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Development and Optimization of Self-Nanoemulsifying tablet dosage form of Nateglinide using Box–Behnken design

Shivaraj B. Katageri, Rambabu Sharma

Department of Pharmaceutical Sciences, Jodhpur National University. Jodhpur, India

ABSTRACT:

The current study was aimed to investigate the potential of solid self-nanoemulsifying drug delivery system (S-SNEDDS) composed of Capmul MCM C8 (oil), Cremophor RH40 (surfactant) and transcutol P (co-surfactant) in improving the dissolution and oral bioavailability of Nateglinide (NTG). Liquid self-nanoemulsifying drug delivery systems (L-SNEDDS) were developed by using rational blends of components with good solubilizing ability for NTG which were selected based on solubility studies, further ternary phase diagram was constructed to determine the self-emulsifying region. The prepared L-SNEDDS formulations were evaluated to determine the effect of composition on physicochemical parameters like rate of emulsification, clarity, phase separation, thermodynamic stability, cloud point temperature, globule size and zeta potential. In vitro drug release studies of optimized L-SNEDDS showed almost 96.76±1.4% within 45 min. The globule size analysis revealed the formation of nanoemulsion (130 ± 1.6nm) from the optimized L-SNEDDS formulation. Optimized L-SNEDDS was incorporated into tabletting excipients to make optimized self-nanoemulsified tablet formulation. A three factor, three-level Box–Behnken design was used for the optimization procedure, with the amounts of X1 (maltodextrin), X2 (Kollidon VA 64), and microcrystalline cellulose (X3) as the independent variables, while Flowability index (Y1), Friability (%) (Y2), Disintegration time (min) (Y3) and Cumulative % of NTG released after 45 min (%) (Y4) as responses. The optimization model predicted 99.48% % release with X1, X2 and X3 levels of 224, 100 and 111, respectively. A new formulation was prepared according to these levels. The observed responses were in close agreement with the predicted values of the optimized formulation.

KEYWORDS: Nateglinide, BCS class II, Self nanoemulifying drug delivery systems, maltodextrin, Kollidon VA 64, Optimization

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For Correspondence:
Shivaraj B. Katageri
Research Scholar,Department of Pharmaceutical Sciences, Jodhpur National University. Jodhpur, India.

(www.jpsbr.org)

INTRODUCTION:

Diabetes mellitus (type II) is growing as a major public health problem throughout the world and is associated with increased cardiovascular mortality, so an attempt has been made towards anti-diabetic treatments. Nateglinide (NTG), 3-phenyl- 2-[(4propan-2ylcyclohexane carbonyl) amino] propanoic acid, is an oral meal time glucose regulator. Nateglinide lowers blood glucose by stimulating the release of insulin from the pancreas by closing ATP-dependent potassium channels in the membrane of the β cells[1, 2]. In contrast to sulfonylureas, nateglinide increases pancreatic β cell sensitivity to ambient glucose without increasing basal insulin secretion. It can be used as monotherapy or in combination with metformin or thiazolidinediones. It has short half-life of 1.5 h, and peak plasma concentration reaches at 0.5–1.0 h. It is metabolized by cytochrome P-450 system to inactive metabolite and eliminated with half-life of 1.4 h. It is freely soluble in methanol, ethanol, and chloroform, soluble in ether,

sparingly soluble in acetonitrile, octanol, and practically insoluble in water[3-5]. Nateglinide is practically insoluble in water leading to poor dissolution and variable bioavailability upon oral administration. So, an attempt was made to increase the solubility of Repaglinide while formulating Self-nanoemulsifying drug delivery system (SNEDDS).

Lipid based formulations were chosen to overcome the above barriers and among them selfnanoemulsifying drug delivery systems (SNEDDS) have recently exhibited an intriguing role in oral delivery of highly lipophilic drugs due to ease of production, practical enhancement of drug solubility and oral bioavailability[6]. SNEDDS are preconcentrates composed of isotropic mixtures of oils, surfactants, co-surfactants which spontaneously form fine oil in water (o/w) emulsion *in situ* upon contact with aqueous medium with a globule size in the range of 20–200 nm[7]. Various other potential features of SNEDDS in enhancing oral bioavailability of lipophilic drugs consists of facilitating transcellular and paracellular absorption, reducing cytochrome-P450 metabolism in the gut enterocytes, promoting lymphatic transport via peyer's patches protects drug from hepatic first pass metabolism[8-11]. The major drawbacks of L-SNEDDS such as chemical instability, precipitation of drugs at storage temperature due to incompatibility of the volatile components of the formulation with gelatin capsule shell, leakage, portability, high production cost[12-14] were overcome by adsorbing them on to highly porous carriers without affecting self-emulsifying properties[16].

An important criterion that governs the quality of the dry adsorbed tablet dosage form is the release rate of the lipid-based formulation. Emulsion release rate is profoundly influenced by the physical and chemical attraction between the formulation and its adsorbing particles. Formulation ingredients, i.e. maltodextrin, Crospovidone and Microcrystalline cellulose (MCC), used in the preparation of the adsorbed tablet dosage form would have a great effect on emulsion release rate. To optimize the level of these ingredients, response surface methodology was used in this study for its effectiveness in demonstrating the interactions between these factors on producing the optimum dry adsorbed tablet dosage form. The statistical optimization designs have been documented for the formulation of many pharmaceutical solid dosage forms [16-18].

Crospovidone paste ground with suitable excipients produces granules of good flow properties that are readily available for direct compression. Maltodextrin was found to be a good excipient for its solubility, particle size and acceptable adsorbing properties. When compressed, however, given granules produce soft compacts, therefore, directly compressible microcrystalline cellulose (MCC) was blended with the granules to increase the hardness of the tablets. MCC is often regarded as one of the best excipients for direct compression[19]. Extragranular MCC was shown to increase dissolution rates and compressibility of tablets made by high shear granulation[20].

The objectives of the present work were (1) to investigate self-nanoemulsifying drug delivery system (SNEDDS), as potential drug delivery system for poorly water soluble drug Nateglinide (NTG) (2) to prepare and evaluate an optimized NTG self-nanoemulsified based solid dosage form. As part of the optimization process, the main effects, interaction effects and quadratic effects of the formulation ingredients were investigated.

MATERIALS AND METHODS

Materials

Nateglinide (NTG) was supplied by Intas Pharmaceuticals Ltd. (Ahmedabad, India). Among the vehicles, polyglycolyzed glycerides such as Capryol 90 (Propylene glycol monocaprylate), Labrafac CC (medium chain triglycerides), Labrafil M 1944 CS (Oleoyl macrogol-8 glycerides), Labrafil M 2125 CS (Linoleoyl macrogolglycerides), Labrasol (Caprylocaproyl macrogol-8 glycerides EP), Lauroglycol FCC (Propylene glycol monolaurate-type-I EP) and Transcutol P (Diethylene glycol monoethyl) were obtained as gift samples from Gatteffose (Saint-Priest Cedex, France). Acconon-E (Polyoxypropylene 15 stearyl ether), Capmul MCM C8 (Glyceryl monocaprylate), Capmul MCM L8 (Glyceryl Mono-dicaprylate1,2,3-propanetriol decanoic acid monoester), Capmul PG8NF (Propylene glycol monocaprylate), Caproyl microexpress (a mixture of PEG-6 caprylic/capric triglyceride, glyceryl caprylate/caprate, polyglycerol-6 dioleate,), Captex 200 (Propylene glycol dicaprylocaprate), Captex 355 (Capric triglyceride), and Captex 8000 (Glyceryl tricaprylate) were provided by ABITEC Corporations (Cleveland, USA). Cremophore EL was procured as a generous gift sample from BASF Corp. (Ludwigshafen, Germany). Tween 80 was purchased from

Merck (Mumbai, India). Neusilin US2 (Magnesium aluminometasilicate) was obtained as gift from Fuji Chemical Industry CO., Ltd. (Toyama, Japan). Dialysis membrane (DM-70; MWCO 10000) was purchased from Hi-media (Mumbai, India). All other chemicals used in this study and solvents were of analytical or HPLC grade respectively. Freshly collected double distilled water was used throughout the study.

HPLC analysis

HPLC analysis of NTG was determined using a reverse-phase isocratic Shimadzu HPLC system (Shimadzu, Kyoto, Japan) equipped with SPD-10 AVP UV/Vis detector (sensitivity of 0.005 absorbance units full scale, AUFS) and LC-10 AT solvent delivery unit. Chromatographic data were collected and processed using Agilent Chemstation software, The separation was conducted at ambient temperature, on a reversed phase ACE C18 column (150 x 4,6 mm; 5 µm particle size). All experiments were employed in the isocratic mode. The mobile phase was prepared by mixing acetonitrile and 0.05% trifluoroacetic acid (25:25, v/v) at a flow rate of 1.5 mL/min. The mobile phase was filtered through Millipore 0.45 µm membrane filter and degassed by sonication. Injection volume was set to 10 µL for the assay method. UV detection of the analytes was carried out at 210 nm[21].

Solubility studies

The saturation solubility of NTG in various vehicles (oils, surfactants, co-surfactants) was assessed using shake flask method (22). Briefly an excess amount of drug was mixed with 1 gm of chosen vehicles (Acconon E, Capmul PG 8 NF, Capmul MCM L8, Capmul MCM C8, Captex 355, Captex 200, Captex 8000, Caproyl 90, Caproyl Microexpress, Cremophor RH40, Labrafil M 1944 CS, Labrafil M 2125 CS, Labrasol, Lauroglycol, Labrafac CC, Transcutol-P and Tween 20, 80) in 5 ml clean glass vials with vortexing to aid the proper mixing of NTG with the vehicle. Then the stoppered vials were agitated for 48h at 37^0C in a shaking water bath. After equilibration all the samples were centrifuged at 10000 rpm for 15 min to remove the un-dissolved NTG from saturated solutions. Accurately measured quantities of supernatants were appropriately diluted with methanol and NTG concentration was quantified by HPLC system.

Preparation of L-SNEDDS

Based on the saturation solubility studies, vehicles with good solubilization capacity for NTG were selected as components (oil, surfactant and co-surfactant) of the L-SNEDDS formulation. A series of L-SNEDDS (Table 1) were prepared by varying oil, surfactant and co-surfactant composition. Accurately weighed quantities of oil, surfactant and co-surfactant were vortex mixed in a glass vial for 30 s to get a clear homogenous mixture. To this mixture, 60 mg of NTG was added in small increments with continuous vortex mixing to form a monophasic system. Then L-SNEDDS were stored in screw capped clean glass vials at room temperature until further evaluation.

Construction of ternary phase diagram

Various ratios of selected oil, surfactant and co-surfactant were plotted on a ternary-phase diagram to establish the stable spontaneous self-emulsification zone. A visual test reported by Craig et al., 1995 [22,23] with minor adaptation was conducted to assess the self-emulsification properties of prepared L-SNEDDS and ternary phase diagram was constructed using Tri plot v1-4 software based on the tendency to form emulsion, clarity, phase separation, coalescence of droplets and drug precipitation. In brief, L-SNEDDS (600 µL) was dropped in small quantities into distilled water (37°C; 300 mL) in a glass beaker with continuous mixing on a magnetic stirrer (100 rpm). Then the stability of formed emulsions was determined by visual observations such as extemporary emulsification, phase separation, drug precipitation, cracking of the emulsion on storage (48h) at room temperature. Poor or no emulsion formation with immediate coalescence of droplets with phase separation and drug precipitaion indicates formation of unstable emulsion. Further L-SNEDDS which formed stable clear emulsions were subjected to increasing dilutions (10, 100 and 1000) using distilled water and 0.1 N hydrochloric acid as mediums to evaluate the effect of dilution on stability of formed emulsions, which mimics *in vivo* gastric condition.

Thermodynamic stability studies and cloud point measurement

Stability of the prepared L-SNEDDS formulations at various stress conditions was evaluated by heating cooling cycles (4°C and 40°C) and freeze thaw cycles (-21^0C and +25^0C) with storage at specified temperature for 48h. For centrifugation stress, the L-SNEDDS

formulations were diluted with distilled water (1:100) and centrifuged at 3500 rpm for 15 min and visually observed for any phase separation[24]. The cloud point temperature of the diluted L-SNEDDS formulation (10 mL) was determined by gradual heating on a water bath and the temperature at which cloudiness appears was denoted using thermometer [25].

Determination of globule size and zeta potential

Mean globule size, polydispersibility index (PDI) and zeta potential of the emulsion formed from stable L-SNEDDS formulations (100 µL) on dilution with double distilled water (100 mL) were determined by photon correlation spectroscopy (PCS) using a Zetasizer (Nano ZS90; Malvern instruments Ltd., UK) with a 50 mV laser at a fixed angle of 90^0 at room temperature. The measurement time was 2 min and each run underwent 12 sub-runs. All the data obtained was the average of three determinations.

In Vitro Drug Release Studies

In vitro dissolution profile of liquid SNEDDS formulation were carried out using USP type II dissolution apparatus in 1000 mL of 0.01 N HCl with 0.5% (w/v) SLS maintained at 37°C ± 1°C and 50 rpm. At predetermined time intervals (10, 20, 30 and 45 min), aliquot (5 mL) samples were collected with replacement, filtered, diluted, and analyzed using HPLC method. Similarly, dissolution study was also conducted on pure drug in an analogous manner. A plot was made between cumulative percentage drug releases with respect to time (minute)[26].

Preparation of the solid-state self-nanoemulsified dosage form

The solid SNEDDS were formulated as per the experimental design employing a three-factor, three-level 3^3 Box– Behnken design (BBD) using Design-Expert 8.0.5 software (Stat-Ease Inc., Mineapolis, USA) by selecting the Amount of maltodextrin added (mg) (X_1), amount of Kollidon VA 64 (mg.), (X_2), and Amount of microcrystalline cellulose added (mg) (X_3) as independent variables, while Flowability index (Y_1), Friability (%) (Y_2), Disintegration time (min) (Y_3) and Cumulative % of NTG released after 45 min (%) (Y_4) as responses. Table 2 illustrates the factor levels selected from the phase diagram for the BBD.Response surface analyses were carried out to identify the effect of different independent variables on the observed responses. Nanoemulsion adsorbed granular material was obtained from a mixture of SNEDDS paste, Kollidon VA 64, Glucidex IT 12 and Avicel PH-112. SNEDDS was initially mixed with Kollidon VA 64 using mortar and pestle until a semisolid waxy paste was obtained. The mixture was then ground with Glucidex IT 12 in the mortar for 1 min to obtain the dry nanoemulsion based granules. Finally, Avicel PH-112 was added to the granules and blended in a V-blender for 5 min. The amount of copolyvidone, maltodextrin and MCC, added in each of the 17 formulations, to make a tablet containing 150 mg. of SNEDDS are given in Table 3.

Carr's flowability index

The flow properties of the solid state powdered emulsion were determined by the Carr's method.

The following four tests were measured: (1) compressibility; (2) angle of repose; (3) angle of spatula and (4) uniformity coefficient or cohesion. The flowability index (FI) was then calculated with the point scores as described [27].

Compressibility

The granular powder (10 g) was poured lightly into a 25 ml graduated cylinder. The powder was tapped until no further change in volume was observed. Powder bulk density and powder tapped density were calculated as the weight of the powder divided by its volume before and after tapping, respectively. Percentage compressibility was computed from the following equation:

% compressibility=100 (tapped density – bulk density) / tapped density.

Angle of repose

Angle of repose was measured using a protractor for the heap of granules formed by passing 10

g of the sample through a funnel at a height of 10 cm from the horizontal surface.

Uniformity coefficient

Uniformity coefficient was obtained by sieve analysis of 10 g of the powdered material using a sieve shaker. The sieve shaker was fitted with eight US standard sieves ranging in size from 0.075 to 1.7 mm and vibrated at a setting of 80 for 120 s. Uniformity coefficient was measured as the numerical value arrived at by dividing the width of the sieve opening that will pass 60% of the

sample by the width of sieve opening that will pass 10% of the sample.

Compaction of the solid state self-nanoemulsified dosage form

Nanoemulsion adsorbed compacts were prepared using rotary multistation tablet compression machine (Cadmach Ltd., Ahmedabad, India). Tablets were made by compressing the powder between the faces of the punch at a compaction pressure of 35 MPa. It was passed through sieve (24#) to achieve the uniformly free flowing self-nanoemulsifying granules (SNEGs). Finally, the SNEGs were compressed into tablets by direct compression using 8-mm flat circular punch, by addition of various tableting excipients like MCC as filler and disintegrant, Crospovidone as binder and Maltodextrin as solubility enhancer. The formulation composition of different batches of SNEGs and S-SNEDDS prepared are shown in Table 3.

Characterization of S-SNEDDS

The S-SNEDDS tablets prepared from different SNEGs were evaluated for hardness, weight variation, friability and disintegration time. Hardness measurement was carried out by Pfizer tester. Weight variation test was carried out using 20 tablets and determining their weight with the help of electronic balance. Friability was calculated by taking 20 tablets with the help of Roche's friability tester. Disintegration test was carried out in USP disintegration test apparatus using 1000 mL of 0.01 N HCl with 0.5% (w/v) SLS.

Comparative in Vitro Drug Release Studies

The comparative in vitro dissolution profile studies were carried out for S-SNEDDS, each containing NTG equivalent to 60 mg. The dissolution studies were carried out by using USP type II dissolution apparatus in 1000 mL of 0.01 N HCl with 0.5% (w/v) SLS maintained at 37°C ± 1°C and 75 rpm. At predetermined time intervals (10, 20, 30 and 45 min), aliquot (5 mL) samples were collected with replacement, filtered, diluted, and analyzed using HPLC method. A plot was made between cumulative percentage drug releases with respect to time (minute).

RESULTS AND DISCUSSION

HPLC analysis

For the development of the chromatographic method, the relevant assay for nateglinid tablets existing in USP Pharmacopoeia was used. However, by using the column reported in the pharmacopoeial method, retention time for nateglinide peak was reported as 10 minutes. By changing the brand of column (Waters to ACE) and mobile phase composition (acetonitrile and 0.05% trifluoroacetic acid ratio (23:27) to (25:25), (v/v)) of the method improved the retention time the peak was obtained at 7 minutes (Figure 1). Analysis was carried out in a shorter analysis time.

Solubility study

In the present study, non-ionic surfactants which were reported [28] to be less toxic compared to ionic surfactants, greater compatibility with biological tissues, less affected by change in pH and ionic strength throughout the GI tract were selected as vehicles. The solubility of NTG was determined in the screened vehicles to choose a suitable vehicle with maximum drug loading capacity and the results were shown in the Table 4. Apart from the drug solubility in the vehicles, mutual solubility of the selected vehicles is a crucial factor in the formation of stable L-SNEDDS formulation. Among the tested vehicles Capmul MCM C8 (312.43 ± 1.24 mg/ml), Cremophor RH40 (434.36 ± 2.12 mg/ml) and Transcutol P (376.76 ± 3.35 mg/ml) showed the highest drug solubilization capacity for NTG.

Based on the solubility results, Capmul MCM C8 was selected as the oil phase, Cremophor RH40 (a non-ionic solubilizer and emulsifying agent; Polyoxyl 40 hydrogenated castor oil) as surfactant and Transcutol-P (Diethylene glycol monoethyl ether) as co-surfactant.

Previous reports demonstrate that medium chain monoglycerides (polar lipids) like Capmul MCM C8 shows good solvent capacity for hydrophobic drugs and also promote water penetration and self dispersibility of lipid formulations upon hydration. Further, Capmul is likely to increase the interfacial fluidity of surfactant boundaries in the micelles because of the entrapment of Capmul in high HLB surfactant enhances the emulsification process upon dilution with aqueous medium [29]. The combination of surfactant and cosurfactant with high and low hydrophilic lipophilic balance (HLB) values results in the rapid formation of stable emulsion with fine emulsion globule size upon dispersion in water [30]. Hence

Cremophor RH40 (HLB 14 - 16) and Transcutol-P (HLB 4) were chosen as surfactant mixture in this study. Cremophor RH 40 is a non-ionic solubilizer and emulsifying agent obtained by reacting hydrogenated castor oil with ethylene oxide. It conforms to the current Ph. Eur./USP requirements. Transcutol-P used as co-surfactant forms more stable interfacial film with surfactants. It also decreases the fluidity of hydrocarbon region of the interfacial film and modify the film curvature, which promotes drug loading into the LSNEDDS, self-dispersibility properties and possesses penetration enhancement effect [31,32].

Construction of pseudo ternary phase diagrams

Based on the results of solubility studies, ternary phase diagram (Figure 2) of the Capmul MCM

C8 (oil), Cremophor RH40 (surfactant) and Transcutol-P (co-surfactant) was constructed to evaluate the self-emulsifying properties of the compositions and to determine the concentration range of components for formation of a clear nanoemulsion. In ternary phase diagram, the concentration of components was expressed as percent weight/weight (%w/w). The enclosed area in the phase diagram represents the region of self-emulsification. All the L-SNEDDS compositions exhibited good spontaneity of emulsification with emulsification time less than 60 sec. The colored region in the enclosed area indicates the formation of clear translucent fine oil in water emulsion upon gentle agitation. However L-SNEDDS compositions S1, S2, S6, S7 and S12 upon dispersion produced milky emulsions without any signs of drug or excipient precipitation. A higher concentration of surfactant mixture or lower concentration of oil resulted in formation of clear translucent emulsions with nanosized globules. This may be due to higher HLB value of Cremophor RH40 and solubilizing effect of Transcutol P. Right mixture of surfactants favorably adsorbed at interface and produces thermodynamically stable nanoemulsion by reducing the interfacial energy as well as providing a mechanical barrier to coalescence. The translucent emulsions formed were visually evaluated for clarity and stability after 48h at room conditions. All tested emulsions remained clear transparent even at the end of 48h. L-SNEDDS which produced stable clear transparent emulsions spontaneously were diluted with distilled water and 0.1 N HCl to 10, 100 and 1000 times. The resultant emulsions were also clear transparent without any phase separation and precipitation with both the media indicating stability of formed emulsions at various dilutions and pH conditions which mimics *in vivo* situation.

Thermodynamic stability studies and cloud point measurement

Thermodynamic stability study was conducted to identify and avoid the metastable LSNEDDS formulation. The L-SNEDDS formulations which produced translucent emulsions upon dispersion in distilled water and their emulsions were tested for stability at different temperatures and centrifugal stress conditions. All the tested L-SNEDDS formulations passed the thermodynamic stability studies without any signs of phase separation and precipitation during alternative temperature cycles (4°C and 40°C), freeze thaw cycles (-21^{0}C and +25^{0}C) and centrifugation at 3500 indicating good stability of formulations and their emulsions.

The cloud point is an essential parameter in the selection of a stable L-SNEDDS particularly when composed with non-ionic surfactants. The cloud point temperature (lower consolute temperature) indicates the temperature at which the transparent monophasic system was transformed into cloudy biphasic system as dehydrated surfactant molecules associated together as precipitate, which can affect the formulation adversely. Therefore, the cloud point for SNEDDS should be higher than body temperature (37^{0}C), which will avoid phase separation occurring in the gastrointestinal tract. The cloud point temperature of the tested L-SNEDDS was found to be in the range of 75-85^{0}C. Therefore, it would suggest that the developed formulation do not requires a precise storage temperature and it develops a stable emulsion upon administration at physiological temperature in vivo.

Globule size analysis and zeta potential

Globule size and size distribution of the emulsion are key parameters which influences in vivo stability of emulsion developed from L-SNEDDS after oral administration. Globule size of the emulsion also affects rate of drug release and absorption, as drug diffusion is faster from smaller globules with large surface area. Globule sizes and poly dispersity index values of the L-SNEDDS formulations which produced translucent emulsions upon hydration with double distilled water are summarized in Table 5. Globule sizes were found to be in the range of 50-250 nm, which indicated that globules are in nanometric size range. In conventional self-

emulsifying drug delivery systems, the amount of free energy required to form an emulsion is very low, thereby allowing the spontaneous formation of an interface between emulsion globules and the water. The variation in fatty acid carbon chain lengths of oil, surfactant and their degree of un-saturation plays a significant role in rapid self-emulsification with small globule size and stability of formed emulsion. Among the tested formulations, globule size of the emulsion developed from S5 formulation was 130 ± 1.6 nm with significantly very low PDI value (0.28 ± 0.015), indicating the narrow size distribution of the globules in the developed emulsion. Hence, L-SNEDDS (S5) was selected as optimized formulation for further evaluation and development of SSNEDDS. Zeta potential values of the emulsions produced upon dilution with double distilled water were found to be in the range of - 7 to -10.6 mV. Both the surfactant and cosurfactant used in this present study are non-ionic in nature and didn't contribute any charge to emulsion globules. However, the small negative zeta potential values of L-SNEDDS could be due to the ionization of free fatty acids and glycols present in the oil and surfactants which improves stability by preventing globule coalescence.

In-vitro drug release studies

In vitro drug release profile from pure drug dispersion and L-SNEDDS was observed using modified dialysis method. Figure 3 shows the highest drug release form L-SNEDDS formulation compared to pure NTG within 45 min. The release of drug from drug dispersion was significantly lower and showed only 18.96 ± 1.12% drug release in 45 min. Whereas L-SNEDDS showed almost 96.76±1.4% within 45 min. The optimized batch of L-SNEDDS showed remarkable improvement in dissolution rate as compared to pure drug, could be attributed to decrease in particle size and decrease in drug crystallinity. Such a pattern of drug release from L-SNEDDS by carrying entrapped drug in the form of fine emulsion droplets to the site of absorption is advantageous in increasing bioavailability, by enhancing release of poorly water soluble drug.

Preparation of the solid-state self-nanoemulsified dosage form

Experimental design

For the response surface methodology based on the Box–Behnken design, 17 experiments were required. Based on the experimental design, the factor combinations resulted in different NTG release rates. The range of the responses Y_4, the cumulative percent of NTG released from the self-nanoemulsified tablet dosage form and emulsified into the dissolution medium within 45 min, was 99.98% in formulation No. 6 (maximum) and 56.31% in formulation No. 9 (minimum). Mathematical relationship in the form of polynomial equation for the measured responses obtained with the statistical package Design expert (version 8) is listed in Table 6.

A total 17 formulations were prepared as per the experimental design and characterized for various dependent variables like Flowability index, Friability (%),Disintegration time (min) and Cumulative % of NTG released after 45 min (%) as shown in Table 2. The response surface analysis was carried out to understand the effect of selected independent variables on the observed responses. The mathematical relationships were established and coefficients of the second order polynomial equation Eq. 1, generated for responses were found to be quadratic in nature with interaction terms. The coefficients of the polynomials fit well to the data, with the values of R^2 ranging between 0.7874 and 0.9778 ($p<0.05$ in all the cases).

$$Y_i = b_0 + b_1 X_1 + b_2 X_2 + b_3 X_3 + b_{12} X_1 X_2 + b_{13} X_1 X_3 + b_{23} X_2 X_3 + b_{11} X_1^2 + b_{22} X_2^2 + b_{33} X_3^2 \quad \text{---------- (1)}$$

Finally, the model was observed for ANOVA ($p<0.005$), which revealed that the model terms for main effects and interaction effects were statistically significant. The ANOVA results are enumerated in Table 7.

Effect of formulation ingredients on dissolution rate

Emulsion release rate and the cumulative percent of NTG dissolved into the aqueous medium are important criteria that govern the quality of the solid-state self-emulsified dosage form. The extent of dissolution, however, is dependent on the reversible attraction and surface adsorption of NTG and the oily formulation onto the adsorbing powder. Therefore, physical properties of the ingredients used to prepare the solid compacts have a profound effect on the emulsion release rate. This relationship between the formulation ingredients (independent variables) and emulsion release rates (dependent variables) was elucidated using contour and response surface plots. The effect of X_1 (maltodextrin), X_2

(Kollidon VA 64) and their interaction on Y_4 (the cumulative percent of NTG dissolved in 45 min) at a fixed level of X_3 (250 mg of microcrystalline cellulose) are given in Figure 4 and 5.

At low levels of X_1, Y_4 is increasing from 59.58 to 74.52% when the amount of Kollidon VA 64 added (X_2) is decreasing from 300 to 100 mg. Similarly, at high levels of X_1, Y_4 is increasing from 60.32 to 80.32% when X_2 is decreasing from 300 to 100 mg. The decline in the efficacy of the tablets to release the self-emulsified formulation at high levels of Kollidon VA 64 can be explained as follows. Kollidon VA 64 holds the oily formulation by forming 'wax-like' granules that entrap the formulation within its matrix base rather than by surface adsorption. This is crucial in preventing emulsion separation, especially when formulating with eutectic based system as in SNEDDS. Eutectic-based delivery systems require close association of the eutectic agent with the drug. Increasing the amount of Kollidon VA 64 effectively reduces the concentration of oil, which was used as the eutectic agent, per unit area of the matrix. Besides, as the amount of Kollidon VA 64 increases it become less effective in absorbing the oily formulation and becomes incapable of producing matrix granules. This is similar to the aqueous-based granulation. Efficient granulation requires optimum amount of granulating fluid. In this case, the oily formulation acts as the granulating agent in what could be termed an 'oil based granulation'. As a consequence, an increasing amount of the absorbed emulsion becomes exposed to the subsequent layers of excipients and subjected to surface adsorption. Surface adsorption onto maltodextrin particles during the granulation process disrupts the emulsion and explains the decline in emulsion release rate.

At low levels of X_2, the amount of the formulation emulsified after 45 min is decreasing from 74.52 to 560.21% as X_1 is increasing from 150 to 450 mg. Similar trend was observed for the effect of X_2 and X_3 (amount of MCC added) and their interaction on Y_4. As seen from Figures 6 and 7, at low levels of X_3, Y_4 is decreasing from 94.65 to 80.65% as X_2 is increasing from 100 to 300 mg.

Similarly, at high levels of X_3, Y_4 is decreasing from 80.45 to 71.45 as X_2 is increasing from 100 to 300 mg. A decline in emulsion release rate was also observed with an increase in the amount of MCC (X_3) added to the formulations (Figs. 6 and 7). At low levels of X_2, Y_6 is decreasing from 99.98 to 71.65% as X_3 is increasing from 100 to 300 mg. MCC however, was not used during the granulation process. Rather, it was blended with the granules at a later stage in an attempt to increase the hardness of the compacts. Compaction of the powdered material and the

'squeeze-out' effect explains the decline in emulsion release rate with an increase in either X_1 or X_3. Any traces of the self-nanoemulsified formulation released from the granular matrix during

tableting will be adsorbed onto the surfaces of the fine MCC particles added to the formulation. Hydrophobic NTG particles that exist in their crystalline form within the eutectic formulation forms tight bonds with the hydrophobic surfaces of the insoluble MCC particles. Irreversible hydrophobic attraction between NTG and MCC during powder compaction causes variable release rates where the oily components of the formulation are emulsified into the aqueous medium at a faster rate compared to the release of NTG. During compaction however, 'squeezed out' formulation will be adsorbed on extragranular maltodextrin as well. This relationship between X_1 (maltodextrin) and X_3 (MCC) and their effect on Y_4 is given in Figures 8 and 9.

As previously discussed, surface adsorption onto insoluble MCC particles explains the decrease in Y_4, at low levels of X_1, from 94.65 to 60.2% as X_3 increases from 100 to 300 mg. Similarly, at high levels of X_1, Y_4 is decreasing from 80.65 to 64.98 % as X3 is increasing from 100 to 300 mg. Maltodextrin, however, is soluble in water. Therefore, the effect of maltodextrin on emulsion release rate is less significant compared to the effect of MCC. This explains the decline in Y_4 at low levels of X_3, from 94.65 to 80.45% as X_1 increases from 150 to 450 mg. At high levels of X_3 however, MCC becomes the dominant adsorbing agent during powder compaction. This explains the release of only 59.65% of the formulation at low levels of X_1. Increasing the amount of maltodextrin added at high levels of X_3 diverts some of the exuded formulation onto the soluble maltodextrin particle, thereby increasing the amount of NTG released to 64.98%.

Optimization of the formulation ingredients

After generating the polynomial equations relating the dependent and independent variables, the process was

optimized for the response Y_4. Optimization was performed to obtain the levels of

X_1–X_3, which maximize Y_4 at constrained conditions of Y_1 through Y_3. Formulation ingredients were optimized to obtain compacts that would maximize the amount of the self-nanoemulsified formulation released within 45 min. Constraints were made in an effort to obtain an optimized formulation with an improved flow, friability, disintegration and compaction properties. Finally, the optimized formulation was selected by numerical optimization method from the Design-Expert 8.0.5 having the desirability value as 0.992. The composition of the optimized formulation was found to be amount of Glucidex IT 12 (224 mg.), Amount of Kollidon VA 64 (100 mg.) and Amount of Avicel PH-112 (111 mg.) respectively and the values of dependent variables obtained are 47.65 Flowability index, 0.249 % Friability, 10.56 min. Disintegration time and 99.48% Cumulative % of NTG released after 45 min. with the total weight of tablet is 585 mg.

CONCLUSIONS:

Liquid self-emulsifying drug delivery systems (L-SNEDDS) composed of Capmul MCM C8 (16.6%), Cremophor RH40 (41.7%) and Transcutol-P (41.7%) was selected as optimized formulation as it has produced clear translucent nanoemulsion (136 nm) upon dispersion with water. Optimization of the solid self-nanoemulsified formulation of NTG was performed using Box–Behnken design. The amount of added maltodextrin (X_1), Kollidon VA 64 (X_2) and microcrystalline cellulose (X_3) showed a significant effect on the dissolution and release rate of the self-nanoemulsified formulation from their solid compacts, as well as on the physical and compaction properties of the dry emulsion-based tablet dosage form. The quantitative effect of these factors at different levels was predicted by using polynomial equations. Response surface methodology was then used to predict the levels of the factors X_1, X_2 and X_3 required to obtain an optimum formulation with minimum weight, friability and disintegration time and with a maximum flowability index value. A new formulation was prepared according to these levels. Observed responses were in close agreement with the predicted values of the optimized formulation, thereby demonstrating the feasibility of the optimization procedure in developing self-nanoemulsified based tablet dosage forms.

REFERENCES

1. Pathare D, Jadhav A, Shingare M. A Validated Stability Indicating LC Method for Nateglinide, Drug Development and Industrial Pharmacy, 2007;33(5):551-557.
2. McLeod J. Clinical Pharmacokinetics of Nateglinide, Clinical Pharmacokinetics, 2004;43(2):97-120.
3. Parfitt K, Martindale W. *Martindale* The complete drug reference. London, UK: Pharmaceutical Press; 1999.
4. Kinneary J, Budavari S, Heckelman P, Smith A. The Merk index, White House station, NJ: Merk Research Laboratories; 1996.
5. http://pubchem.ncbi.nlm.nih.gov/compound/nateglinide#section=Computed-Properties
6. Elnaggar Y, Abdallah, Gohar, Elsheikh. Nanoemulsion liquid preconcentrates for raloxifene hydrochloride: optimization and in vivo appraisal, International Journal of Nanomedicine, 2012:3787.
7. Porter C, Pouton C, Cuine J, Charman W. Enhancing intestinal drug solubilisation using lipid-based delivery systems, Advanced Drug Delivery Reviews,2008;60(6):673-691.
8. Porter C. Intestinal lymphatic drug transport: an update, Advanced Drug Delivery Reviews, 2001;50(1-2):61-80.
9. Balakrishnan P, Lee B, Oh D, Kim J, Hong M, Jee J et al. Enhanced oral bioavailability of dexibuprofen by a novel solid Self-emulsifying drug delivery system (SEDDS), European Journal of Pharmaceutics and Biopharmaceutics, 2009;72(3):539-545.
10. Date A, Desai N, Dixit R, Nagarsenker M. Self-nanoemulsifying drug delivery systems: formulation insights, applications and advances, Nanomedicine, 2010;5(10):1595-1616.
11. Balakumar K, Raghavan C, selvan N, prasad R, Abdu S. Self nanoemulsifying drug delivery system (SNEDDS) of Rosuvastatin calcium: Design, formulation, bioavailability and pharmacokinetic evaluation, Colloids and Surfaces B: Biointerfaces, 2013;112:337-343.
12. Oils and fats. Nutrition & Food Science, 2011;41(5).
13. Tuleu C, Newton M, Rose J, Euler D, Saklatvala R, Clarke A et al. Comparative bioavailability study in dogs of a self-emulsifying formulation of progesterone presented in a pellet and liquid form compared with an aqueous suspension of progesterone, Journal of Pharmaceutical Sciences, 2004;93(6):1495-1502.

14. Franceschinis E, Voinovich D, Grassi M, Perissutti B, Filipovic-Grcic J, Martinac A et al. Self-emulsifying pellets prepared by wet granulation in high-shear mixer: influence of formulation variables and preliminary study on the in vitro absorption, International Journal of Pharmaceutics, 2005;291(1-2):87-97.
15. Beg S, Jena S, Patra C, Rizwan M, Swain S, Sruti J et al. Development of solid self-nanoemulsifying granules (SSNEGs) of ondansetron hydrochloride with enhanced bioavailability potential, Colloids and Surfaces B: Biointerfaces, 2013;101:414-423.
16. Karnachi A. Box-behnken design for the optimization of formulation variables of indomethacin coprecipitates with polymer mixtures, International Journal of Pharmaceutics, 1996;131(1):9-17.
17. Singh S, Reddy I, Khan M. Optimization and characterization of controlled release pellets coated with an experimental latex: II. Cationic drug, International Journal of Pharmaceutics, 1996;141(1-2):179-195.
18. Wehrle P, Korner D, Benita S. Sequential Statistical Optimization of a Positively-Charged Submicron Emulsion of Miconazole, Pharmaceutical Development and Technology, 1996;1(1):97-111.
19. Lahdenp E, Niskanen M, Yliruusi J. Crushing strength, disintegration time and weight variation of tablets compressed from three Avicel PH grades and their mixtures, European Journal of Pharmaceutics and Biopharmaceutics, 1997;43(3):315-322.
20. Li J, Rekhi G, Augsburger L, Shangraw R. The Role of Intra- and Extragranular Microcrystalline Cellulose in Tablet Dissolution, Pharmaceutical Development and Technology,1996;1(4):343-355.
21. http://www.drugfuture.com/Pharmacopoeia/USP35/data/v35300/usp35nf30s0_m1927.html
22. Wang L, Dong J, Chen J, Eastoe J, Li X. Design and optimization of a new self-nanoemulsifying drug delivery system, Journal of Colloid and Interface Science, 2009;330(2):443-448.
23. Kallakunta V, Bandari S, Jukanti R, Veerareddy P. Oral self emulsifying powder of lercanidipine hydrochloride: Formulation and evaluation, Powder Technology, 2012;221:375-382.
24. Desai J, Khatri N, Chauhan S, Seth A. Design, development and optimization of selfmicroemulsifying drug delivery system of an anti-obesity drug, Journal of Pharmacy and Bioallied Sciences, 2012;4(5):21.
25.http://www.accessdata.fda.gov/scripts/cder/dissolution/dsp_SearchResults_Dissolutions.cfm?PrintAll=1
26. Schwedes J. Measurement of flow properties of bulk solids, Powder Technology, 1996;88(3):285-290.
27. Subramanian N, Ray S, Ghosal S, Bhadra R, Moulik S. Formulation Design of Self-Microemulsifying Drug Delivery Systems for Improved Oral Bioavailability of Celecoxib, Biol Pharm Bull, 2004;27(12):1993-1999.
28. Taha E, Al-Saidan S, Samy A, Khan M. Preparation and in vitro characterization of self-nanoemulsified drug delivery system (SNEDDS) of all-trans-retinol acetate, International Journal of Pharmaceutics, 2004;285(1-2):109-119.
29. Craig D. An investigation into the mechanisms of self-emulsification using particle size analysis and low frequency dielectric spectroscopy, International Journal of Pharmaceutics, 1995;114(1):103-110.
30. Craig D, Lievens H, Pitt K, Storey D. An investigation into the physico-chemical properties of self-emulsifying systems using low frequency dielectric spectroscopy, surface tension measurements and particle size analysis, International Journal of Pharmaceutics, 1993;96(1-3):147-155.
31. Cui S, Zhao C, Tang X, Chen D, He Z. Study on the bioavailability of puerarin from self-microemulsifying drug-delivery systems and tablets in rabbits by liquid chromatography-mass spectrometry, Biomed Chromatogr, 2005;19(5):375-378.
32. Ghosh P, Murthy R. Microemulsions: A Potential Drug Delivery System, Current Drug Delivery, 2006;3(2):167-180.

Figures:

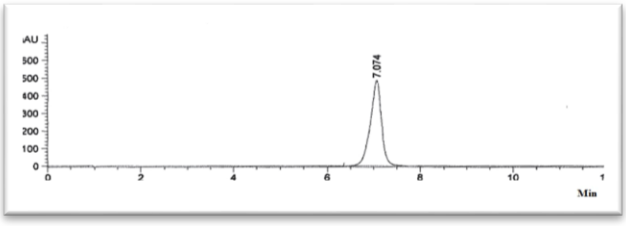

Figure 1: Typical Chromatogram of Nateglinide

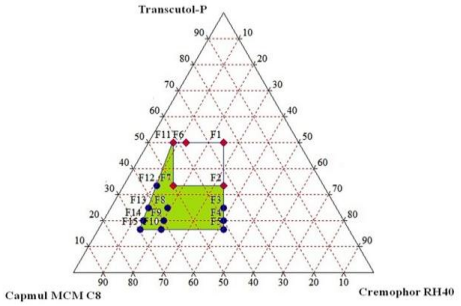

Figure 2: Pseudoternary phase diagram of liquid self-nanoemulsifying drug delivery systems (LSNEDDS)

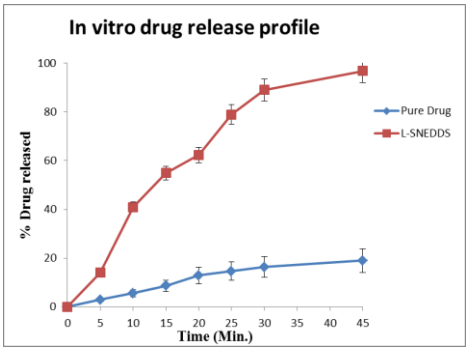

Figure 3: Compression of In vitro drug release profile from pure drug and L-SNEDDS

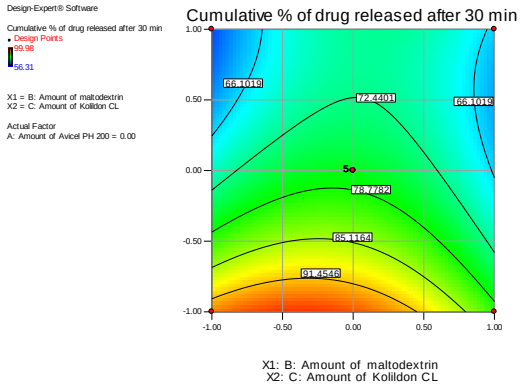

Figure 4: Contour plot showing the effect of the amount of maltodextrin (X_1) and Kollidon VA 64 (X_2) added on the response Y_4

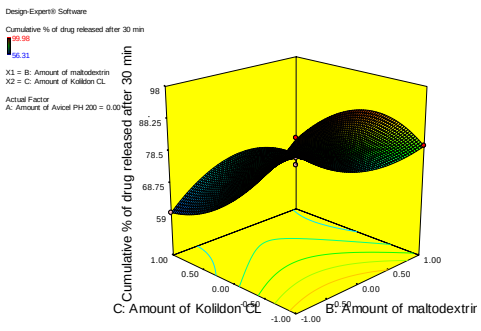

Figure 5: Response surface plot (3D) showing the amount of maltodextrin (X_1) and Kollidon VA 64 (X_2) added on the response Y_4

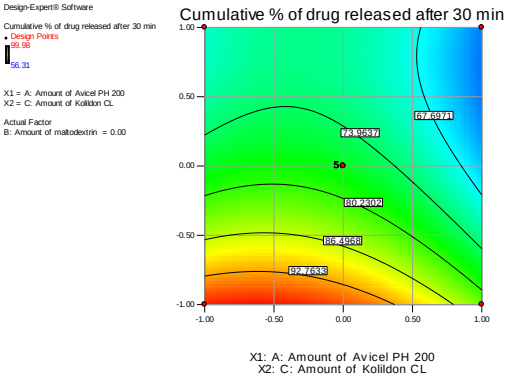

Figure 6: Contour plot showing the effect of the amount of Kollidon VA 64 (X_2) and microcrystalline cellulose (X_3) added on the response Y_4

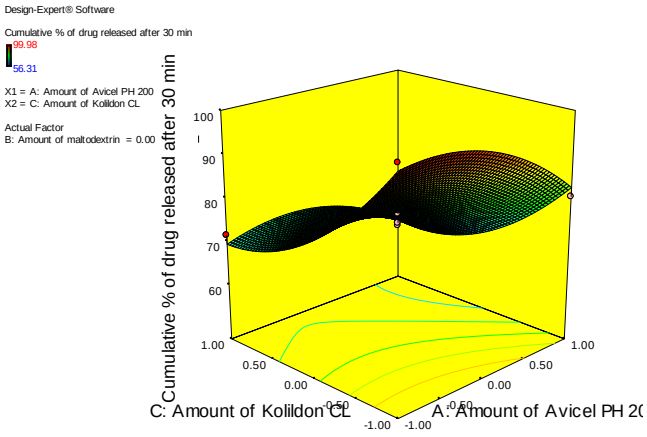

Figure 7: Response surface plot (3D) showing the effect of the amount of Kollidon VA 64 (X_2) and microcrystalline cellulose (X_3) added on the response Y_4

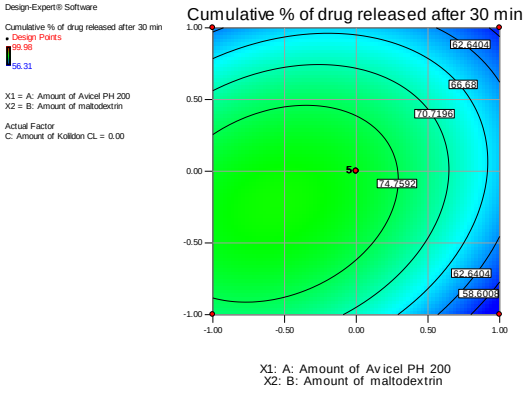

Figure 8: Contour plot showing the effect of the amount of maltodextrin (X_1) and microcrystalline cellulose (X_3) added on the response Y_4

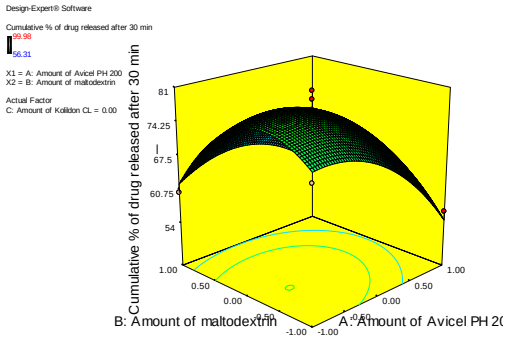

Figure 9: Response surface plot (3D) showing the effect of the amount of maltodextrin (X_1) and microcrystalline cellulose (X_3) added on the response Y_4

Tables:

Table 1: Composition of the NTG loaded liquid self-nanoemulsifying drug delivery systems (L-SNEDDS; % w/w) and evaluation parameters

Batch No	Ratio					
	S/CS	O/Smix	O(%)	S(%)	CS(%)	Appearance
S1	1:1	1:1	50	25	25	M
S2	1:1	1:2	33.3	33.3	33.3	M
S3	1:1	1:3	25	37.5	37.5	T
S4	1:1	1:4	20	40	40	T
S5	**1:1**	**1:5**	**16.6**	**41.7**	**41.7**	**T**
S6	3:1	1:1	50	37.5	12.5	M
S7	3:1	1:2	33.3	50	16.7	M
S8	3:1	1:3	25	56.3	18.7	T
S9	3:1	1:4	20	60	20	T
S10	3:1	1:5	16.6	62.6	20.8	T
S11	5:1	1:1	50	41.7	8.3	M
S12	5:1	1:2	33.3	55.6	11.1	T
S13	5:1	1:3	25	62.5	12.5	T
S14	5:1	1:4	20	66.6	13.3	T
S15	5:1	1:5	16.6	69.5	13.9	T

Table 2: Ranges of the Factors Investigated Using Box–Behnken Experimental Design

	Range		
Independent variables (factors)	**Low (-1)**	**Medium (0)**	**High (+1)**
X_1 = Amount of Glucidex IT 12 added (mg)	150	300	450
X_2 = Amount of Kollidon VA 64 added (mg)	100	200	300
X_3 = Amount of Avicel PH-112 added (mg.)	100	200	300

Table 3: Experimental Runs Obtained from Box–Behnken Design and Observed Responses

Run	X_1 Amount of Glucidex IT 12 added (mg)	X_2 Amount of Kollidon VA 64 added (mg)	X_3 Amount of Avicel PH-112 added (mg)	Y_1 Flowability index	Y_2 Friability (%)	Y_3 Disintegration time (min)	Y_4 Cumulative % of NTG released after 45 min (%)
1	-1	0	1	59	0.1	20.6	71.65
2	0	-1	-1	66.5	0.09	14.8	94.65
3	1	0	-1	58	0.28	16.35	80.65
4	1	1	0	56	1.8	21.36	59.58
5	1	0	1	57	1.65	6.1	60.32
6	-1	0	-1	35.6	0.09	10.3	99.98
7	0	-1	1	59.4	0.13	3.85	59.65
8	0	0	0	55	0.21	13.6	74.52
9	1	-1	0	60	0.26	12.64	56.31
10	0	0	0	52	0.22	12.5	76.65
11	0	0	0	54	0.16	18.65	74.54
12	-1	1	0	36	0.13	22.65	60.21
13	0	1	-1	55	0.12	17.85	80.45
14	-1	-1	0	28.9	0.09	15.64	71.45
15	0	1	1	54.6	0.56	8.67	64.98
16	0	0	0	52.65	0.3	11.56	78.65
17	0	0	0	56.35	0.29	12.34	80.32

Table 4: Solubility of NTG in various oils, surfactants and cosurfactants

Oils	Solubility (mg/g)	Surfactants
Paceol	198.12 ± 2.44	Labrafac CC
Lauroglycol FCC	87.54 ± 3.65	Labrafil M 1944 CS
Arachis oil	43.52 ± 2.62	Labrafil M 2125 CS
Captex 200	62.52 ± 1.65	Labrasol
Captex 355	45.65 ± 3.76	Acrysol K140
IPM	72.65 ± 2.14	Cremophor EL
Oilve oil	56.98 ± 2.63	**Cremophor RH40**
Castor oil	67.65 ± 3.44	Solutol HS15
Capryol 90	249.12 ± 1.62	Acrysol K140
Oleic acid	87.12 ± 1.15	Acrysol EL135
Miglyol 812	92.56 ± 3.75	Tween 20
Sefsol 218	78.32 ± 1.36	Tween 80
Coconut oil	62.89 ± 1.22	
Palm oil	43.12 ± 1.32	
Capmul MCM	**312.43 ± 1.24**	

Table 5: Evaluation parameters of selected batches of L-SNEDDS

Batch	Evaluation parameters			
	Cloud point (^{0}C)	Globule size (nm)	PDI	Zeta potential (mV)
S3	80	247 ± 1.12	0.32 ± 0.012	-7.5
S4	83	201 ± 1.54	0.56 ± 0.015	-8.3
S5	85	130 ± 1.6	0.28 ± 0.015	-10.6
S8	81	178 ± 2.34	0.13 ± 0.054	-9.3
S9	78	102 ± 2.5	0.76 ± 0.025	-7.0
S10	79	50 ± 1.23	0.87 ± 0.087	-7.8
S12	82	221 ± 1.87	0.42± 0.034	-8.9
S13	84	187 ± 1.6	0.34 ± 0.065	-8.3
S14	83	98 ± 1.98	0.71± 0.023	-9.54
S15	84	52 ± 3.32	0.67 ± 0.018	-10.2

Table 6: Regression equations for the responses

$Y_1 = 54 + 8.94X_1 - 1.65X_2 + 1.86X_3 - 2.77 X_1X_2 - 6.10 X_1X_3 + 1.68X_2X_3 - 7.63X_1^2 - 1.15X_2^2 + 6.02X_3^2$
$Y_2 = 0.24 + 0.45X_1 + 0.26X_2 + 0.23X_3 + 0.37X_1X_2 + 0.34X_1X_3 + 0.1X_2X_3 + 0.32X_1^2 + 0.015X_2^2 - 0.026X_3^2$
$Y_3 = 13.73 - 1.59X_1 + 2.95X_2 - 2.51X_3 + 0.43X_1X_2 - 5.14X_1X_3 + 0.44X_2X_3 + 3.19X_1^2 + 1.15X_2^2 - 3.59X_3^2$
$Y_4 = 76.94 - 5.80X_1 - 2.10X_2 - 12.39X_3 + 3.63X_1X_2 + 2.00X_1X_3 + 4.88X_2X_3 - 5.92X_1^2 - 9.13X_2^2 + 7.13X_3^2$

Table 7: ANOVA Results of Various Responses Using Experimental Design

ANOVA parameters	Y_1	Y_2	Y_3	Y_4
SS	276.61	0.26	60.79	28.22
df	3	3	3	3
MS	92.20	0.086	20.26	9.41
F value	29.88	25.20	2.50	1.44
P value	0.0034	0.0047	0.0019	0.0095
Std. deviation	6.42	0.20	3.65	2.78
R^2 value	0.8140	0.9369	0.7874	0.9778