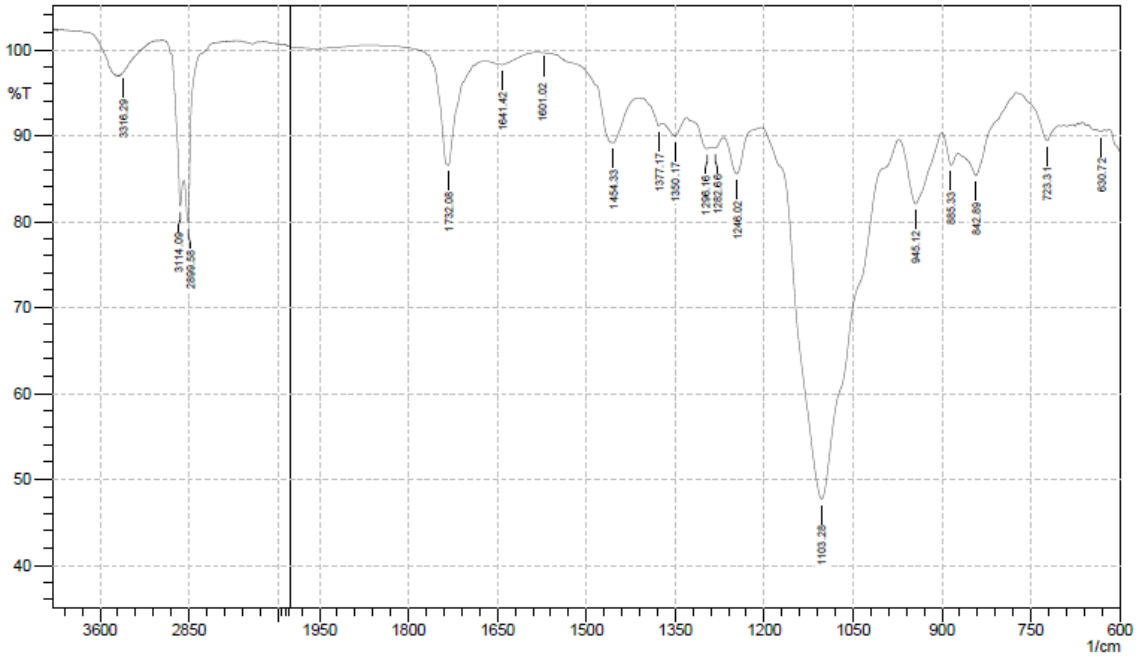


Figure 4.6.10: FTIR spectra of NISO SMEDDS

The FTIR spectra of DE SMEDDS and NISO SMEDDS showed all characteristic peaks of drug indicating absence of any form of chemical interaction between the respective formulation excipients and individual pure drugs. Hence, it was be confirmed that there was no incompatibility among respective drugs and their formulation excipients.

4.7 Fabrication of SMEDDS

4.7.1. Selection of oil phase by solubility studies

One of the critical steps in the formulation of SMEDDS is selection of oil phase as it determines the amount of drug that can be solubilized in the system [5]. Depending on the molecular nature of the triglycerides, oil can solubilise noticeable amount of lipophilic drug and can increase the fraction of lipophilic drug transported via the intestinal lymphatic system, thereby increasing absorption from the GIT [6]. Moreover, the presence of lipids in the GIT increases drug solubilisation and extent of dissolution [7] via a number of potential mechanisms such as an increased secretion of bile salts and endogenous biliary lipids [8]. Medium chain triglycerides (MCT) are commonly used in the SMEDDS formulation. They passively diffuse from the GIT to the portal system without requirement for modification like long chain fatty acids or very long chain fatty acids do [9]. Solubility studies were conducted to identify a suitable oily phase for the development of the DE and NISO SMEDDS so as to achieve optimum drug loading [10] to ultimately increase solubility and bioavailability of the drug via oral route. Optimal bioavailability enhancement is generally provided by lipids in which the drug is most soluble [11]. Therefore, oils which had maximum solubilizing capacity for both the drugs were selected. Among various oils that were screened, maximum solubility of DE was observed in Capmul MCM C8 (150.12 ± 0.28 mg/mL) while NISO showed maximum solubility in Peceol (60.57 ± 1.94 mg/mL) (Table 4.7.1). Both the oils are medium chain triglycerides with HLB 3-4. Capmul MCM C8 (HLB 4), a medium chain monoglyceride was selected as an oil component which promote water penetration, self dispersibility and have good solvent capacity for lipophillic drugs. Furthermore, it has partial aqueous solubility which may help in easy dispersion of drug in aqueous medium [12]. Increase in oral bioavailability by using Peceol is potentially associated with the medium chain fatty acids present in its composition and selective absorption of highly lipophilic drugs by the lymphatic transport system reducing hepatic first-pass metabolism [13]. Hence, Capmul MCM C8 and Peceol were selected as the oil phase for further development of the DE SMEDDS and NISO SMEDDS formulations in order to achieve desired drug loading and to avoid precipitation of the drug upon dilution in the gut lumen *in vivo*.

Table 4.7.1: Solubility of DE and NISO in various oils

S.No.	Oil	DE Solubility# (mg/mL)	NISO Solubility# (mg/mL)
1	Capmul MCM	60.83 ± 0.56	29.71 ± 1.16
2	Capmul MCM C8	150.12 ± 0.28	32.69 ± 0.99
3	Oleic Acid	20.87 ± 0.67	34.77 ± 1.67
4	Isopropyl Myristate	10.14 ± 1.10	5.59 ± 1.10
5	Peceol	55.22 ± 0.84	60.57 ± 1.94
6	Captex 500	23.76 ± 1.04	32.61 ± 1.04
7	Labrafac Lipophile WL1349	13.34 ± 0.56	4.97 ± 1.56
8	Labrafac PG	11.88 ± 0.77	4.94 ± 1.77
9	Castor oil	2.78 ± 0.37	23.01 ± 1.34

#Data expressed as mean± SD (n = 3)

4.7.2. Selection of surfactant and cosurfactant by emulsification and solubility test

To observe the role of surfactant and cosurfactant in drug solubilization, solubility studies of DE and NISO were performed in different surfactants as shown in Table 4.7.2 and 4.7.3 respectively. Similarly, solubility data for DE and NISO in different cosurfactants are shown in Table 4.7.4 and 4.7.5 respectively. Surfactants can form a thin film at the water/oil interface, lessen the interfacial strain of two phases and provide a mechanical barrier to coalescence [1]. Moreover, it was considered as an additional advantage to prevent drug precipitation during storage of formulation [14] and dilution in GIT due to lowering of solvent capacity if surfactant or co-surfactant contributes to drug solubilization [15]. Generally, nonionic surfactants with high HLB values are used for formulating SMEDDS due to their low toxicities and high emulsifying properties. Therefore, they are usually accepted for oral ingestion [16]. Emulsification study clearly distinguish the ability of surfactants to emulsify oil and in present study the testing of emulsification capacity revealed that Cremophor EL was most efficient for emulsifying Capmul MCM C8 as well as Peceol and required least number of flask inversions to yield homogenous emulsion among several surfactants (Table 4.5.8 and 4.5.9). It was observed that the surfactants with high HLB values showed better solubilization (Table 4.5.8 and 4.5.9). This may be due to the hydrophilicity of the surfactants which enables rapid and easy dispersion of the oil in the aqueous phase as a very fine oil-in-water emulsion [17]. Cremophor EL and Cremophor RH 40 showed almost similar solubility

of DE but their emulsions had difference in % Transmittance. Moreover, the transmittance values obtained were not exactly in the order of the HLB values of surfactants. This indicates that emulsification was also influenced by other factors which may include the structure and chain length of the surfactant [17, 18]. Cremophor EL was selected as surfactant for further development of the formulation since its emulsion had high % transmittance among all surfactants studied and required lesser number of inversions to make stable microemulsion [1]. In case of NISO, drug showed good solubility in Labrasol and Cremophor RH 40 but Cremophor EL was selected as surfactant owing to its high emulsifiying ability, better transmittance and less no. of inversions.

Table 4.7.2: Data for selection of surfactants for DE SMEDDS

Surfactant	HLB value	Solubility# of DE (mg/mL)	% Transmittance# of surfactant in Capmul MCM C8 (at 650 nm)	No. of Inversions# for emulsification
Labrasol	12	16.55±1.10	40.76±1.30	11-12
Tween 80	15	30.21±0.65	71.25±2.46	8-9
Tween 20	16.7	41.16±0.47	73.36±2.10	7-8
Tween 60	14.9	25.10±1.02	70.21±2.09	8-9
Caproyl 90	6	16.84±0.31	75.52±1.08	9-10
Lauroglycol 90	3	10.29±0.46	80.09±0.89	6-7
Brij 35	16.9	42.64±0.87	85.20±1.75	6-7
Solutol HS 15	14.0-16.0	36.27±0.98	72.65±2.45	6-7
Cremophor EL	12.0-14.0	48.19±0.16	98.23±0.21	2-3
Caproyl PGMC	5	13.02±0.99	52.36±1.42	10-11
Cremophor RH 40	14.0-16.0	48.07±1.06	95.50±0.37	3-4

Table 4.7.3: Data for selection of surfactants for NISO SMEDDS

Surfactant	HLB value	Solubility# of NISO (mg/mL)	% Transmittance# of surfactant in Peceol (at 650 nm)	No. of Inversions# for emulsification
Labrasol	12	109.03±5.26	90.55±2.22	5-6
Tween 80	15	74.40±3.33	80.21±3.54	8-9
Tween 20	16.7	39.58±1.87	71.16±2.47	9-10
Tween 60	14.9	40.21±2.64	75.10±3.02	8-9
Caproyl 90	6	72.22±3.54	66.84±2.95	6-7
Lauroglycol 90	3	30.20±2.79	50.29±3.88	12-13
Brij 35	16.9	76.05±2.26	82.64±2.36	11-12
Solutol HS 15	14.0-16.0	10.09±3.51	76.27±2.56	15-16
Cremophor EL	12.0-14.0	122.65±1.62	99.19±2.76	1-2
Caproyl PGMC	5	48.42±2.31	63.02±3.42	7-8
Cremophor RH 40	14.0-16.0	119.14±2.11	97.07±2.25	3-4

After selection of oil and surfactant, various co-surfactants were screened for improving the emulsification ability and spontaneity of microemulsion formation. Assimilation of co-surfactant in the formulations containing surfactant were also reported to obtain/provide better dispersibility and drug absorption from the formulations [19]. The co-surfactant penetrates into the interface causing void spaces for water penetration. This increases interfacial fluidity that facilitates spontaneous formation of emulsion [20]. The incorporation of suitable co-surfactant lowers the interfacial tension, fluidizes the hydrocarbon region of the interfacial film, and decreases the bending stress of the interface, resulting in the improvement in spontaneity of emulsification, reduction in emulsion droplet size and polydispersity [15]. Among the various cosurfactants that were screened for both the drugs, maximum solubility, % transmittance and minimum number of inversions required were observed for Transcutol HP. Data for various co-surfactants in combination with Cremophor EL as surfactant, Capmul MCM C8 as oil for DE and Peceol as oil for NISO were noted in Table 4.7.4 and 4.7.5. The data clearly exemplified that selected oil phase viz. Capmul MCM C8 for DE and Peceol for NISO underwent highest emulsification with Transcutol HP as cosurfactant as compared with surfactant alone. This explained the importance of cosurfactant addition to SMEDDS

[21]. Co-surfactant (Transcutol HP) with a shorter molecular chain length (C6) is considered to be more efficient and has better ability to promote water penetration. Hence, Transcutol HP was selected as cosurfactant for DE and NISO SMEDDS.

Table 4.7.4: Data for selection of cosurfactants for DE SMEDDS

Cosurfactant	Solubility# of DE (mg/mL)	% Transmittance# of surfactant in Capmul MCM C8 (at 650 nm)	No. of Inversions# for emulsification
Transcutol HP	306.85±1.36	99.27±0.30	1-2
Ethanol	124.36±2.01	96.39±0.46	7-8
Proylene Glycol	27.70± 0.86	97.75±0.70	8-9
PEG 200	28.34±0.74	85.24±1.09	7-8
PEG 400	32.87±0.81	80.65±1.08	8-9
Lauroglycol FCC	31.45± 0.68	89.27±1.00	6-7

#Data expressed as mean± SD (n = 3)

Table 4.7.5: Data for selection of cosurfactants for NISO SMEDDS

Cosurfactant	Solubility# of NISO (mg/mL)	% Transmittance# of surfactant in Peceol (at 650 nm)	No. of Inversions# for emulsification
Transcutol HP	209.88±5.19	99.74±1.21	1-2
Ethanol	99.97±3.14	92.21±1.63	6-7
Proylene Glycol	68.85± 2.64	56.63±1.18	11-12
PEG 200	89.59±4.68	78.84±2.01	8-9
PEG 400	120.77±5.24	87.22±1.47	7-8
Lauroglycol FCC	71.41± 2.25	72.27±1.58	9-10

#Data expressed as mean± SD (n = 3)

4.7.3. Establishment of pseudo ternary phase diagram

The foremost step towards the formulation development was to determine the feasibility of the microemulsion formation. The boundaries of the microemulsion domains were determined by plotting pseudoternary phase diagrams for the components screened from solubility studies and emulsification test.

Pseudo ternary phase diagrams were constructed to identify self-emulsifying regions which would help to choose the proper concentration of oil, surfactant and co-surfactant

in the SMEDDS formulations to produce emulsions with good stability [22]. On the basis of solubility and emulsification studies, Capmul MCM C8 and Peceol were selected as oil for DE SMEDDS and NISO SMEDDS respectively. Cremophor EL was selected as surfactant and Transcutol HP as cosurfactant for both DE and NISO SMEDDS. A visual observation was made to confirm spontaneity of emulsification, clarity, phase separation and precipitation.

For DE SMEDDS, Figure.4.7.1 depicts almost similar self- microemulsifying region at a S_{mix} ratio of 3:1 and 2:1, while minimum area was observed with 1:1 combination. It is well known that high amount of surfactant in formulation is toxic to GIT and may cause moderate reversible changes in intestinal wall permeability [23]. Therefore, 2:1 ratio of S_{mix} was selected for DE SMEDDS. The usual surfactant concentration in self-emulsifying formulations required to form and maintain an emulsion state in the GIT ranges from 30 to 60% w/w of the final formulation. Cosurfactants are helpful when forming a microemulsion at a proper concentration range. However, an excessive amount of the co-surfactant will make the system less stable because its intrinsic high aqueous solubility will lead to increase in droplet size as a result of the expanding interfacial film [24]. Hence, 2:1 was selected as the optimal ratio of surfactant to co-surfactant for the fabrication of DE SMEDDS .

For NISO SMEDDS, Figure.4.7.2. depicts that maximum microemulsion region was observed at 1:1 ratio of S_{mix}. Further increase in surfactant to 2:1 and 3:1 showed decrease in formation of stable microemulsion region indicating that best possible emulsification was achieved at 1:1 ratio of S_{mix}. Hence, 1:1 ratio was considered as optimum surfactant: co-surfactant ratio for the preparation of NISO SMEDDS.

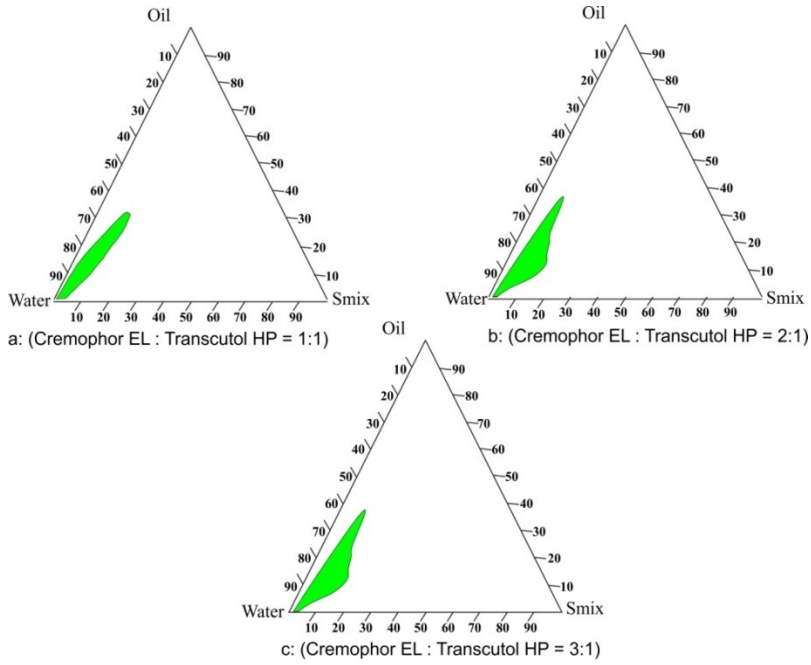


Figure 4.7.1. Pseudo ternary phase diagrams of DE SMEDDS formulations composed of oil, S_{mix} and water

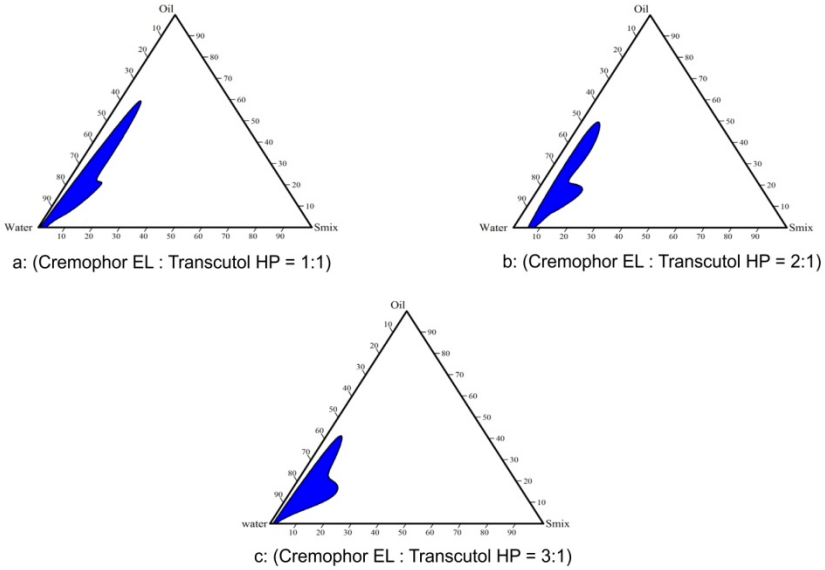


Figure 4.7.2. Pseudo ternary phase diagrams of NISO SMEDDS formulations composed of oil, S_{mix} and water

4.7.4. Optimization of DE SMEDDS using D-optimal design

The D-optimal mixture design, a subtype of mixture design, is one of the most popular response surface methodologies for optimizing formulation of a SMEDDS. The D-optimal mixture design minimizes the variance associated with evaluation of coefficients in a model and produces the best possible subset by considering the criteria for maximizing information matrix determinants. The D-optimal mixture design considers the total system as 100%. D-optimal design is particularly appropriate in formulation optimization where the total quantity of the different excipients under consideration must be constant. In this design, the response is assumed to be only dependent on the proportions of excipients [25].

A three-factor, two-level D-optimal statistical experimental design was used to optimize the formulation variables of DE SMEDDS. The globule size (GS) and % Transmittance (T) were chosen as responses because these are considered as critical factors for development of SMEDDS formulation. The observed responses are shown in Table 4.7.6. A small droplet size allows better drug absorption since it provides an increased surface area and allows faster drug release [26] while transparency confirms the microemulsion formation. All responses were simultaneously fitted to linear, quadratic, special cubic and cubic models by using the Design-Expert software version 7.0. The cubic model showed maximum R-squared value among all fitted models and was suggested as the fitting mathematical model for both Y1 and Y2. Several statistical parameters, such as sequential p-value, lack of fit p-value, standard deviation, R-squared (R^2), adjusted and predicted R-squared (R^2) values (Table 4.7.7) also justified the model. Model F-value for both the responses implied that the models were significant. The Predicted R-Squared for both the responses were in reasonable agreement with the Adjusted R-Squared indicating that the selection and model fitting were acceptable.

Table 4.7.6: Results for D-Optimal Design for DE SMEDDS

S. No.	Conc. of oil (X_1)	Conc. of surfactant (X_2)	Conc. of co-surfactant (X_3)	GS (nm) (Y_1)	T (%) (Y_2)
1	10.135	60.000	29.865	70.2± 2.1	99.1± 2.5
2	17.204	46.936	35.860	217.3± 5.6	75.2± 3.6
3	10.251	49.749	40.000	768.6± 4.3	32.3± 3.3
4	13.313	53.453	33.234	101.3± 3.6	90.2± 2.9
5	20.000	40.010	39.990	585.5± 5.2	45.8± 4.2
6	19.994	60.000	20.006	320.8± 3.4	60.1± 3.5
7	20.000	51.183	28.817	265.5± 2.9	63.8± 4.1
8	10.135	60.000	29.865	71.6± 2.2	99.6± 3.3
9	14.763	45.237	40.000	336.6± 3.1	55.8± 3.4
10	19.994	60.000	20.006	330.1± 2.8	59.9± 2.7
11	10.251	49.749	40.000	772.1± 2.7	30.2± 4.4
12	20.000	55.810	24.190	321.1± 3.4	58.9± 3.1
13	15.121	60.000	24.879	93.3± 4.4	92.1± 2.9
14	20.000	40.010	39.990	580.2± 2.8	42.6± 3.1
15	20.000	51.183	28.817	268.5± 4.9	61.5± 3.2
16	16.007	55.461	28.532	171.2± 4.2	84.8± 2.6

The results are presented as mean± standard error of the mean. The GS and %T values for all 16 batches showed a wide variation from 70.2 to 772.1 nm and 30.2 to 99.6 % respectively. This variation can be seen in cubic polynomial equations in terms of U_Pseudo Components in eq.4.7.1 and eq. 4.7.2 for GS and %T respectively. The p-value and t-stat demonstrated the significance of each coefficient [27].

$$Y_1 = 7502.47X_1 + 583.77X_2 + 324.25X_3 - 12867.86X_1X_2 - 15300.01X_1X_3 - 802.58X_2X_3 + 14040.69X_1X_2X_3 - 4635.34X_1X_2(X_1 - X_2) - 8911.96X_1X_3(X_1 - X_3) + 836.89X_2X_3(X_2 - X_3) \quad \text{.....Eq.4.7.1}$$

$$Y_2 = -251.89X_1 + 44.14X_2 + 60.16X_3 + 529.40X_1X_2 + 777.85X_1X_3 + 48.73X_2X_3 - 319.49X_1X_2X_3 + 139.44X_1X_2(X_1 - X_2) + 378.80X_1X_3(X_1 - X_3) + 72.50X_2X_3(X_2 - X_3) \quad \text{.....Eq.4.7.2}$$

Table 4.7.7: Model Statistics for Y1 and Y2 responses for DE SMEDDS using D-optimal design

Response	Model	Model F-value	R-Squared	Adjusted R-Squared	Predicted R-Squared	Lack of Fit p-value	Std. Dev.
Y1	Cubic	1147.29	0.999	0.999	0.990	0.0707	4.85
Y2	Cubic	45.14	0.998	0.995	0.959	0.2965	2.75

Influence of independent variables on droplet size for DE SMEDDS using D-optimal design

The magnitude of coefficient indicates its contribution to the respective response. The coefficients of X_1 and its interaction terms had high magnitude, indicating that X_1 was a critical factor for determining droplet size. Coefficient X_1 was positive, indicating that X_1 positively influenced the response Y_1 (equation 4.7.1). In other words, globule size decreased with decrease in oil content. Together X_2 and X_3 gave negative effect on Y_1. Response Y_1 negatively influenced by the interactive effect of surfactant and cosurfactant in combination. This suggested that a smaller amount of oil and higher amount of surfactant and cosurfactant in the DE SMEDDS formulation resulted in decreasing the droplet size.

Influence of independent variables on % Transmittance for D-optimal design

As per equation 4.7.2, X_1 was negatively while X_2 and X_3 positively influenced the response for transmittance. On decreasing the concentration of oil and increasing surfactant and cosurfactant concentrations, transmittance increased. Since the design selected is mixture design, the interactions among different variables had significant effect on their respective responses.

Contour plots and response surface analysis

The relationship among variables and responses was exemplified by contour plots and response plots for both the responses. For each response, globule size and % Transmittance, contour plots were generated between X_1, X_2 and X_3 as shown in Figure 4.7.3. Globule size decreased upon decreasing the oil concentration while sharp increase was observed upon increasing the surfactant concentration. Similar observations were noted for response Y_2. Response surface plots show the relationship between these variables even more clearly when plotted between X_1, X_2 and X_3 (Figure 4.7.4).

Minimum globule size and maximum transmittance were observed when X_1 was in mid of A(0) and A (20), X_2 was near to B(60) and X_3 was in middle of C (20) and C(40).

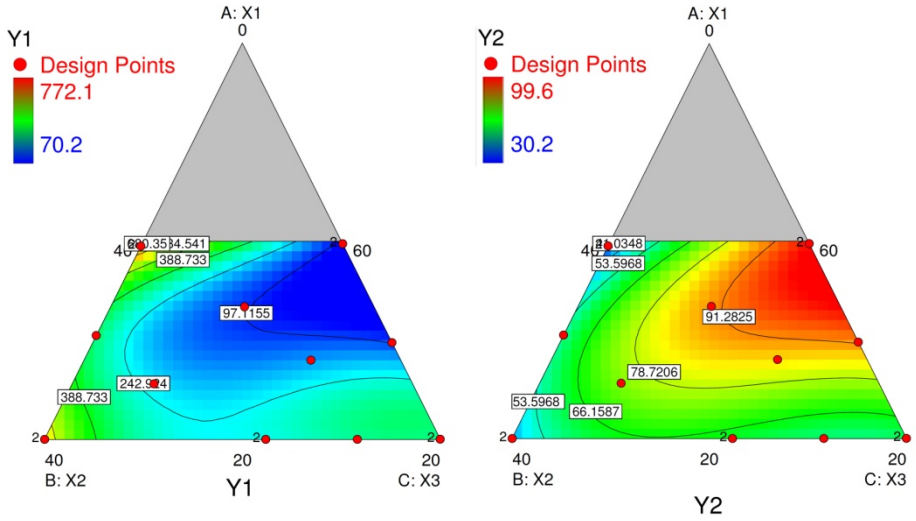


Figure 4.7.3: Contour plots showing effect of X_1, X_2 , X_3 on Y_1 and Y_2 for DE SMEDDS

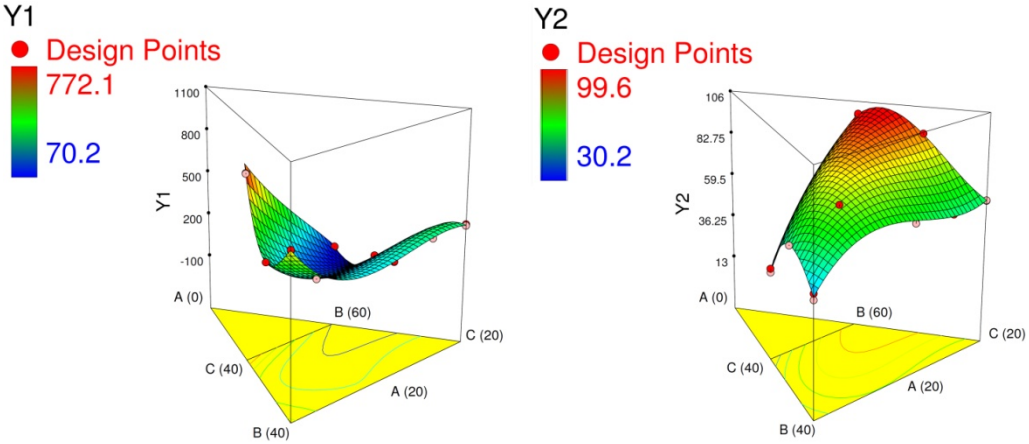


Figure 4.7.4: Response surface plot showing effect of X_1, X_2 , X_3 on Y_1 and Y_2 for DE SMEDDS

Check point analysis/desirability function

The independent variables were concurrently optimized for both the responses using desirability function. The optimum formulation was selected based on the criteria of attaining the minimum value of GS and the maximum value of %T. Response Y_1 was set to be minimum and Y_2 to be maximum. The desirability function is a transformation of the response variable from 0 to 1 scale. Value of 0 represents a completely undesirable response and 1 represents the most desirable response [27]. Based on this, 7 different

solutions were predicted with the desirability of 1. Figure 4.7.5 shows the overlay plot for the effect of different variables on the two responses.

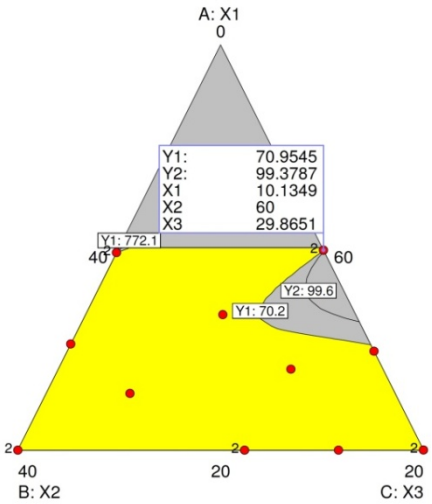


Figure 4.7.5: Overlay plot of desirable DE -SMEDDS for D-optimal design

The experimental and predicted results were compared for the three different formulation obtained from check point analysis. Data analysis using student's t-test showed that there was no statistically significant difference ($p < 0.05$) between experimentally obtained values and predicted values (Table 4.7.8) indicating that the measured responses can be accurately predicted by the contour and response plots.

Table 4.7.8: Check point analysis with 't' test

Sr. No.	Conc. of oil (X_1)	Conc. of surfactant (X_2)	Conc. of co-surfactant (X_3)	Globule size (nm) (Y_1)		Transmittance (%) (Y_2)	
				Experimental (Mean)	Predicted	Experimental (Mean)	Predicted
1	10.425	60.251	29.962	72.254	70.684	96.124	99.392
2	11.579	57.366	31.056	71.634	72.193	98.732	96.465
3	12.307	56.203	31.490	70.912	71.837	97.314	95.139
$t_{calculated}$				0.995		0.859	
$t_{tabulated}$				4.302		4.302	

From the preliminary and optimization studies, the final composition for DE SMEDDS comprised of DE (3.08%), Capmul MCM C8 (9.69%), Cremophor EL (58.15%) and Transcutol HP (29.08%) was selected for further characterization.

4.7.5. Optimization of NISO SMEDDS using 3 level factorial design

Factorial design has frequently been applied to optimize the formulation variables with fundamental requirement of understanding interactions of independent variables [28]. This design is a very useful tool for the identification of critical parameters and to optimize the respective composition and process conditions [29]. A factorial design for two factors at three levels each was selected to investigate the effect of two factors viz. concentration of oil and mass ratio of surfactant to co-surfactant (Km) on the response of the variables like GS and % T. Table 4.7.9 shows the data obtained for the experimental trials after 3^2 factorial design.

Table 4.7.9: Results for 3^2 factorial design for NISO SMEDDS

S.No.	Concentration of oil, (X_1)	Km (X_2)	GS (nm)	%T (%)
1	20	2	211.1± 2.1	72.1± 1.8
2	20	1	133.6± 1.8	73.9± 2.3
3	30	2	396.1± 3.2	55.7± 1.1
4	10	3	132.6± 1.1	90.2± 3.1
5	10	2	153.7± 1.3	90.8± 3.2
6	30	3	264.8± 2.2	74.2± 2.6
7	20	2	191.5± 2.5	72.2± 2.1
8	30	1	342.3± 3.1	52.7± 1.4
9	20	2	204.6± 2.7	73.9± 1.6
10	20	2	215.0± 2.6	70.1± 1.8
11	10	1	16.7± 1.0	99.4± 2.7
12	20	3	92.2± 1.1	80.8± 2.1
13	20	2	190.0± 1.8	76.2± 1.9

Thirteen different batches were prepared using 3^2 factorial design varying the two independent variables. The quality of formulation can be improved by optimizing the formulation systematically. GS and %T showed a wide variation from 16.7 to 396.1nm and 52.7 to 99.4 % respectively (Table 4.7.9). This variation is reflected in full model

eqn. 4.7.3 and eqn. 4.7.4 for GS and %T respectively. A second order polynomial regression equation was generated to determine the influence of independent variables on globule size and % Transmittance. A positive sign in equation indicates synergistic effect while the negative sign indicates antagonistic effect of the variables on the response.

$$Y_1 = 201.92 + 116.70X_1 - 0.50X_2 - 48.35X_1X_2 + 74.27\ X_1^2 - 87.73X_2^2 \quad \text{... Eq. 4.7.3}$$

$$Y_2 = 72.70 - 16.30X_1 + 3.20X_2 + 7.68X_1X_2 + 1.04X_1^2 + 5.14X_2^2 \quad \text{... Eq. 4.7.4}$$

For both Y_1 and Y_2, quadratic model was obtained. The p-value demonstrated the significance of each coefficient. Values of 'Prob > F' less than 0.0500 indicate that the model terms are significant and the terms having coefficients with p>0.05 are least contributing in the prediction of response. In case of globule size, X_1, X_1X_2 , X_1^2, X_2^2 are significant model terms (Table 4.7.10) while X_1, X_2, X_1X_2 , X_2^2 are the significant terms in case of % transmittance for NISO SMEDDS (Table 4.7.11). Lack of fit is not significant for both the responses which signifies good model fitting.

Table 4.7.10: Results of ANOVA for response surface quadratic model for globule size of NISO SMEDDS

Source	Sum of Squares	df	Mean square	F Value	p-value prob>F	
Model	1.177E+005	5	23542.88	91.23	<0.0001	Significant
X_1	81713.34	1	81713.34	316.65	<0.0001	
X_2	1.50	1	1.50	5.813E-003	0.9414	
X_1X_2	9350.89	1	9350.89	36.24	0.0005	
X_1^2	15232.92	1	15232.92	59.03	0.0001	
X_2^2	21259.32	1	21259.32	82.38	<0.0001	
Residual	1806.37	7	258.05	--		
Lack of Fit	1294.52	3	431.51	3.37	0.1355	Not Significant

Model statistics for both the responses are shown in Table 4.7.12. For response Y_1, the 'Predicted R-Squared' of 0.8837 is in reasonable agreement with the 'Adjusted R-Squared' of 0.9741 and for Y_2 , the 'Predicted R-Squared' of 0.9556 is in reasonable agreement with the 'Adjusted R-Squared' of 0.9770.

Table 4.7.11: Results of ANOVA for response surface quadratic model for % Transmittance of NISO SMEDDS

Source	Sum of Squares	df	Mean square	F Value	p-value prob>F	
Model	1993.10	5	398.62	102.83	< 0.0001	Significant
X_1	1594.14	1	1594.14	411.23	*< 0.0001*	
X_2	61.44	1	61.44	15.85	0.0053	
X_1X_2	235.62	1	235.62	60.78	0.0001	
X_1^2	2.98	1	2.98	0.77	0.4100	
X_2^2	72.91	1	72.91	18.81	0.0034	
Residual	27.14	7	3.88	--		
Lack of Fit	6.28	3	2.09	0.40	0.7605	Not Significant

Table 4.7.12: Model Statistics for Y1 and Y2 responses for 3^2 Factorial design of NISO SMEDDS

Response	Model	Model F-value	R-Squared	Adjusted R-Squared	Predicted R-Squared	Lack of Fit p-value	Std. Dev.
Y1	Quadratic	91.23	0.9849	0.9741	0.8837	0.1355	16.06
Y2	Quadratic	102.83	0.9866	0.9770	0.9556	0.7605	1.97

Influence of independent variables on globule size of NISO SMEDDS

The magnitude of coefficient indicates its contribution to the respective response. The coefficients of X_1 and its interaction terms had high magnitude, indicating that X_1 was a critical factor for determining globule size. Coefficient X_1 was positive, indicating that X_1 directly influenced the response Y_1. In other words, globule size increased with increase in oil content. This might be due to the fact that at higher oil concentration the surfactant concentration was unable to reduce interfacial tension sufficiently which led to coalescence and ultimately increased globule size [12]. However, coefficient X_2 had negative effect, indicating that X_2 negatively influenced the response Y_1 i.e. as surfactant concentration increased, globule size decreased. This might be attributed to more amount of surfactant which may provide closely packed interfacial surfactant film,

thereby stabilizing the oil droplets. However as ratio of surfactant to co-surfactant (Km) increased above level 1, an increase in globule size was observed. This might be due to the fact that above a certain concentration of surfactant + cosurfactant mixture, micelle formation begins which may lead to increase in globule size. Similar result was also achieved with that of ternary phase diagram (section 4.7.3). Together, X_1 and X_2 showed negative effect on Y_1. Response Y_1 was negatively influenced by the interactive effect of concentration of oil and Km in combination. This suggested that a higher amount of oil and higher Km in the NISO SMEDDS formulation obtained using the 3^2 factorial design resulted in decreased globule size.

Influence of independent variables on % Transmittance of NISO SMEDDS

Coefficient of X_1 was negative while X_2 and combination of X_1 and X_2 were positive. On decreasing the concentration of oil and increasing Km, transmittance increased. This effect was similar to that obtained with globule size but in opposite direction. Smaller the globule size, higher is the transmittance. This might be attributed to the fact that higher globule size may reduce the transparency and thereby decrease the value of % transmittance.

Contour plots and response surface analysis for 3^2 factorial design

The two-dimensional contour plots and three-dimensional response surface plots are graphical representations of the regression equations and express two independent variables at once against the selected responses. The statistically significant relationship between the dependent and independent variables was further deduced by using response surface analysis. For both the responses, contour plots were generated between the two variables, concentration of oil (X_1) and mass ratio of surfactant to cosurfactant, Km (X_2) as shown in Figure 4.7.6. In both the cases, the contour plot formed parabolic shape. For responses Y_1 and Y_2, contour plots showed increase in globule size and % Transmittance with increase in X_1 and decrease in X_2 respectively.

The relationship between these variables was seen even more clearly when plotted between X_1 and X_2 for both the responses. The 3D response surface plots showed similar results as obtained from contour plots (Figure 4.7.7) i.e., at low level of oil concentration (10 %) and Km (1), minimum globule size (16.7 ± 1.0 nm) and maximum % transmittance (99.4± 2.7 %) was obtained. Thus, the formed system was optically clear which is requirement for good microemulsion.

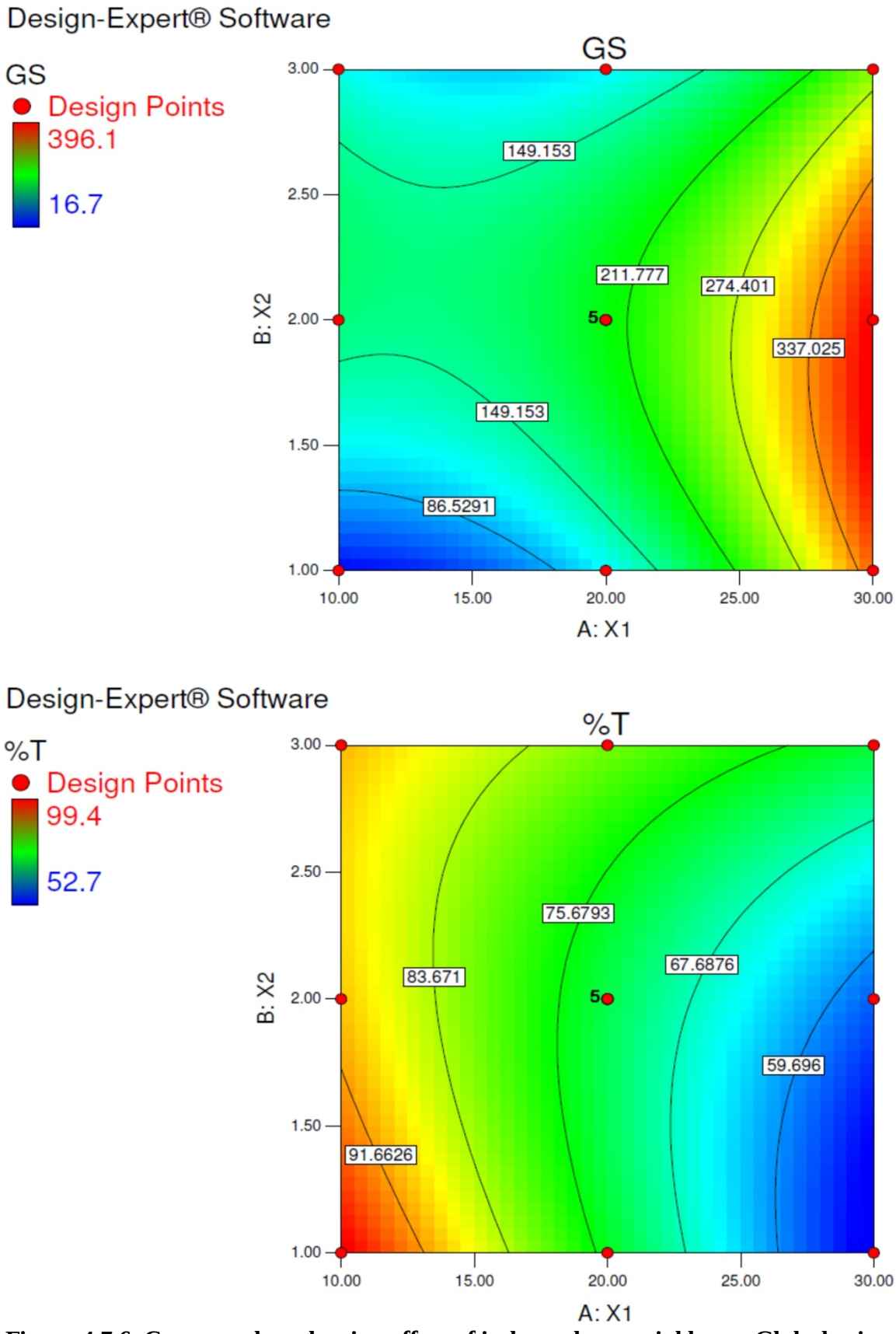


Figure 4.7.6. Contour plots showing effect of independent variables on Globule size and %Transmittance for NISO SMEDDS

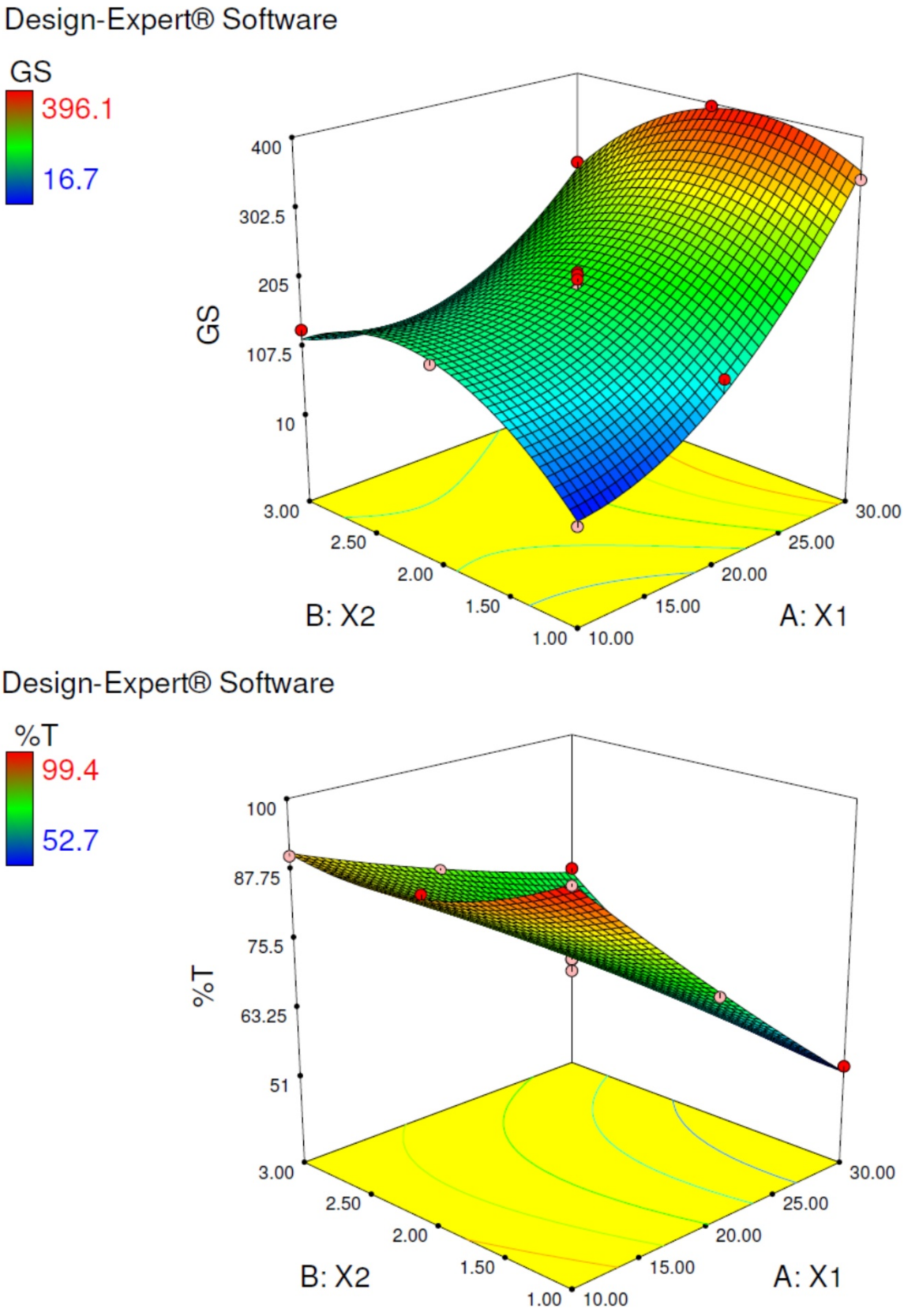


Figure 4.7.7. Response surface plots showing effect of independent variables on Globule size and %Transmittance for NISO SMEDDS

Check point analysis/desirability function

The optimum formulation was selected based on the criteria of attaining the minimum value of globule size and maximum value of % transmittance. Priority levels were set as '+++' for both the responses [30]. Three different solutions were predicted with the desirability of 1. Out of them, two check point formulations were selected, and the experimental and predicted results were compared. Data analysis using student's t-test showed that there was no statistically significant difference ($p < 0.05$) between experimentally obtained values and predicted values (Table 4.7.13). Experimental values were found to be in close proximity to the predicted values and the low values of standard deviations confirmed the reproducibility of the results. The experimental values were also found to be in close agreement with predicted value for both the responses with lower percent prediction error which suggested suitability of the design applied. The desirability for the selected quadratic model was found to be 1 (Figure 4.7.8) indicating accurate and reliable approaches in the optimization process.

Table 4.7.13: Predicted and experimental responses for check point analysis of NISO SMEDDS with 't' test

Sr. No.	Conc. of oil (X_1)	Km (X_2)	Globule size (nm) (Y_1)		Percent Prediction Error	Transmittance (%) (Y_2)		Percent Prediction Error
			Experimental (Mean)	Predicted		Experimental (Mean)	Predicted	
1	10.00	1.02	13.216	12.917	2.26	99.3743	99.212	0.16
2	10.04	1.01	10.5302	10.185	3.27	99.4139	99.135	0.28
$t_{calculated}$			0.055			0.164		
$t_{tabulated}$			12.706			12.706		

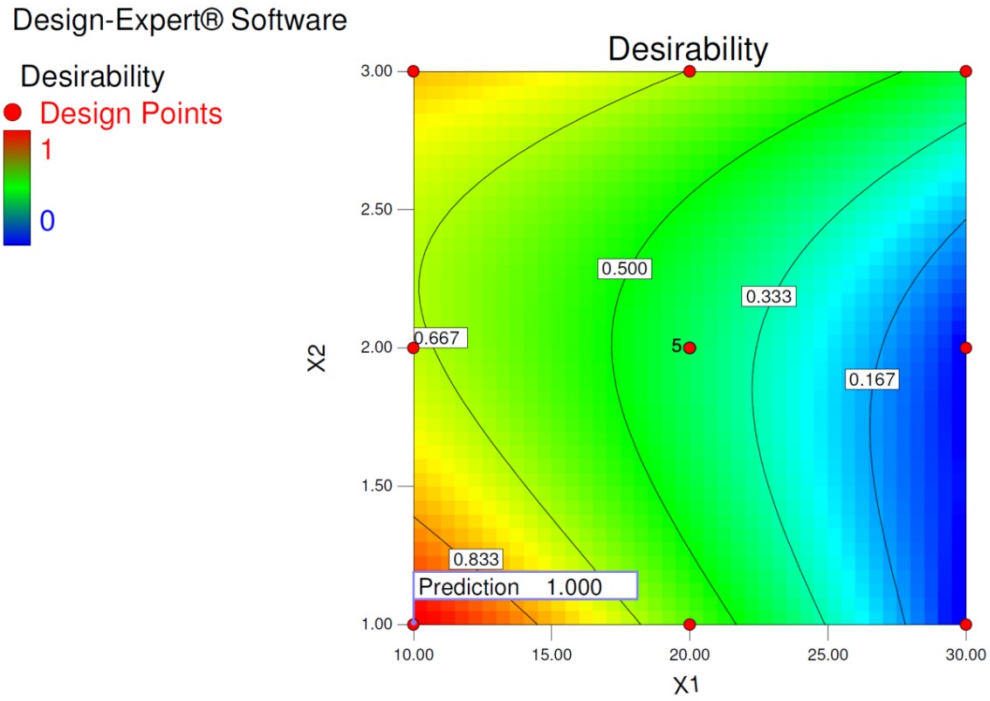


Figure 4.7.8: Desirability plot of optimized batch of NISO SMEDDS

From the preliminary and optimization studies, the final composition for NISO SMEDDS comprised of NISO (2.34%), Peceol (9.77%), Cremophor EL (43.95%) and Transcutol HP (43.95%) was selected for further characterization.

4.8. Characterization of DE SMEDDS and NISO SMEDDS

4.8.1. Robustness to dilution test

Robustness to dilution is important for SMEDDS to ensure that the emulsions formed have similar properties at different dilutions to achieve uniform globule size, drug release profile and to ensure that the drug will not get precipitated at higher dilutions *in vivo* which may significantly retard the absorption of the drug from the prepared formulation [31]. Uniform microemulsions should form upon self-emulsification of SMEDDS at different dilution conditions. Dilutions may affect globule size, transparency, drug release etc. if drug gets precipitated or any phase separation is observed [17]. The effect of extent of dilution on globule size and % Transmittance of optimized batch of DE SMEDDS and NISO SMEDDS were evaluated as shown in Table 4.8.1 and 4.8.2 respectively. All the diluted batches of both the formulations exhibited a globule size of <100 nm and transmittance above 90% irrespective of type and volume of dilution medium. Therefore, the optimized SMEDDS formulations were considered to be robust

against dilution as neither precipitation of the drug nor any phase separation was observed even after 24 h [14]. Furthermore, it also indicates the probability of uniform *in vivo* drug release profile when the optimized formulations come across measured dilution [32].

Table 4.8.1: Effect of dilution and media on globule size and % transmittance of optimized batch of DE SMEDDS

Dilution Factor	Distilled water#		0.01 N HCl#		Phosphate buffer pH 6.8#	
	Globule Size (nm)	Transmittance (%)	Globule Size (nm)	Transmittance (%)	Globule Size (nm)	Transmittance (%)
50 times	51.5 ± 1.3	96.6± 1.3	65.3± 1.2	96.2 ± 2.1	85.6 ± 2.1	96.8 ± 2.1
100 times	49.8 ± 1.7	96.8± 2.1	56.3 ± 0.6	99.1 + 1.1	77.7 ± 1.2	99.5 ± 2.0
250 times	55.5 ± 2.1	99.4± 2.2	52.1 ± 0.7	99.4 ± 2.0	81.0 ± 0.9	98.1 ± 2.1
1000 times	58.3 ± 1.9	99.1± 1.1	61.3 ± 1.4	97.8 ± 2.1	98.6 ± 1.3	91.2 ± 2.1

#Data expressed as mean± SD (n=3)

Table 4.8.2: Effect of dilution and media on globule size and % transmittance of optimized batch of NISO SMEDDS

Dilution Factor	Distilled water		0.1 N HCl		Phosphate buffer pH 6.8 with 0.5%SLS	
	Globule Size (nm)	Transmittance (%)	Globule Size (nm)	Transmittance (%)	Globule Size (nm)	Transmittance (%)
50 times	21.5 ± 2.0	96.6 ± 1.5	20.3± 1.1	97.4± 2.7	32.6 ± 2.9	97.3 ± 1.6
100 times	29.3 ± 1.8	97.6 ± 3.1	16.3 ± 1.2	98.9+ 2.9	27.4 ± 2.0	98.8 ± 1.2
250 times	25.7 ± 2.4	99.2 ± 2.6	18.2 ± 1.1	99.7± 2.7	36.5 ± 2.3	99.5 ± 1.7
1000 times	28.1 ± 2.1	99.1 ± 1.1	19.5 ± 1.4	97.8± 2.2	39.4 ± 1.5	95.2 ± 1.3

The values are mean of n=3

4.8.2. Thermodynamic stability studies

Microemulsions are known to be thermodynamically stable systems which are formed at a particular concentration of oil, surfactant and water, with no effect of temperature

variations on physical stability [7]. Optimized DE SMEDDS and NISO SMEDDS were subjected to different stress tests like heating cooling, centrifugation and freeze-thaw cycle stress tests to evaluate its thermodynamic stability. Neither phase separation nor any precipitation was observed upon centrifugation, indicating the stability of the microemulsion thus formed after self-emulsification of both the formulated SMEDDS. Hence, the optimized SMEDDS formulations of DE and NISO were found to be stable in these conditions.

4.8.3. Globule size, polydispersity index (PDI) and zeta-potential

Globule size is a key factor in determining self-emulsification performance as it determines the rate and extent of drug release, absorption as well as the stability of the emulsion [33]. The globule size of the optimized batch of DE SMEDDS and NISO SMEDDS were found to be 73.24± 1.10 nm with 0.085± 0.008 PDI and 16.78± 0.97 with PDI 0.121± 0.024 indicating unimodal globule distribution (Figure 4.8.1 and 4.8.2). Small PDI revealed narrow size distribution of microemulsion. The smaller globule size was particularly observed at low concentrations of lipid, adequate concentration of surfactant and intermediate concentrations of cosurfactant in SMEDDS formulation. This small globule size will provide high surface area which will assist in the absorption of drug and thus augment its bioavailability [32]. Zeta potential measurement was done to identify the charge on the surface of droplets. High zeta potential values, either positive or negative, avoids the attraction forces and resists particle aggregation. The extent of surface hydrophilicity can then be predicted from the values of zeta potential [34]. Zeta potential of the optimized batch of DE SMEDDS and NISO SMEDDS were found to be -22.4± 0.1mV and -28.6± 1.3mV respectively (Figure 4.8.3 and 4.8.4). These values indicated that the formulations were negatively charged due to existence of free fatty acids of oil and repulsive forces predominates which makes the system stable by resisting aggregation of emulsion droplets. Zeta potential should usually reach an absolute value ±30 mV to obtain stable emulsion by preventing flocculation and coalescence of nanosized droplets [31]. Such results inferred towards enhanced synergism of components used in the development of SMEDDS for obtaining desired globule size and zeta potential.

Results

		Size (d.nm):	% Intensity	Width (d.nm):
Z-Average (d.nm): 73.24	Peak 1:	73.24	100.0	26.73
PdI: 0.085	Peak 2:	0.000	0.0	0.000
Intercept: 0.924	Peak 3:	0.000	0.0	0.000
Result quality : Good				

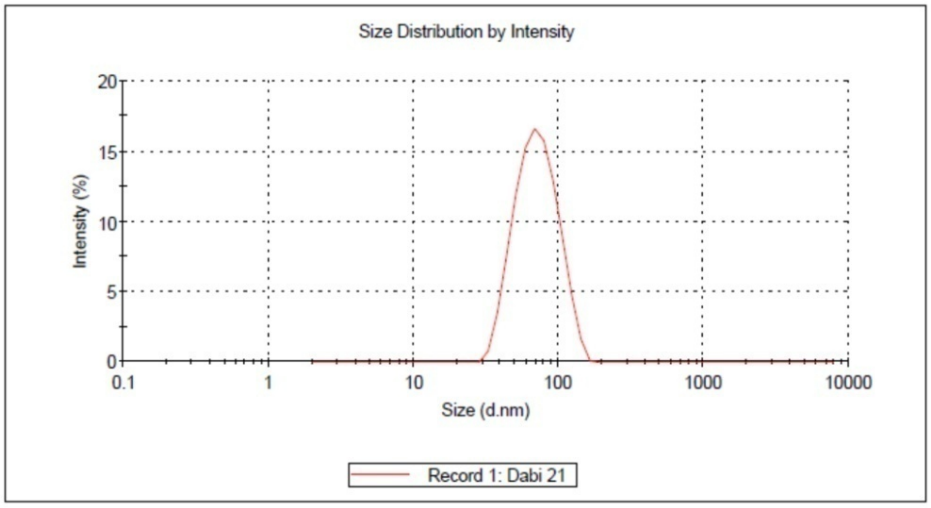


Figure 4.8.1: Globule size of optimized batch of DE SMEDDS

Results

		Size (d.nm):	% Intensity	Width (d.nm):
Z-Average (d.nm): 16.78	Peak 1:	16.78	100.0	4.690
PdI: 0.121	Peak 2:	0.000	0.0	0.000
Intercept: 0.936	Peak 3:	0.000	0.0	0.000
Result quality : Good				

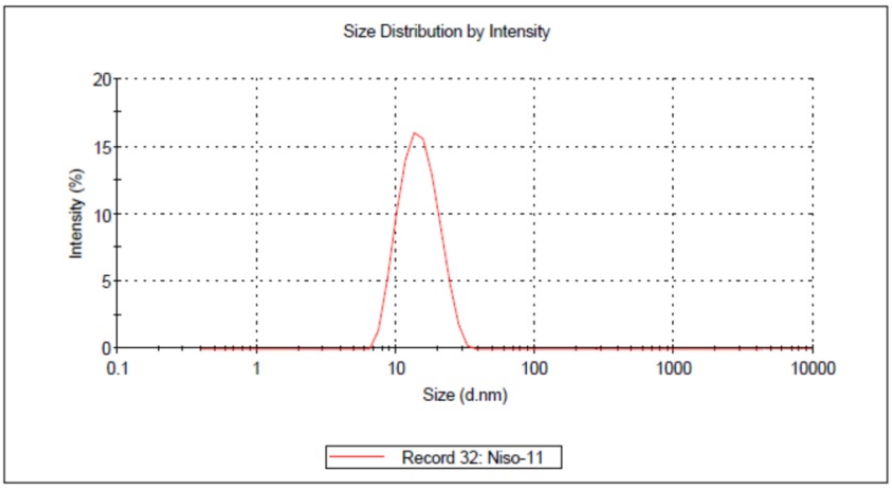


Figure 4.8.2: Globule size of optimized batch of NISO SMEDDS

Results

		Mean (mV)	Area (%)	Width (mV)
Zeta Potential (mV): -22.4	**Peak 1:**	-22.4	100.0	7.72
Zeta Deviation (mV): 7.72	**Peak 2:**	0.00	0.0	0.00
Conductivity (mS/cm): 0.0431	**Peak 3:**	0.00	0.0	0.00

Result quality : Good

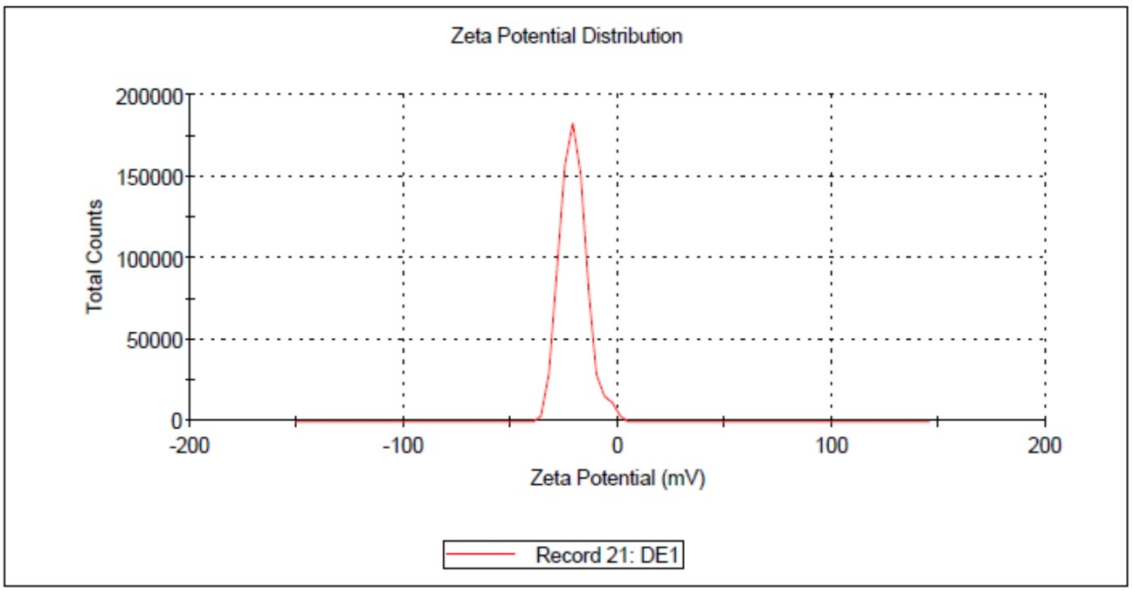


Figure 4.8.3: Zeta potential of optimized batch of DE SMEDDS

Results

		Mean (mV)	Area (%)	Width (mV)
Zeta Potential (mV): -28.6	**Peak 1:**	-28.6	100.0	11.6
Zeta Deviation (mV): 11.6	**Peak 2:**	0.00	0.0	0.00
Conductivity (mS/cm): 0.0520	**Peak 3:**	0.00	0.0	0.00

Result quality : Good

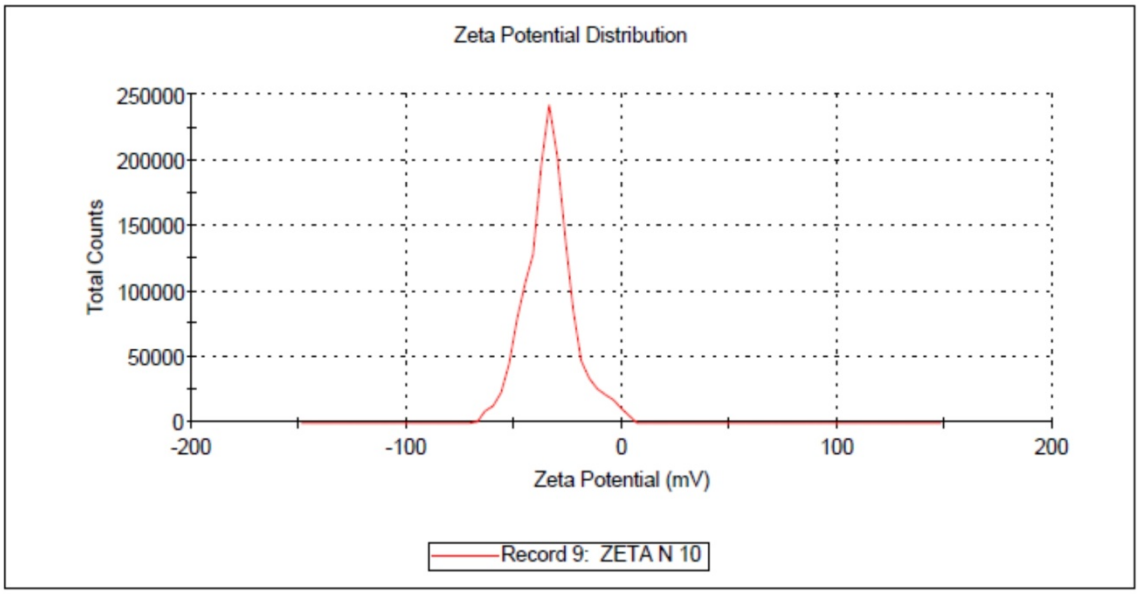


Figure 4.8.4: Zeta potential of optimized batch of NISO SMEDDS

4.8.4. % Transmittance

The optimized batch of both DE SMEDDS and NISO SMEDDS were visually found to be transparent and without any turbidity. % Transmittance of both the formulations upon dilution with different media and at different dilution factors are as shown in Table 4.8.1 and 4.8.2. A transmittance value of >80% indicates good microemulsification. However, the value closer to 100% indicated that the formulation was isotropic in nature [25]. Thus, the prepared formulation showed desired transmittance for fabrication of good product and along with this the higher transmittance values of optimized formulations were accredited to low amount of oil and adequate amount of surfactant and cosurfactant used [35].

4.8.5. Cloud point measurement

The cloud point is a crucial parameter in SMEDDS to decide integrity of the emulsion at elevated temperature particularly in formulations consisting of non-ionic surfactants. The temperature above which a clear formulation turns cloudy is known as the cloud point. At temperatures higher than the cloud point, an irreversible phase separation occurs due to dehydration of polyethylene oxide moiety of the non-ionic surfactant, which may affect the formulation adversely by disturbing drug absorption [20]. Hence, to avoid this phenomenon, the cloud point for SMEDDS should be above body temperature (37 °C) [36]. The cloud points for DE SMEDDS and NISO SMEDDS were 77.5± 2.9 and 71.4± 2.2 °C respectively which were much higher than body temperature, indicating that they will form stable microemulsion at physiological temperature i.e. *in vivo* and during storage without any phase separation. The reason for obtaining higher cloud point temperature might be attributed to high solubility of drug in oil and surfactant system, use of optimized ratio of S_{mix} or surfactants with higher HLB values [12]. Additionally, this also implied good thermal stability of the prepared optimized SMEDDS [37]. No turbidity was observed visually and this was further confirmed by high % transmittance in different media as shown earlier in Table 4.8.1 and 4.8.2 for DE and NISO SMEDDS respectively.

4.8.6. Viscosity

SMEDDS can be formulated either into tablet or capsule dosage forms. However, challenges like leaching of oil onto the surface are encountered in the tablet dosage form. A much simpler way to overcome this problem is filling of SMEDDS into hard or soft gelatin/HPMC capsule shells. In such case, viscosity is crucial in determining its ability to be filled in hard or soft gelatin capsules [17]. If the system has very low viscosity, there

may be probability of leakage from the capsule while the system with very high viscosity may create problem in pourability [38]. The viscosity of the DE SMEDDS and NISO SMEDDS at 25°C were found to be 124.80± 4.01 and 80.68± 3.44 cps respectively. As the value of viscosity for both the formulations was less than 10,000 cps, it implied that the developed SMEDDS can be filled in capsule shells by commercial liquid filling equipments [17].

4.8.7. Self emulsification time and precipitation assessment

The time of emulsification is an important parameter to assess efficiency of self emulsification since it is indicative of faster solubilization of the drug in the gastrointestinal fluid [39] and a prerequisite for SMEDDS to disperse quickly and completely when subjected to dilution under mild agitation. Two minutes has been considered as an index for evaluating the emulsification process [1]. The time of emulsification of DE SMEDDS in 0.01 N HCl and NISO SMEDDS in 0.1N HCl +0.5% HCl at 37.5°C was found to be 26.0± 2.0 and 19.0± 2.0 sec respectively which indicated the spontaneity of emulsification of the prepared SMEDDS. Moreover, the resultant microemulsions appeared to be clear (transparent or isotropically clear) which indicated desirable emulsification efficiency owing to complete miscibility of lipids in the aqueous phase by micellar solubilization [39].

4.8.8. Morphological examination using Transmission Electron Microscopy (TEM)

The morphology of the 100 fold diluted DE SMEDDS (Figure. 4.8.5) and NISO SMEDDS (Figure. 4.8.6) when examined using TEM revealed discrete, spherical oil globules of less than 100 nm size. The globules were found to be of uniform size distribution. The globule size were found to be in the range of 45-65nm for DE SMEDDS and 15-35 nm for NISO SMEDDS which are in accordance with results observed earlier . As the oil globules were discrete and non-aggregated, we can state that the microemulsions formed spontaneously and were physically stable for both the formulations.

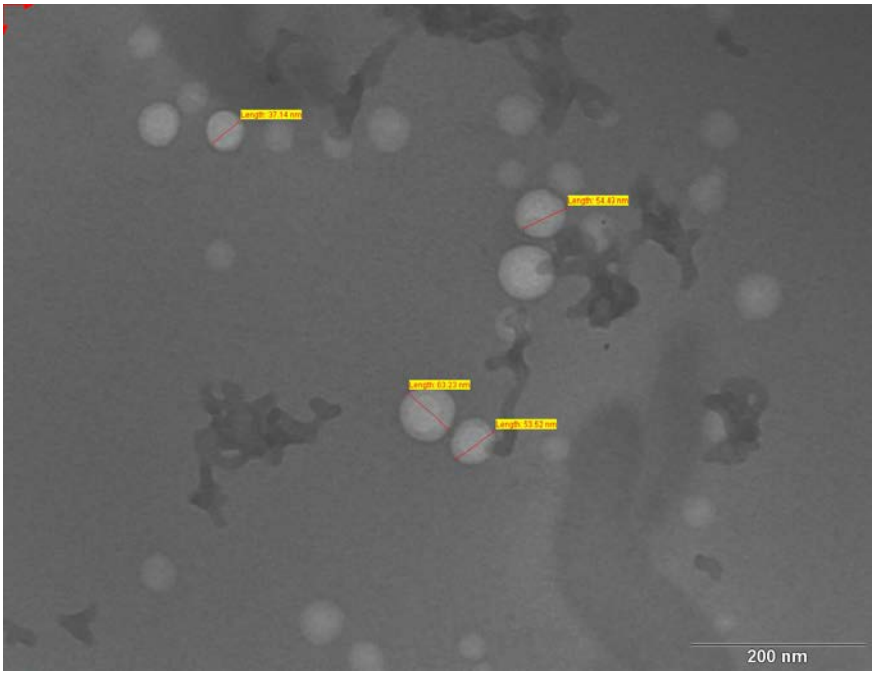


Figure 4.8.5:TEM image of optimized batch of DE SMEDDS

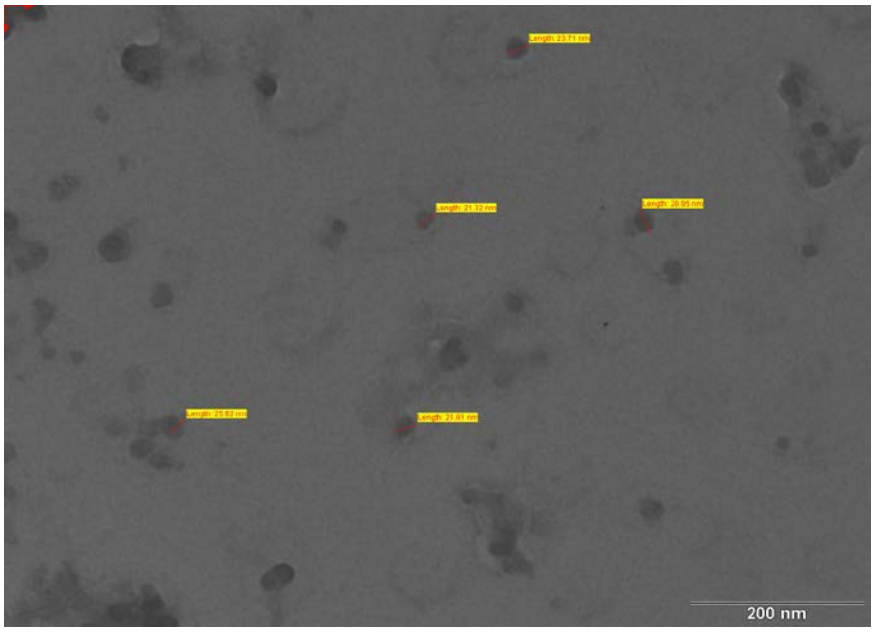


Figure 4.8.6:TEM image of optimized batch of NISO SMEDDS

4.8.9. Drug content

The drug content of the optimized batch of DE SMEDDS and NISO SMEDDS were found to be 97.78 ± 2.02 and 98.21± 1.68%, indicating uniform dispersion of drug and high entrapment in the oil phase in both the formulations.

4.8.10. Fourier transform infrared (FTIR) spectroscopy

The FTIR spectra of optimized formulation of DE and NISO SMEDDS, pure drugs and placebo mix were recorded. The results are as shown in section 4.6.2.2. The FTIR spectra of both the optimized formulations showed all characteristic peaks of individual pure drugs indicating absence of any form of chemical interaction (incompatibility) with the respective formulation excipients.

4.8.11. Drug release studies

4.8.11.1. *In vitro* dissolution study

4.8.11.1.1. *In vitro* dissolution study of DE SMEDDS

In vitro dissolution studies of DE SMEDDS and pure drug suspension were performed in 0.01N HCl and pH 6.8 phosphate buffer (Figure 4.8.7). In 0.01N HCl, 98.74± 3.72% drug was released after 60 min from DE SMEDDS while only 39.86± 2.41% drug was released from pure drug suspension. This suggests that the formulated SMEDDS led to enhancement in solubility due to reduction in particle size by micro emulsification [40]. Thus, this greater availability of dissolved DE from the SMEDDS formulation could lead to better absorption and better oral bioavailability.

In case of pH 6.8 phosphate buffer, cumulative drug release was 70.42± 2.93% in 60 min from DE SMEDDS and 7.47± 2.84% from drug suspension (Figure 4.8.7). Almost complete drug release was achieved in 0.01N HCl than in pH 6.8 phosphate buffer. This slower rate of dissolution in pH 6.8 phosphate buffer was due to lower solubility of DE at higher pH [41]. Reason for higher dissolution rate in 0.01N HCL is the high solubility of drug in acidic conditions as compared to neutral and alkaline conditions. Overall, results of *in vitro* dissolution studies indicated that the formulation of DE in the form of SMEDDS enhanced its dissolution properties.

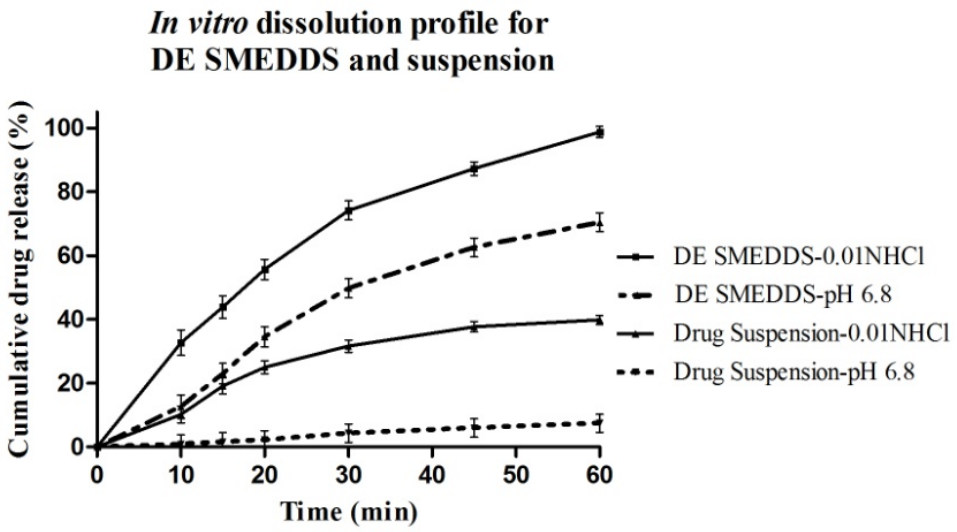


Figure 4.8.7 *In vitro* dissolution profile of DE SMEDDS and drug suspension in 0.01N HCl and pH 6.8 phosphate buffer

4.8.11.1.2. *In vitro* dissolution study of NISO SMEDDS

The dissolution of NISO SMEDDS and pure drug suspension were performed in 0.1N HCl with 0.5% SLS and pH 6.8 phosphate buffer with 0.5% SLS as shown in figure 4.8.8. In case of NISO SMEDDS, the cumulative percent drug released in 0.1N HCl+ 0.5% SLS after 60 min was 98.90± 2.01% while in case of pure drug suspension it was 11.43± 3.22%. In pH 6.8 Phosphate buffer with 0.5% SLS, 97.41± 1.71% drug was released from NISO SMEDDS and 10.11± 2.23% from drug suspension. Higher release of NISO from SMEDDS formulation might be due to high affinity of drug for the oil phase (Peceol) used in fabrication of formulation. This might be attributed to high polarity of Peceol towards NISO and high polarity always promote a rapid release of drug into the aqueous phase [42]. Thus, significant amount of drug will be carried to the intestine via the fine microemulsion globules [24] which in turn will help in enhancing oral bioavailability via lymphatic uptake. The small globule size will provide high surface area, which will permit fast drug release when compared with plain drug suspension.

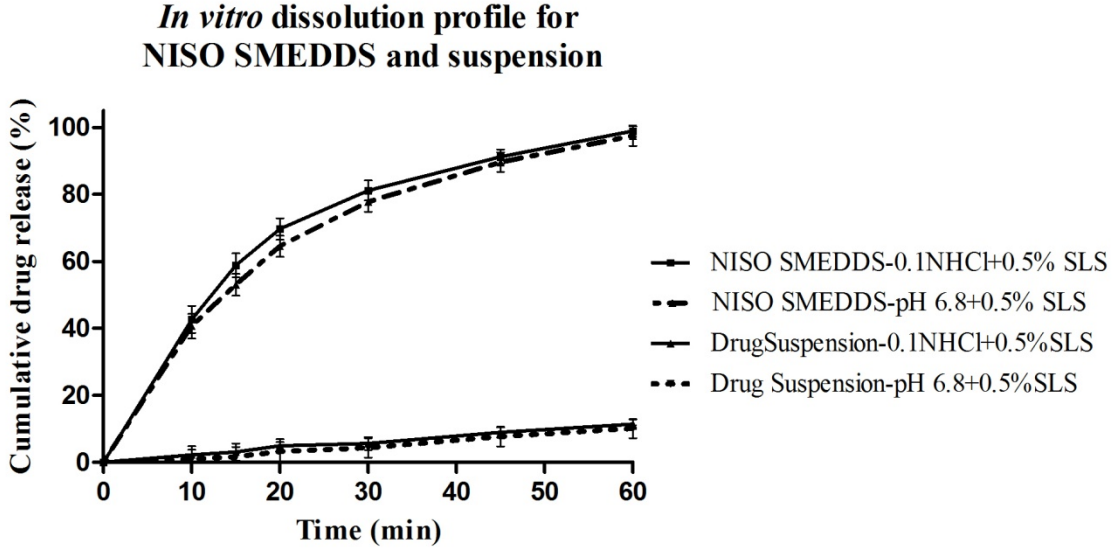


Figure 4.8.8 *In vitro* dissolution profile of NISO SMEDDS and drug suspension in 0. 1N HCl +0.5% SLS and pH 6.8 phosphate buffer +0.5% SLS.

4.8.11.2. *In vitro* diffusion study

4.8.11.2.1. *In vitro* diffusion study of DE SMEDDS

The *in vitro* release pattern was also studied through dialysis bag in which drug diffused through a semi permeable membrane of 12000 Daltons and pore size of 2.4 nm. In case of 0.01N HCl, the cumulative percent drug released was 98.05± 2.09% after 300 min from DE SMEDDS and 26.68± 2.24% from DE suspension. Similar results were observed in pH 6.8 phosphate buffer with 68.84± 2.71% release from DE SMEDDS but only 11.33± 1.28% drug diffused from DE suspension (Figure 4.8.9). The higher amount of drug diffused from SMEDDS as compared to plain drug suspension can be attributed to the increased solubility and dissolution rate of SMEDDS [10]. *In vitro* drug diffusion profiles are strong indicators of bioavailability. The data from *in vitro* diffusion study (for both 0.01N HCl and pH 6.8 phosphate buffer) was fitted to various mathematical models to determine the best-fit model (Table 4.8.3). The r^2 values were found to be highest for Higuchi model (r^2 = 0.999) in both 0.01N HCl and in pH 6.8 phosphate buffer (Figure 4.8.10 and 4.8.11). Since the drug release is from oil globules, so its geometry was considered to be sphere and the type of drug release mechanism was defined. The value for release component 'n' was between 0.43 and 0.85, indicating Non- Fickian diffusion release kinetics [43].

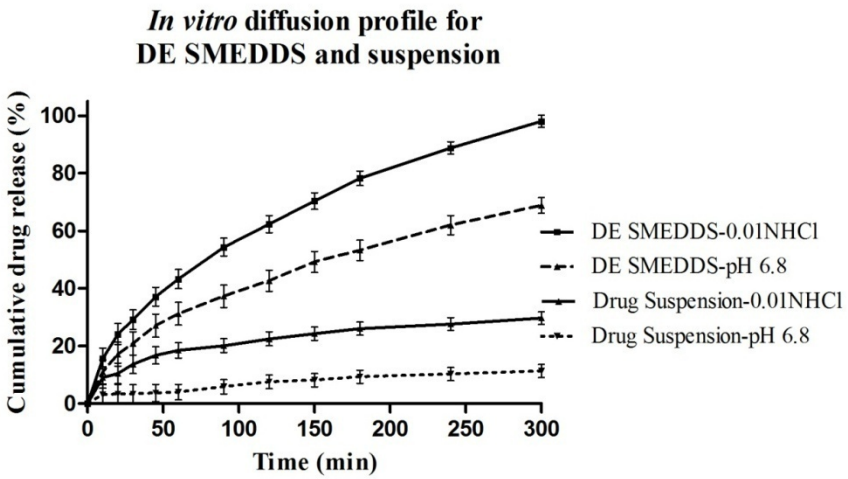


Figure 4.8.9. *In vitro* diffusion profile of DE SMEDDS and drug suspension in 0.01N HCl and pH 6.8 phosphate buffer

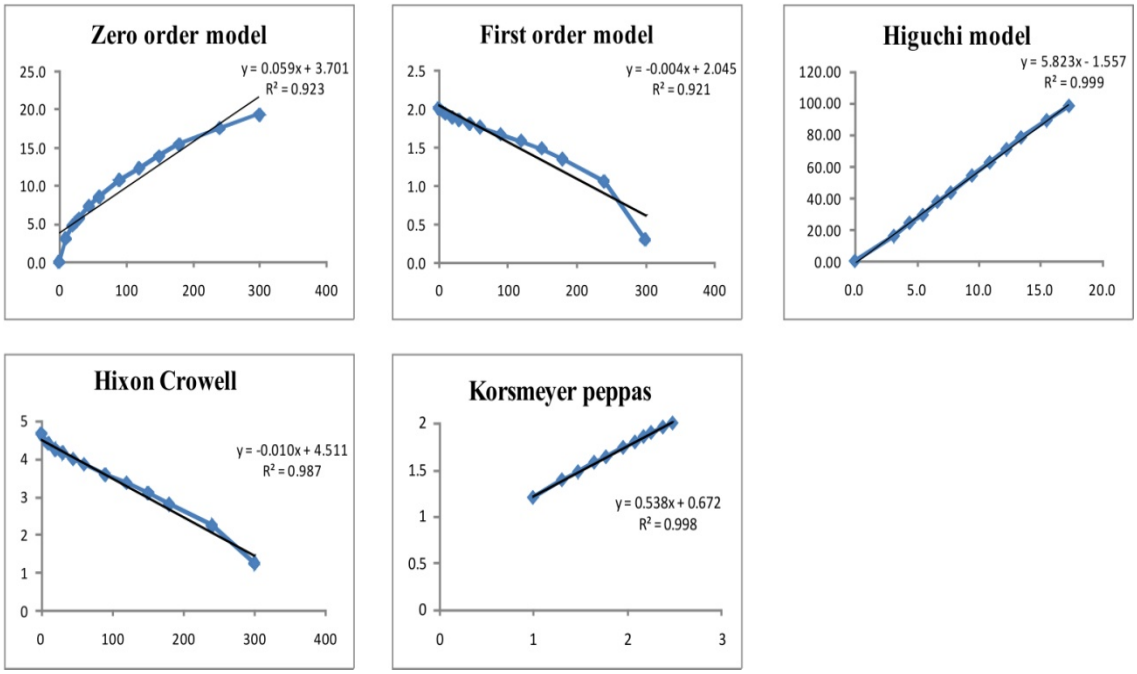


Figure 4.8.10. Release kinetics curves for DE SMEDDS in 0.01N HCl

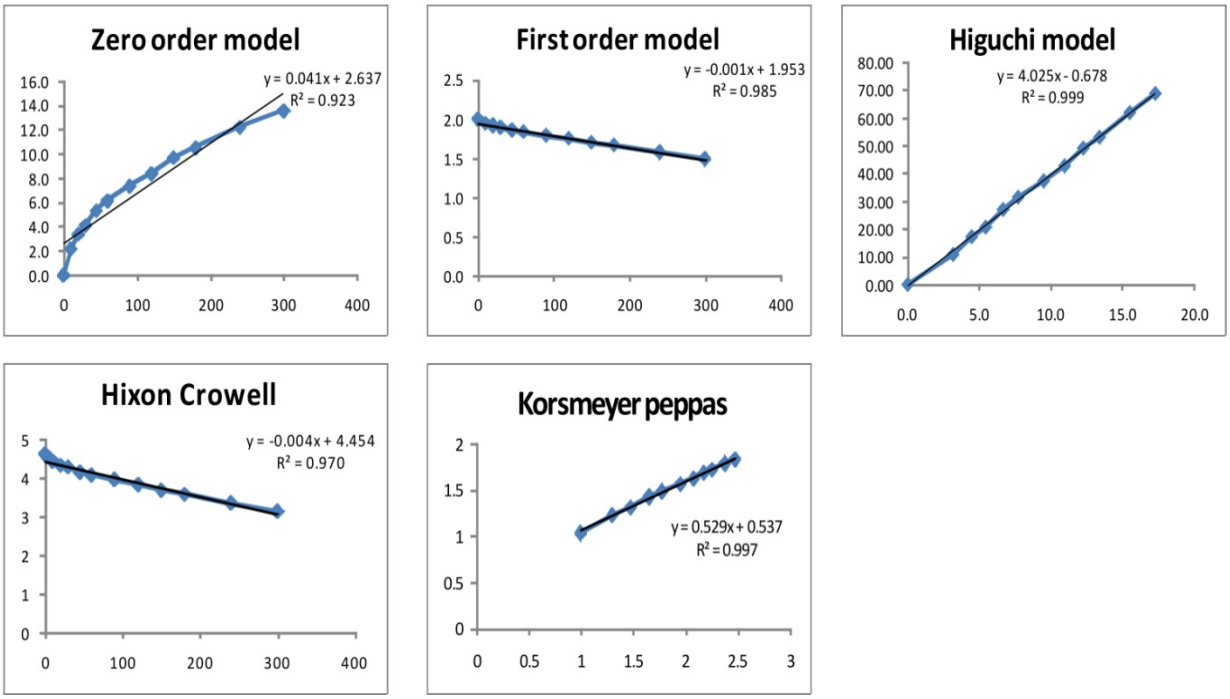


Figure 4.8.11. Release kinetics curves for DE SMEDDS in pH 6.8 phosphate buffer

Table 4.8.3. Regression coefficient of various *in vitro* release models for DE SMEDDS

Release models	0.01N HCl	pH 6.8 Phosphate Buffer	n' values for sphere geometry	Drug release mechanism
	R^2			
Zero order	0.923	0.923	0.43	Fickian diffusion
First order	0.921	0.985		
Hixson-Crowell	0.987	0.970	0.43<n<0.85	Non-fickian (anomalous) diffusion
Higuchi	0.999	0.999		
Korsmeyer-Peppas (Release component -'n')	0.998 (0.538)	0.997 (0.529)	0.85	Case-II transport

4.8.11.2.2. *In vitro* diffusion study of NISO SMEDDS

The *in vitro* diffusion profiles of NISO SMEDDS and plain drug suspension are as given in Figure.4. 8.12. The *in vitro* release studies showed significant increase in drug release as compared to plain drug suspension. In case of 0.1N HCl + 0.5% SLS the cumulative

percent drug released after 300 min was 97.71± 1.30% and 26.62± 2.91% from NISO SMEDDS and drug suspension respectively. This could be attributed to enhanced solubility and dissolution rate of NISO which in turn can be due to low globule size and surface properties of the prepared SMEDDS. Similarly, for pH 6.8 Phosphate buffer + 0.5% SLS, 98.84± 2.44% and 27.54± 3.21% drug was released from NISO SMEDDS and drug suspension respectively. The release profiles were then fitted into different exponential equations such as Zero order, First order, Higuchi, Hixon Crowell and Korsmeyer- Peppas to characterize the release. It was found that drug release from NISO SMEDDS in 0.1N HCl with 0.5% SLS and in pH 6.8 phosphate buffer with 0.5% SLS follows Higuchi (r^2=0.995 and r^2=0.993 respectively) more than other models as shown in figure 4.8.13 and 4.8.14 respectively. Value of *'n'* indicates that plain drug suspension and NISO SMEDDS formulation followed fickian diffusion as the release component values were between 0.43<n<0.85 (Table 4.8.4).

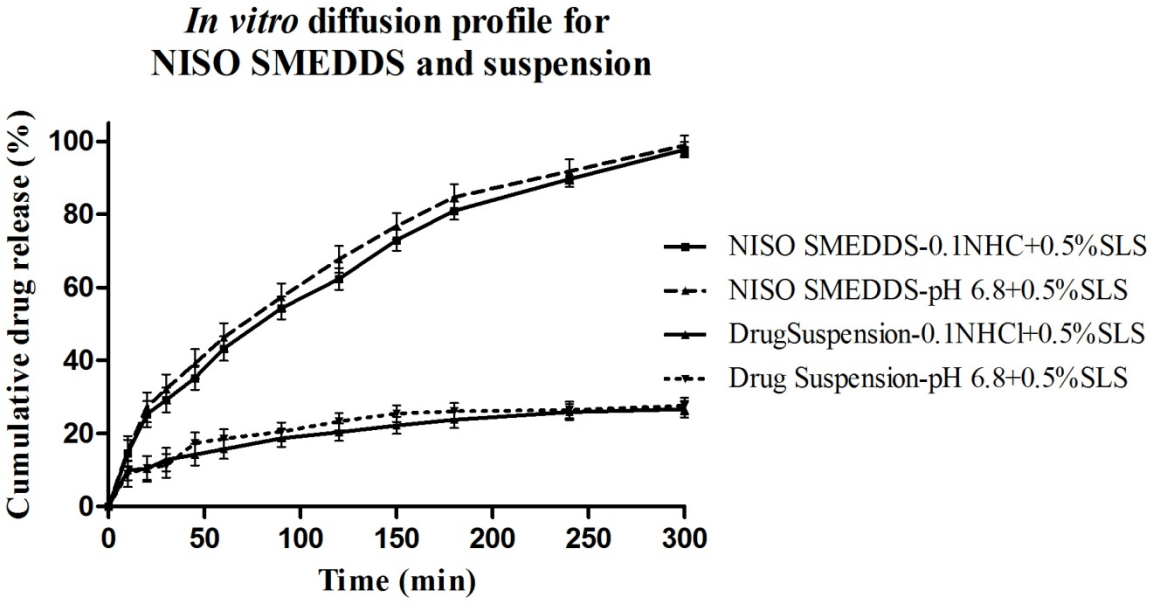


Figure 4.8.12 *In vitro* diffusion profile of NISO SMEDDS and drug suspension in 0.1N HCl with 0.5% SLS and pH 6.8 phosphate buffer with 0.5% SLS

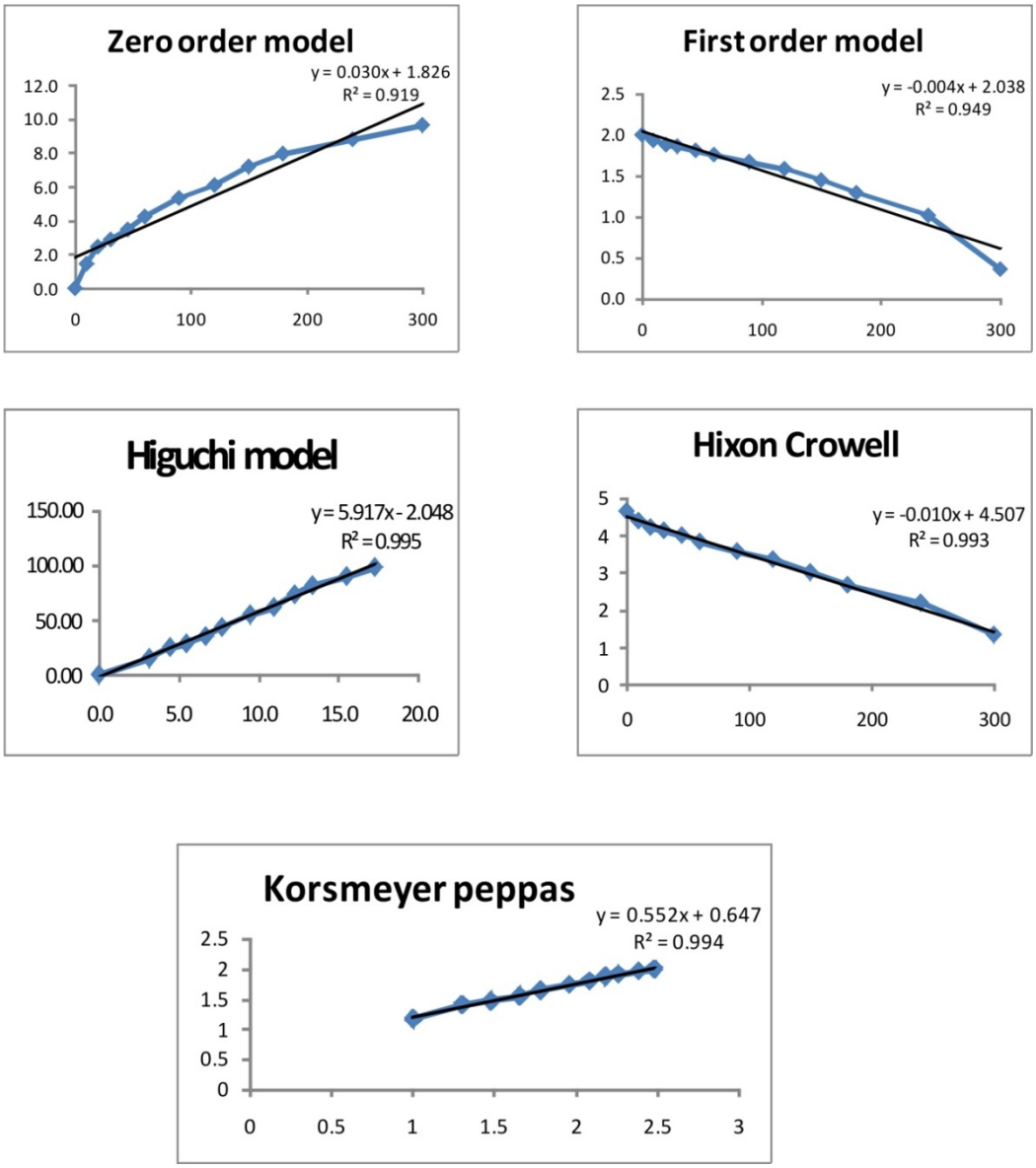
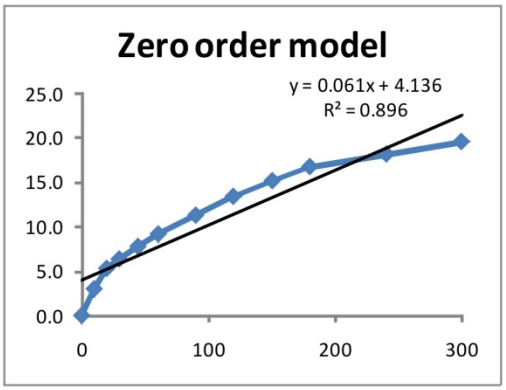
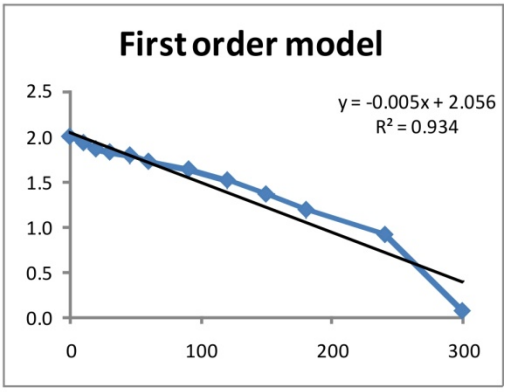
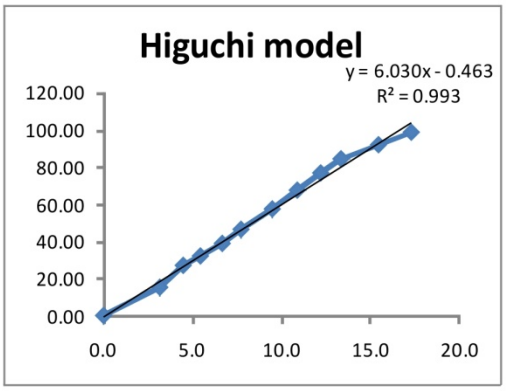
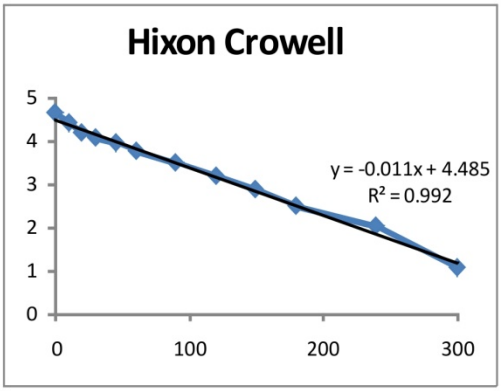


Figure 4.8.13. Release kinetics curves for NISO SMEDDS in 0.1N HCl with 0.5% SLS









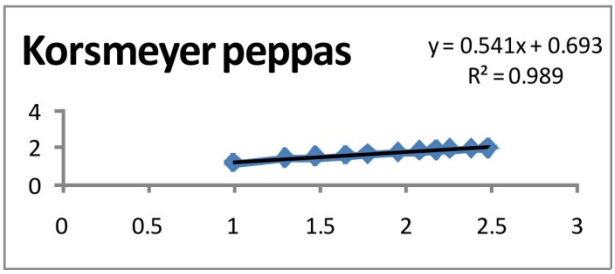


Figure 4.8.14. Release kinetics curves for NISO SMEDDS in pH 6.8 Phosphate buffer with 0.5% SLS

Table 4.8.4. Regression coefficient of various *in vitro* release models for NISO SMEDDS

Release models	0.1N HCl+ 0.5% SLS	pH 6.8 Phosphate Buffer + 0.5% SLS	n' values for sphere geometry	Drug release mechanism
	R^2			
Zero order	0.919	0.896	0.43	Fickian diffusion
First order	0.949	0.934		
Hixson-Crowell	0.993	0.992	0.43<n<0.85	Non-fickian (anomalous) diffusion
Higuchi	0.995	0.993		
Korsmeyer-Peppas (Release component -'n')	0.994 (0.552)	0.989 (0.541)	0.85	Case-II transport

4.8.11.3. *Ex vivo* release study

4.8.11.3.1. *Ex vivo* release study of DE SMEDDS

The cumulative % drug release of DE SMEDDS and DE suspension from rat stomach and intestine are shown in Figure 4.8.15. It was observed that 98.63± 3.21% drug diffused from the SMEDDS formulation in stomach after 300 min while only 31.38± 2.74% drug diffused from plain drug suspension. Thus, the amount of the drug diffused through the biological membrane increased when it was formulated as a SMEDDS. In Intestine, the drug diffusion was relatively slower than from stomach i.e. 69.34 ± 2.76% drug was diffused from the SMEDDS formulation and 10.65± 1.87% through plain drug suspension. This could be attributed to the higher solubility of DE (weak base) in acidic conditions [41].

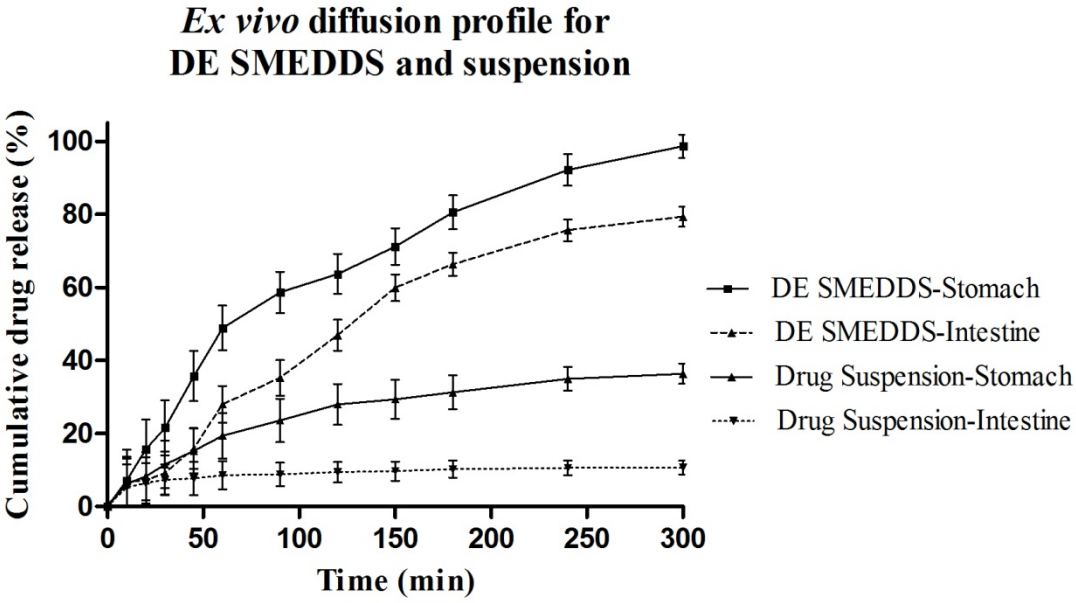


Figure 4.8.15 *Ex vivo* release study of DE SMEDDS and drug suspension in 0.01N HCl and pH 6.8 phosphate buffer

4.8.11.3.2. *Ex vivo* release study of NISO SMEDDS

The *ex vivo* release studies in stomach and intestine were performed to simulate the gastric emptying time and to study the release pattern of drug from SMEDDS in GI environment. Graphically, the release values are represented in Figure 4.8.16. In case of NISO SMEDDS, 96.23± 5.87% of drug was diffused through stomach while almost similar type of release was observed through intestine i.e. 95.14± 4.93% indicating that NISO had same release from the formulation irrespective of GI pH. But the release was found very less from plain drug suspension from stomach (14.54 ± 2.42%) as well as from intestine (11.25± 2.11%) when compared to prepared formulation. Hence, it can be noted that permeation of the drug was enhanced with SMEDDS, which fulfilled objective of increasing intestinal permeability for enhancing the bioavailability of drug.

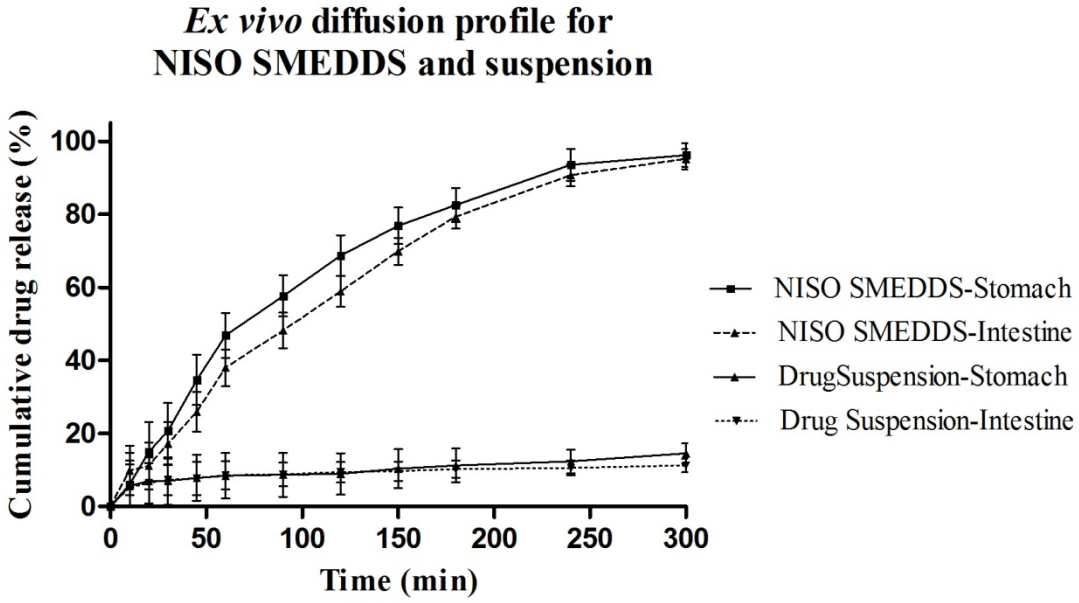


Figure 4.8.16. *Ex vivo* release study of NISO SMEDDS and drug suspension in 0.1N HCl and pH 6.8 phosphate buffer

4.9 Stability study

The optimized formulations of DE SMEDDS (Table 4.8.5) and NISO SMEDDS (Table 4.8.6) showed negligible change under the conditions of storage for parameters in terms of globule size, % Transmittance and drug content. The data suggested that the formulation was stable for 6 months at 25 ± 2^0C/60 ± 5% RH and 40 ± 2^0C/75 ± 5% RH conditions.

Table 4.8.5: Results of stability studies for DE SMEDDS

Time (months)	Long term study (25 ± 2°C/60% ± 5% RH)			
	Physical Description	**Globule size (nm)**	**%Transmittance**	**Drug content (%)**
Initial	clear liquid	74.68± 2.13	99.73± 1.14	96.58± 1.88
1 month	clear liquid	79.85± 2.13	99.62± 2.41	97.11±2.55
3 months	clear liquid	71.54± 2.45	99.11±2.12	96.78±2.42
6 months	clear liquid	76.36± 2.32	99.42± 3.11	95.34± 1.97
	Accelerated Study (40 ± 2°C/75% ± 5% RH)			
1 Month	clear liquid	75.34± 1.12	99.61± 2.05	97.21± 2.13
2 months	clear liquid	75.26± 1.32	99.31± 2.76	98.46±2.35
3 months	clear liquid	71.54± 2.46	99.12± 2.12	96.74± 2.42
6 months	clear liquid	72.36± 3.22	99.32± 1.89	95.97± 1.96

Table 4.8.6: Results of stability studies for NISO SMEDDS

Time (months)	Long term study (25 ± 2°C/60% ± 5% RH)			
	Physical Description	**Globule size (nm)**	**%Transmittance**	**Drug content (%)**
Initial	clear yellow liquid	16.78± 0.91	99.23± 2.68	98.21±1.68
1 month	clear yellow liquid	21.54± 1.21	99.32± 2.18	97.54± 1.89
3 months	clear yellow liquid	15.54± 1.43	99.22±2.37	97.25±1.55
6 months	clear yellow liquid	16.82± 2.31	99.43±2.55	98.12±1.43
	Accelerated Study (40 ± 2°C/75% ± 5% RH)			
1 Month	clear yellow liquid	25.34± 1.12	98.51± 3.14	95.42± 2.53
2 months	clear yellow liquid	19.46± 1.32	99.75± 2.96	98.76±1.99
3 months	clear yellow liquid	21.54± 2.44	98.91± 1.89	96.79± 2.47
6 months	clear yellow liquid	18.36± 3.23	99.32± 2.02	94.77± 1.86

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