International Journal for Pharmaceutical Research Scholars (IJPRS)

V-4, I-3, 2015 ISSN No: 2277 - 7873

RESEARCH ARTICLE

Development, Optimization and Characterization of Oral Solid Self-Nanoemulsifying Drug Delivery Systems (S-SNEDDS) of Repaglinide Tablets for Type – II Diabetes

Sharma R*, Katageri SB

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

Manuscript No: IJPRS/V4/I3/00167, Received On: 31/08/2015, Accepted On: 07/09/2015

ABSTRACT

The present studies entail formulation development of novel solid self-nanoemulsifying drug delivery systems (S-SNEDDS) of repaglinide for successful oral delivery, and evaluation of their in vitro. Preliminary solubility studies were carried out and pseudoternary phase diagrams were constructed using blends of oil (Caprypol 90), surfactant (Labrasol), and cosurfactant (Transcutol P). The SNEDDS were systematically optimized by response surface methodology employing 3^3-Box–Behnken design. The prepared SNEDDS were characterized for viscosity, refractive index, globule size, zeta potential, and TEM. Optimized liquid SNEDDS were formulated into free flowing granules by adsorption on the porous carriers like Aerosil 200, Sipernat 22S, Sylysia 350, Zeopharm 600, Neusilin US2, Neusilin UFL2 and compressed into tablets. In vitro dissolution studies of S-SNEDDS revealed 2.5 – 5-fold increased in dissolution rate of the drug due to enhanced solubility. Solid-state characterization of S-SNEDDS using FTIR, DSC and powder XRD studies confirmed lack of any significant interaction of drug with lipidic excipients and porous carriers. Further, the accelerated stability studies for 6 months revealed that S-SNEDDS are found to be stable without any change in physiochemical properties. Thus, the present studies demonstrated the solubility and may be bioavability enhancement potential of porous carriers based S-SNEDDS for a BCS class II anti diabetic drug, repaglinide.

KEYWORDS

Repaglinide, BCS Class II, Self Nanoemulifying Drug Delivery Systems, Porous Carrier, Solid State Characterization

INTRODUCTION

The oral medication is generally considered as the first avenue of investigation in drug discovery and development of pharmaceutical formulations predominantly because of patient acceptance, convenience in administration and cost effective manufacturing process. However, oral drug delivery may also get hampered for some of drug molecules that exhibit poor aqueous solubility.[1]

***Address for Correspondence:**
Shivaraj B. Katageri
Research Scholar, Department of Pharmaceutical Sciences,
Jodhpur National University. Jodhpur, India.
E-Mail Id: shivarajbkatageri@gmail.com

Nowadays, the use of high throughput screening in drug discovery has led to large proportions of new drug candidates having poor water solubility and hence poor and highly variable oral bioavailability. Majority of new drug candidates have poor aqueous solubility.[2] According to an FDA survey conducted between 1995 and 2001, only 9% of the new drug molecules belonged to BCS Class I category. By adopting different strategies, such as complexation with cylcodextrin, solid dispersion, and self-emulsifying drug delivery system, solubility and

thereby bioavailability of drugs can be improved.[3-6]

In recent years, considerable attention has been paid to develop lipid-based pharmaceutical preparations that amend the aqueous solubility and oral bioavailability of poorly water-soluble drugs. Self-nanoemulsifying drug delivery system (SNEDDS), a lipid based formulation is defined as isotropic mixtures of natural or synthetic oils, surfactants/solvents and co-surfactants/co-solvents.[7,8] SNEDDS is a promising technology that conveniently develops emulsion with gentle agitation, presents a high surface area for interaction between the formulation and the gastrointestinal fluid, offers a large solubilisation capacity and produces a small droplet size, which could facilitate permeation across the GI membrane.

The nanosized drug-loaded droplets of SNEDDS provide a large interfacial area thereby promote the rapid release of drugs. Regardless of this, SNEDDS are still liquid formulations with several disadvantages such as incompatibilities of drug with capsule material, low drug stability, drugs leakage and capsule ageing. SNEDDS is normally a liquid preparation encapsulated in soft gelatine capsules, which might be sensitive to humidity and results in high production cost. Furthermore, the liquid preparation might cause compatibility issues with the shell of the soft gelatine capsule and capsules that are inconvenient to the patient.[9-11]

In order to overcome potential problems mentioned above and combine advantages of SNEDDS with those of a dosage form, incorporation of liquid SNEDDS into solid carrier can be used. The solid forms of SNEDDS (S-SNEDDS) are able to offer the advantages of SNEDDS in combination with those of solid dosage forms such as production reproducibility, improved stability and patient compliance. In recent years low density porous carriers with large surface area composed of porous silica (Sylysia®) as well as magnesium aluminometasilicate (Neusilin®), precipitated silica, aluminium and calcium silicates (Sipernat 22S) are used in order to improve dissolution and bioavailability of poorly soluble drugs such as carvedilol, indomethacin.[12-15]

Repaglinide[16,17] is antidiabetic drug in the class of medications known as meglitinides.[18] Repaglinide is used to treat type II diabetes. Repaglinide depresses blood glucose concentrations by stimulating the release of insulin from beta cells of pancreatic islet tissue.

This is done by a selective ion channel mechanism. Repaglinide prevents adenosine triphosphate (ATP) potassium channels on the beta cell membrane and potassium efflux. The resultant depolarization and calcium influx induce insulin secretion. Repaglinide is oral antihyperglycemic agent in type II diabetes. Repaglinide is BCS class II drug and its oral bioavailability is 56%. Repaglinide is incompletely absorbed from the gastrointestinal tract and has low oral bioavailability because of the poor solubility. So, an attempt was made to increase the solubility of Repaglinide while formulating SNEDDS.

The aim of this study was to investigate solid self-nanoemulsifying drug delivery system (S-SNEDDS), as potential drug delivery system for poorly water soluble drug Repaglinide (RPG). Thus, the present work embarks upon development of optimized SNEDDS of RPG using rational blend of lipidic excipients, surfactants, and cosurfactants to enhance the oral bioavailability.

The concentration of oil and surfactant/cosurfactant mixture were varied from 10 to 90% v/v at all the Smix ratios (1:1, 1:2, 1:3, 2:1, 2:3, 3:4) to obtain the maximal region and to select best possible formulations with enhanced solubility and bioavailability. Further, drug-loaded SNEDDS were converted into free flowing solid granules (S-SNEDSS) using porous carriers like Aerosil 200, Sipernat 22S, Sylysia 350, Zeopharm 600, Neusilin US2 and Neusilin UFL2 by adsorption technique. The prepared S-SNEDDS formulations were characterized for in vitro physiochemical performance, solid-state compatibility, and stability studies in order to get a best possible formulation.

MATERIAL AND METHODS

Repaglinide was obtained as a generous gift sample from Torrent pharmaceuticals ltd., (Ahmedabad, India). Labrasol, Labrafac PG, and Labrafil M were gifted by Gattefosse (Saint Priest Cedex, France), Capryol 90, Cremophor RH40, Captex 200P, Captex 355, and Capmul MCM were gifted by Abitec (Janesville, USA). Sipernat 22S and Sylysia 350 were obtained as gift samples from from evonic industries and Fuji chemicals (Toyama, Japan), and Neusilin US2, UFL2 from Gangwal Chemicals (Mumbai, India). Various tableting excipients like Avicel 112, Aerosil 200, cross povidone, and magnesium stearate were purchased from FMC Biopolymer (Mumbai, India). Deionized double distilled water was used throughout the study obtained from Milli-Q-water purification system Millipore (Massachusetts, USA), while all other chemicals and reagents used were of analytical reagent grade.

Solubility Studies

The solubility of RPG was measured in numerous oils, surfactants and cosurfactants individually by shake flask method.

An excess amount of drug was introduced into 2mL of each excipient and these mixtures were sealed in glass vials. Each of the samples was subjected to vortex mixing on a vortexer (GeNei, Bangalore, India) for 5 min in order to facilitate initial mixing. Further, vials were charged on an environmental shaker bath (Tempo Instruments and Equipments Pvt. Ltd., Mumbai, India) for a period of 72 h at 37^0C with 300 rpm speed. After equilibrium for additional 72 h at 25^0C temperature, each vial was centrifuged at 10,000 rpm for 10 min using a centrifuge (Remi Laboratory Instruments, Mumbai, India).

The supernant of each sample was filtered through a membrane filter (0.45 mm) to remove any undissolved drug if present. The amount of drug in all samples was determined by their subsequent dilution with suitable solvent using HPLC.[19] The study was repeated in triplicate and their mean values were documented.[20,21]

Construction of Pseudoternary Phase Diagrams

The selected oil, surfactant, cosurfactant on the basis of solubility studies were used to develop the pseudoternary phase diagrams using water titration method. The various surfactant–cosurfactant (Smix) ratios were prepared using different proportions of surfactant and cosurfactant (1:1, 1:2, 1:3 and 2:3) to fulfil HLB value requirement (between 12 and 16) for formation of transparent clear solution. A series of oil/Smix mixtures were prepared at all nine combinations (1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2 and 9:1) and titrated with water to identify the nanoemulsion region. The total water consumed was noted in terms of w/w and during titration oil–Smix ratio and observations were made for phase clarity. The concentration of water at which turbidity-to-transparency and transparency-to-turbidity transitions occur were derived from the weight measurements. These values were used to determine the boundaries of the nanoemulsion region corresponding to the selected value of oil and Smix ratio. Phase diagrams were constructed using PRO SIM Ternary phase diagram software (STRATEGE, Cedex, France).

Formulation and Optimization of SNEDDS

The liquid 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 volume (microliter) of Capryol 90 (X_1), Labrasol (X_2), and Transcutol P (X_3) as independent variables, while self-emulsification time in sec (Y_1), percent drug release after 30 min (Y_2), and globule size in nanometer (Y_3) as responses. Response surface analyses were carried out to identify the effect of different independent variables on the observed responses. Table 1 illustrates the factor levels selected from the phase diagram for the BBD. A fixed dose of the drug was admixed with the oil, surfactant, and cosurfactant at ambient temperature with continuous stirring in a vortex mixer (Remi, Mumbai, India) to achieve complete

solubilization in the formulation components to obtain a homogeneous mixture. Table 2 illustrates the experimental runs obtained from Box Behnken design and their observed responses. The responses were statistically evaluated using ANOVA procedure. Further, the optimum formulation was selected by the numerical optimization procedure using the desirability function.

Table 1: Ranges of the Factors Investigated Using Box–Behnken Experimental Design

Independent variables (factors)	Range		
	Low (-1)	Medium (0)	High (+1)
X_1 = Amount of Capryol 90 (µL)	100	120	140
X_2 = Amount of Labrasol (µL)	150	160	170
X_3 = Amount of Transcutol P (µL)	150	160	170

Characterization of SNEDDS

Dispersibility Studies and Self-Emulsification Time

The dispersibility studies were carried out to observe the self-emulsification efficiency and self-emulsification time (SEF time). One millilitre of each of the SNEDDS was added to 500 mL of distilled water in a glass beaker with gentle agitation by placing it on a magnetic stirrer at 50 rpm with temperature at 37°C. The process of self-emulsification was visually monitored for the rate of emulsification and appearance of produced microemulsion.

Globule Size

One milliliter of the formulation was taken in a volumetric flask and diluted with 20 mL water with gentle mixing. The globule size of the resultant emulsions was determined using Malvern Particle size analyzer (Zetasizer, Model Nano75 Malvern, UK).

In Vitro Drug Release Studies

In vitro dissolution profile of liquid SNEDDS formulation were carried out using USP type II dissolution apparatus in 900 mL of citro phosphate buffer pH 5 maintained at 37°C ± 1°C and 75 rpm. At predetermined time intervals (5, 10, 15, 20 and 30 min), aliquot (5 mL) samples were collected with replacement, filtered, diluted, and analyzed using UV visible spectrophotometer at λmax 283 nm. 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).

Thermodynamic Stability

The liquid SNEDDS were subjected to six refrigerator cycles between temperatures of 4°C to 45°C with storage of not less than 48 h in a heating–cooling incubator (Remi). Formulations stable at these temperatures were further subjected to centrifugation test at 3,500 rpm for 30 min using 12C micro-centrifuge (Remi). Formulations which did not show any phase separation upon centrifugation were taken for freeze thaw stress test by three cycles between −21°C and 25°C temperature for 48 h. Based on the thermodynamic stability studies the SNEDDS formulations were selected.

Optical Birefringence

The optical clarity of the optimized liquid SNEDDS formulation was determined in terms of refractive index using Abbes' refractrometer (Mettler Toledo, Mumbai, India).

Viscosity

The viscosity of the optimized liquid SNEDDS was determined by placing 1 mL of the formulation in a Brookfield viscometer R/S CPS Plus (Brookfield Engineering Laboratories Inc., Middleboro, MA) using spindle C 50–1 at 25±0.5°C temperature. The spindle no. 50 was used at a speed of 70 rpm at shear stress of 430 per min and wait time was kept at 15 min.

Finally, shear rate produced as a parameter of viscocity was noted in terms of centipoise.

Table 2: Experimental Runs Obtained from Box–Behnken Design and Observed Responses

Run	X_1	X_2	X_3	Y_1	Y_2	Y_3
	Capryol 90 (µL)	**Labrasol (µL)**	**Transcutol P (µL)**	**self-emulsification time (SEF) in sec**	**percent drug release after 30 min**	**globule size in nanometer**
1	-1	-1	0	41	92	102
2	1	-1	0	26	88	107
3	-1	1	0	47	99	98
4	1	1	0	61	87	99
5	-1	0	-1	51	90	97
6	1	0	-1	24	94	110
7	-1	0	1	20	88	120
8	1	0	1	36	77	105
9	0	-1	-1	34	96	107
10	0	1	-1	30	90	106
11	0	-1	1	41	87	102
12	0	1	1	70	98	98
13	0	0	0	68	95	90
14	0	0	0	69	96	88
15	0	0	0	72	94	89
16	0	0	0	70	96	94
17	0	0	0	69	95	92

Table 3: Composition of S-SNEDDS Prepared Using SNEGs

Component(s)	**N1**	**N2**	**N3**	**N4**	**N5**	**N6**
SNEDDS (µL)	450	450	450	450	450	450
Aerosil 200 (mg)	**350**	---	---	---	---	---
Sipernat 22S (mg)	---	**220**	---	---	---	---
Sylysia 350 (mg)	---	---	**250**	---	---	---
Zeopharm 600 (mg)	---	---	---	**300**	---	---
Neusilin US2 (mg)	---	---	---	---	**260**	---
Neusilin UFL2 (mg)	---	---	---	---	---	**260**
Component(s) (mg.)	**D1**	**D2**	**D3**	**D4**	**D5**	**D6**
Avicel PH-200	208	338	308	258	298	298
Kollidon CL	30	30	30	30	30	30
Talc	12	12	12	12	12	12
Total (mg/tablet)	**600**	**600**	**600**	**600**	**600**	**600**

Transmission Electron Microscopy

The visual observation of the emulsion droplet was observed by transmission electron microscopy (TEM) (PHILIPS TECNAI 12, The Netherlands). The optimized liquid SNEDDS formulation was diluted with distilled water 1:25 and mixed by slight shaking, and a drop of the sample was placed on copper grid, stained with 1% w/v phosphotungstic acid solution for 30 s and visually observed under microscope.

Preparation of S-SNEDDS

The optimized liquid SNEDDS formulation was transformed into free flowing granules using various porous carriers like Aerosil 200, Sipernat 22S, Sylysia 350, Zeopharm 600, Neusilin US2 and Neusilin UFL2 as adsorbent due to their oil adsorption property.[22] The liquid SNEDDS formulation was poured onto the porous carriers placed in a small stainless-steel bowl, mixed, and wet granulation was performed with hand to obtain the homogeneous mass. 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 in a rotary multistation tablet compression machine (Cadmach Ltd., Ahmedabad, India), by addition of various tableting excipients like Avicel 112 as filler, Crosspovidone as superdisintegrant, and talc, magnesium stearate as lubricant. The formulation composition of different batches of SNEGs and S-SNEDDS prepared are shown in Table 3.

Optimization of S-SNEDDS

The S-SNEDDS prepared using different porous carriers were optimized based upon their oil adsorption capacity, micromeritic properties, and in vitro drug release. Oil adsorption capacity was determined by allowing the self-nanoemulsifying formulations to adsorb onto the porous carriers till it solidifies and estimating the drug content. Various micromeritic properties (bulk density, tapped density, angle of repose, Carr's index, and Hausner's ratio) of the prepared granules were determined. In vitro drug release from prepared S-SNEDDS tablets were determined by dissolution studies.

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 900 mL of citro phosphate buffer pH 5.

Drug Content Estimation

Liquid SNEDDS, SNEGs, and S-SNEDDS containing RPG, each equivalent to 2 mg was dispersed in suitable quantity of methanol. The samples were mixed thoroughly to dissolve the drug in citro phosphate buffer pH 5, centrifuged at 3,000 rpm for 15 min using 12C micro-centrifuge (Remi, Mumbai, India) to separate the undissolved excipients. The supernatant was suitably diluted and analyzed.

Comparative in Vitro Drug Release Studies

The comparative in vitro dissolution profile studies were carried out for pure drug, marketed preparation, optimized liquid SNEDDS and S-SNEDDS, each containing RPG equivalent to 2 mg. The dissolution studies were carried out by using USP type I basket dissolution apparatus in 900 mL of citro phosphate buffer pH 5 maintained at 37°C ± 1°C and 75 rpm. At predetermined time intervals (5, 10, 15, 20 and 30 min), aliquot (5 mL) samples were collected with replacement, filtered, diluted, and analyzed using UV visible spectrophotometer at λmax 283 nm. A plot was made between cumulative percentage drug releases with respect to time (minute).[23]

Fourier Transformed Infrared Spectroscopy

The drug–excipient compatibility study was carried out by Fourier transformed infrared (FT-IR) spectroscopy. The FT-IR spectra of pure RPG, optimized SNEDDS formulation, RPG-loaded self nanoemulsifying granules in Sipernat

22S and optimized S-SNEDDS tablet formulation D2 were carried out using KBr disk. The spectra were recorded employing the Shimadzu IR-affinity-1 FT-IR spectrophotometer (Shimadzu, Japan).

Differential Scanning Calorimetry

The samples of optimized SNEDDS formulation, RPG-loaded self nanoemulsifying granules and pure drug were subjected to differential scanning calorimeter (DSC-60, Shimadzu Corporation, Japan) which was previously calibrated with indium standard. Sample (5–10 mg) was hermetically sealed in an aluminum crucible and subjected to a purging of nitrogen gas at a flow rate of 50 mL/min. The heating was done in between 30 and 300^0C temperature a rate of 10^0C/min.

Powder XRD Studies

Powder X-ray diffraction studies were carried out for solid state characterization and to observe the crystallographic structure of pure drug and SNEGs. X-ray diffraction patterns of samples were recorded on Philips PW 17291 powder X-ray diffractometer (Jeol, Peabody, MA) using Ni-filtered, Cu kV radiation, a voltage of 40 kV and a 25-mA current. The scanning rate employed was maintained at 1°min−1 over the 10–40° 2θ range.

Accelerated Stability Studies

For accelerated stability studies, the optimized liquid SNEDDS and S-SNEDDS were stored at 40°C/75% RH for 6 months. Samples were withdrawn after specified time intervals (0, 1, 2, 3, and 6 months) and observed for SEF time, globule size, drug release in 15 min and disintegration time.

RESULTS AND DISCUSSION

Solubility Studies

One of the critical steps in the formulation of SNEDDS is selection of oil phase, since the oil is digested in the GI tract and may play a major role in determining rate and extent of dissolution.[22] In the present study, selection of oil for the preparation of SNEDDS was done on the basis of their aptitude to solubilize maximum amount of respective drug. This might be attributed to the fact that in SNEDDS drug should be in its dissolved state, as this form have been reported to possess greater concentration of drug. The high concentration gradient provides driving force for the permeation of drug through GI tract.[23] The comparative solubility studies of the drug in various oils, surfactants, and cosurfactants are reported in Table 4 respectively.

As portrayed from the table 4, among the oils, Capryol 90 (230.65 ± 2.65 mg/g), among the surfactants, Labrasol (260.42 ± 3.15 mg/g) and among the cosurfactants, Transcutol P (302.28 ± 4.25 mg/g) showed highest solubility for RPG. Hence, they were selected for phase titration studies for construction of pseudoternary phase diagrams.

Construction of Pseudoternary Phase Diagrams

The pseudoternary phase diagrams were constructed in the absence of drug to identify the self-emulsifying regions and to optimize the concentration of oil, surfactant, and cosurfactant in the SNEDDS formulations. Figures 1 depict the phase diagrams of the systems containing Capryol 90 as oil, Labrasol as surfactant and Transcutol P as cosurfactants.

It was observed that efficiency of emulsification was good when the Smix concentration was more than 70% of SNEDDS formulation. Further, increasing the concentration of surfactant increased the spontaneity of the self-emulsification process. However, it was also observed that emulsification was not efficient with less than 45% of Smix ratio.

The increase in cosurfactant decreases the region of emulsion formation, as cosurfactant have very little effect on reducing the interfacial tension directly rather they help the surfactants to reduce the interfacial tension. Based on the various combinations, Caprypol 90, Labrasol, and Transcutol P (1:1) were selected for formulation development, as the above Smix ratio showed highest area for nanoemulsion formation.

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

Oils	Solubility (mg/g)	Surfactants	Solubility (mg/g)	Cosurfactants	Solubility (mg/g)
Paceol	173.35 ± 1.25	Labrafac CC	63.56 ± 5.65	PG	244.56 ± 2.36
Lauroglycol FCC	56.36 ± 5.65	Labrafil M 1944 CS	95.65± 2.34	PEG 200	253.65 ± 6.2
Arachis oil	36.52 ± 2.62	Labrafil M 2125 CS	123.34 ± 2.34	PEG 400	256.69 ± 2.35
Captex 200	65.52 ± 1.65	**Labrasol**	**260.42 ± 3.15**	PEG 600	262.35 ± 3.36
Captex 355	39.65 ± 3.36	Acrysol K140	224.23 ± 1.26	**Transcutol P**	**302.28 ± 4.25**
IPM	68.65 ± 2.65	Cremophor EL	236.12 ± 1.37	Triacetin	128.65 ± 3.57
Oilve oil	48.98 ± 3.65	Cremophor RH40	243.36 ± 1.12		
Castor oil	53.35 ± 2.41	Solutol HS15	185.65 ± 3.3		
Capryol 90	**230.65 ± 2.65**	Acrysol K140	163.52 ± 2.12		
Oleic acid	98.65 ± 2.12	Acrysol EL135	179.32 ± 1.14		
Miglyol 812	88.56 ± 3.65	Tween 20	209.63 ± 4.5		
Sefsol 218	102.32 ± 3.36	Tween 80	194.65 ± 2.65		
Coconut oil	67.89 ± 2.35				
Palm oil	50.23 ± 3.32				
Capmul MCM	134.26 ± 4.5				

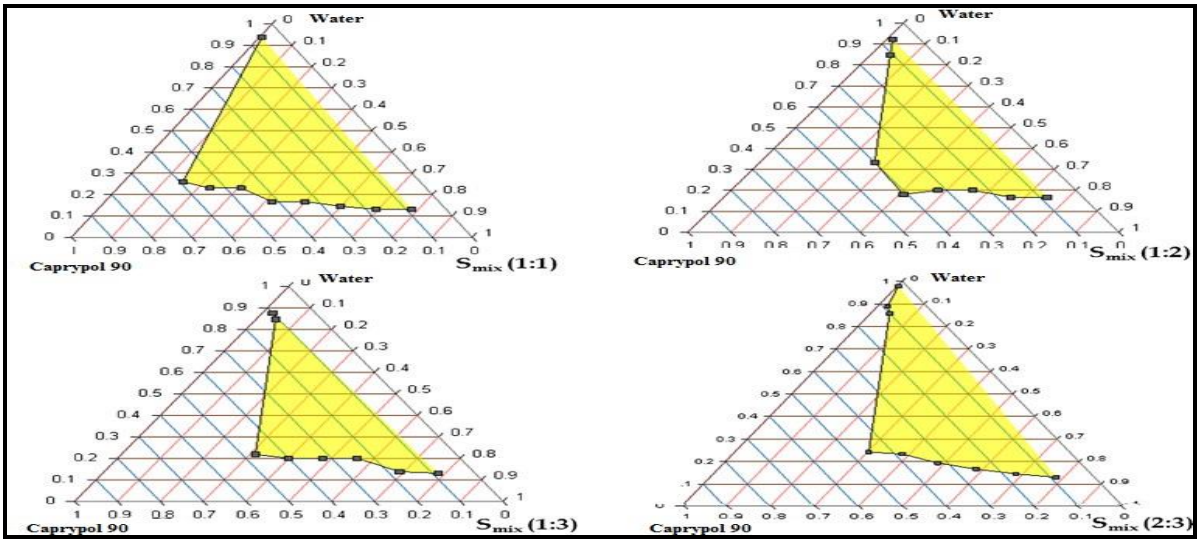

Figure 1: Pseudoternary phase diagram of systems containing 1:1, 1:2, 1:3, 2:3 ratio of S/CoS such as Labrasol and Transcutol P, Caprypol 90 as oil

Optimization of SNEDDS

A total 17 formulations were prepared as per the experimental design and characterized for various dependent variables like SEF time, % drug release in 30 min and globule size 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 SEF time, percent drug release in 30 min and globule size 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 0.9350 and 0.9942 ($p<0.05$ in all the cases).

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

Figure 2 (A–C) depicts the 3D-response surfaces for various response variables. Figure 2A depicts a dome-shaped response surface plot, characterizing initial increase in the SEF time with increasing the concentration of both oil (Capryol 90) and surfactant (Labrasol), followed by a gradual decrease. Hence, it can be revealed that at the intermediate levels of oil and surfactant, the SEF time was found to be maximum. Similarly, Figure 2B depicts a relationship between surfactant and co-surfactant on drug release. It was observed that at low concentration of oil, surfactant, and intermediate concentration of cosurfactant, percent drug release in 30 min was larger. Figure 2C portrays an interaction effect and relationship between oil (Capryol 90) and cosurfactant (Transcutol P) on the globule size as the response variable. A curvilinear plot was observed, where with increasing the amount of Capryol 90 and Transcutol P, a linear increase in the globule size was observed. Hence, at low levels of oil (Capryol 90) and cosurfactant (Transcutol P) and intermediate levels of surfactant (Labrasol), the globule size was ideal. All the response surfaces were best fitted with quadratic polynomial models, and able to predict the interaction effects too. 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 5. Finally, the optimized formulation was selected by numerical optimization method from the Design-Expert 8.0.5 having the desirability value as 0.95. The composition of the optimized SNEDDS formulation was found to be Capryol 90 (131 µL), Labrasol (150.00 µL), Transcutol P (157.50 µL), and RPG 2mg, respectively and the values of dependent variables obtained are 42.72 sec SEF time, 95 % drug release in 30 min. and almost 100 nm. of globule size.

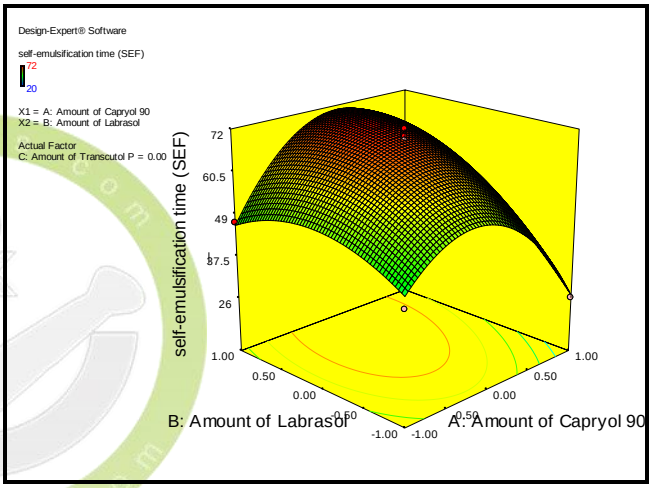

Figure 2A: Response surface graph representing the effect of Caprypol 90 and Labrasol on SEF time of SNEDDS

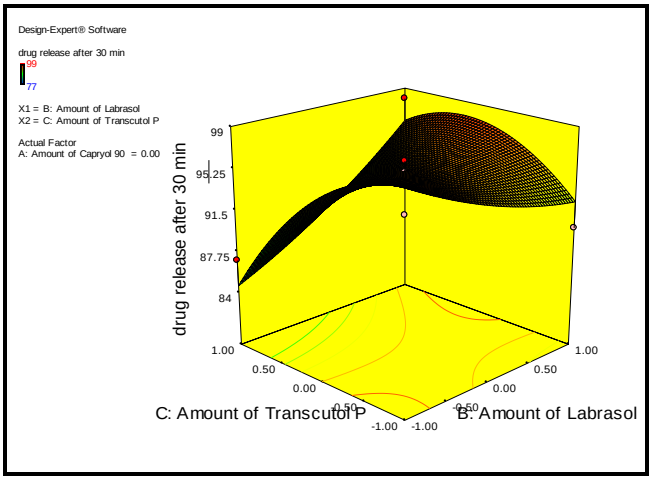

Figure 2B: Response surface graph representing the effect of Labrasol and Transcutol P on % Rel 30 min of SNEDDS

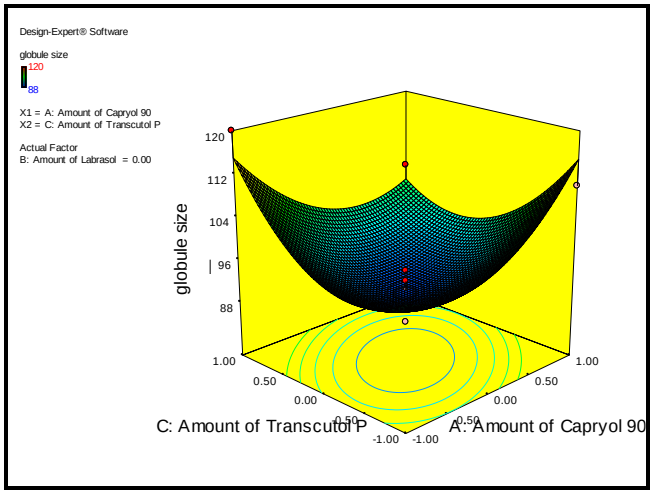

Figure 2C: Response surface graph representing the effect of Caprypol 90 and Transcutol P on globule size of SNEDDS

Table 5: ANOVA Results of Various Responses Using Experimental Design

ANOVA parameters	Y_1 (SEF time)	Y_2 (% drug release after 15 min)	Y_3 (globule size)
SS	5002.86	420.21	991.60
df	9	9	9
MS	555.87	46.69	110.18
F value	6.50	6.10	4.89
P value	0.0109	0.0131	0.0239
Std. deviation	9.24	2.76	4.74
R^2 value	0.9450	0.9417	0.9289

Characterization of SNEDDS

All the liquid SNEDDS formulation prepared as per the experimental design showed good self-emulsification efficiency and forms nanoemulsion immediately after dilution with aqueous phase within 70 sec. In vitro drug release studies showed almost 66% drug release in initial 150 min and complete drug release was observed within 30min. This was attributed due to solubility enhancement by selected lipidic excipients. Figure 3 represents the globule size distribution of liquid SNEDDS were found to be within the nanometric range (88– 120 nm). Refractive index and viscosity were found to be 1.428 ± 0.07 and 38.85 ± 1.57 cPs, which indicated that formulations were clear and transparent with good pour ability. TEM image of the optimized SNEDDS after dilution appeared as dark globules as shown in Figure 4.

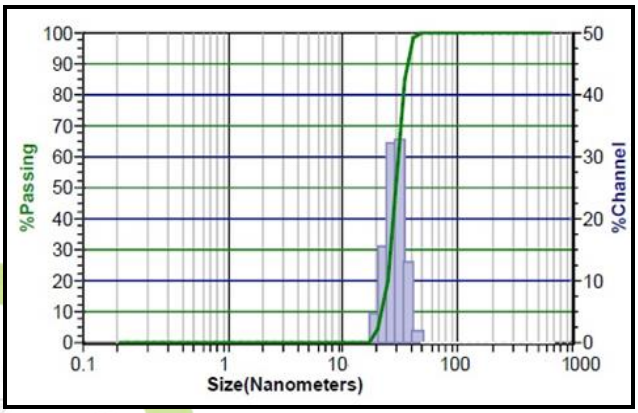

Figure 3: Particle size distribution of optimized liquid SNEDDS formulation

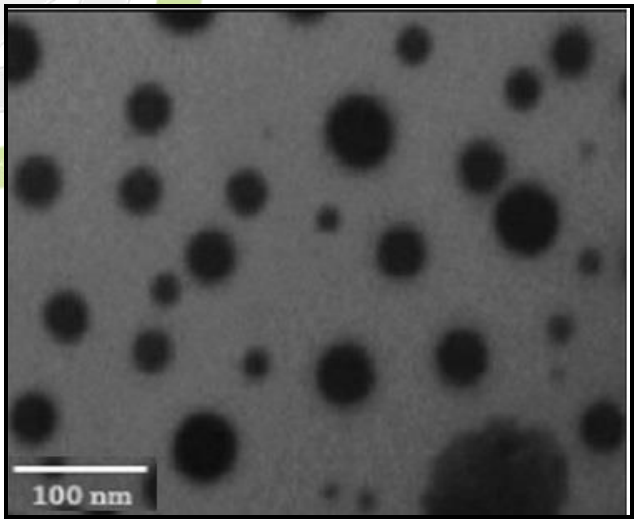

Figure 4: TEM image of the optimized liquid SNEDDS formulation representing the dark emulsion globules

Optimization of S-SNEDDS

The SNEGs prepared using porous carriers were optimized based on their oil adsorption capacity, faster drug release property, and flow characteristics. The micromeritic properties and

drug content of different granules indicating oil adsorption tendency of porous carriers as depicted in Table 6. All the batches of SNEGs showed good flow characteristics with Carr's index between 8.35 and 13.50, Hausner's ratio less than 1.25 and angle of repose (θ) between 26.35 to 34.12. Oil adsorption capacity was determined by drug content estimation showed that Sipernat 22S and Neusilin UFL2 containing granules showed highest oil adsorption tendency due to highly porous nature and presence of larger interparticulate void space in the particles, however, Aerosil 200-containing granules showed lowest oil adsorption tendency. Sipernat 22S is used extensively as a flow and anticaking agent in many applications. It is fine particle silica with high absorption capacity for liquids. Neusilin® UFL2 alone at 0.5% can resolve sticking issues of oily formulations. The in vitro dissolution studies of the S-SNEDDS revealed that all formulations showed faster drug release property with more than 85% drug release in first 15 min and complete drug release within 20 min. Among them Sipernat 22S -containing granules-based tablet formulations, D2 showed faster dissolution with highest drug release up to 100%. Thus, D2 was finally selected as the optimized S-SNEDDS formulation.

Table 6: Micromeritic Properties and Drug Content Estimation of SNEGs

SNEGs	Hausner's ratio	Carr's index	Angle of repose (θ)	% Drug content
N1	1.15 ± 0.056	13.5 ± 2.5	34.12 ± 3.5	76.35 ± 1.25
N2	1.10 ± 0.026	8.35 ± 3.12	26.35 ± 2.35	99.32 ± 0.60
N3	1.13 ± 0.026	11.26 ± 3.12	29.58 ± 1.29	97.56 ± 0.62
N4	1.14 ± 0.012	12.6 ± 1.65	33.26 ± 2.41	82.36 ± 2.26
N5	1.09 ± 0.020	9.18 ± 1.24	32.58 ± 1.34	98.64 ± 0.25
N6	1.07 ± 0.064	10.67 ± 2.31	29.41 ± 1.47	99.14 ± 0.34

Characterization of S-SNEDDS

The tablet hardness was found to be 7 kg/cm^2, friability was less than 1%, disintegration time was less than 1 min, and weight variation was found to be within the pharmacopoeial acceptance limits. This indicated immediate release nature of the prepared tablets due to the porous carriers.

Comparative In Vitro Drug Release Studies

Dissolution profile of pure drug RPG, marketed product, optimized liquid SNEDDS and S-SNEDDS (D2) was carried out using standard conditions mentioned in US Pharmacopoeia. The drug release studies depicted optimized liquid SNEDDS and S-SNEDDS formulations exhibited complete drug release almost up to 100% within 30 min vis-à-vis the pure drug and marketed product showed maximum drug release up to 20.7% and 42.5 % in 30 min, respectively (Figure 5). This confirmed that prepared S-SNEDDS formulations showed 2.5– 5-fold increase in the dissolution rate of RPG due to enhanced solubility. The faster drug release from SNEDDS is attributed due to spontaneous formation of nanoemulsion due to low surface free energy at oil–water interface, which causes immediate solubilization of drug in dissolution medium. During emulsification with water, oil, surfactant, and cosurfactant effectively swells and decreases; the globule size leads to decrease in surface area and surface free energy, thus eventually increases the drug release rate.[24] Further, upon deep investigation it has been revealed that drug release from S-SNEDDS was slightly slower initially in 10 min. compared to SNEDDS, because additional steps like disintegration of tablet into granules desorption of liquid SNEDDS from Sipernat 22S and interparticulate voids of porous carrier slows down the release process.[25] However, the difference in drug release between liquid SNEDDS and S-SNEDDS was not statistically significant ($p>0.1$). This confirmed that S-SNEDDS preserved the property of liquid SNEDDS. The mean dissolution time (MDT) was used to predict dissolution efficiency of prepared formulations. Lower MDT with

SNEDDS (MDT = 6.97 min) and S-SNEDDS (MDT = 7.23 min) indicated higher dissolution efficiency and faster solubilization of drug compared to pure drug (MDT = 45.12 min) and marketed product (MDT = 30.82 min). The in vitro drug release from S-SNEDDS prepared using porous carriers can be explained by the rapid desorption of the liquid SNEDDS from Sipernat 22S surface owing to the stronger physical interactions between silica and dissolution medium compared to Sipernat 22S and liquid SNEDDS.[26] Further, high specific surface area of these particles contributes to improved dissolution compared to pure drug. Additional parameters influencing dissolution rate of S-SNEDDS are the improved wetting of dispersion granules due to accelerated penetration of dissolution medium into the voids and capillaries of these granules. Mathematical modeling of dissolution data revealed that drug release from S-SNEDDS follows non-Fickian zero order kinetic. Other mechanisms including diffusion and convection of liquid SNEDDS when porous carriers come in contact with dissolution medium governs the drug release from S-SNEDDS.[27]

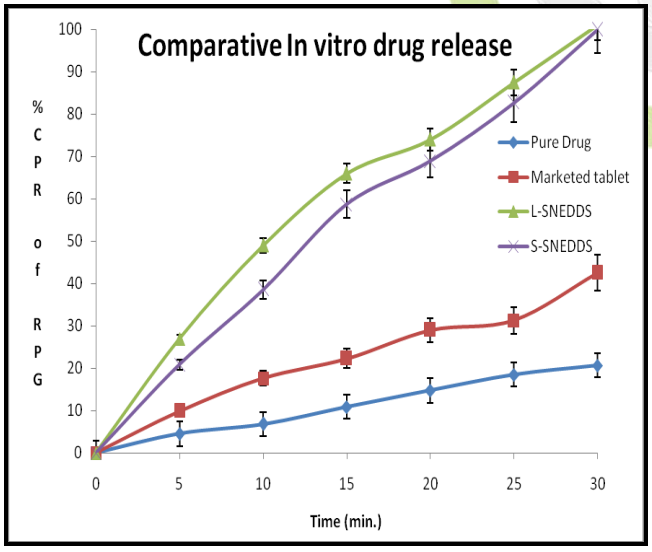

Figure 5: Comparative in vitro drug release profile of pure drug, marketed product, optimized SNEDDS and S-SNDDS formulations containing Sipernat 22S. Data represented are cumulative percent drug release versus time (minute) in terms of mean ± SD. (n = 3)

Fourier Transformed Infrared Spectroscopy

The FT-IR spectra of physical mixture of drug with various excipients observed no specific physiochemical interaction. There was no significant difference found in wave number (per centimeter) or functional group of the drug in all spectra (Figure 6).

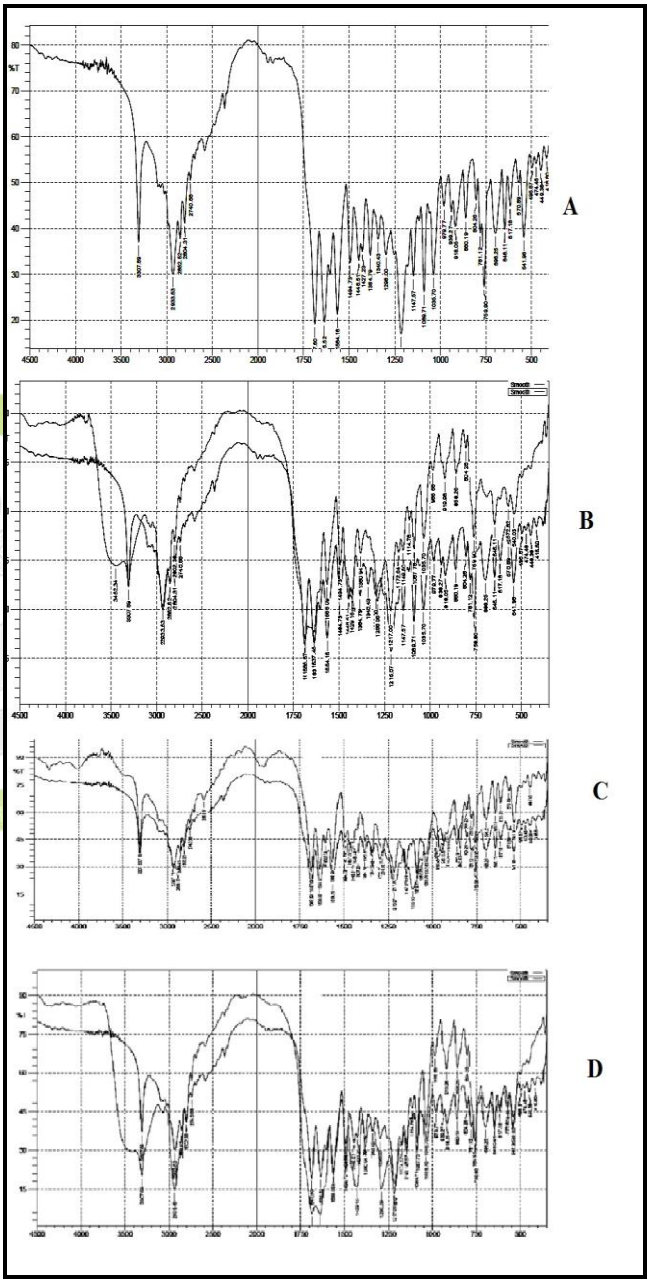

Figure 6: FT-IR spectra of RPG and different formulations: (A) RPG, (B) optimized SNEDDS formulation, (C) RPG-loaded self nanoemulsifying granules in Sipernat 22S, (D) optimized S-SNEDDS tablet formulation D2

Differential Scanning Calorimetry

DSC thermograms for optimized SNEDDS formulation, RPG-loaded self nanoemulsifying granules in Sipernat 22S and pure drug have been summarized in Figure 7. The pure drug samples of RPG had sharp endothermic peak at 132^0C which corresponded to its melting point. SNEDDS-loaded formulations illustrated reduction in the magnitude of endothermic peak which was an indicative of conversion of RPG to its amorphous forms. This might be due to presence of drug molecules in a molecularly dissolved state in SNEDDS formulations. (Figure 7). Moreover, the data also indicate there seems to be no interaction between the drug and polymer. These results were in line with other reports of solid state conversion of SNEDDS.[28,29]

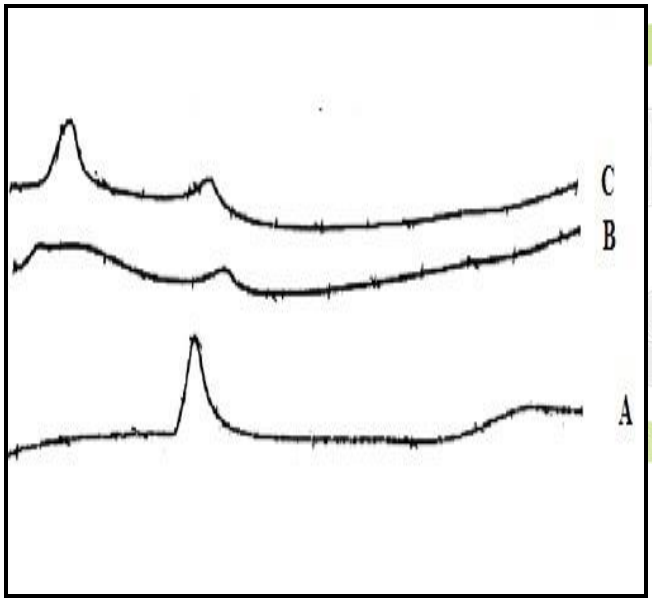

Figure 7: DSC thermograms of (A) RPG and (B) optimized SNEDDS formulation and (C) RPG-loaded self nanoemulsifying granules

Powder XRD Studies

The results of PXRD of SNEDDS-loaded mixture and pure drug samples have been summarized in Figure 8. The X-Ray patterns of pure RPG sample displayed presence of numerous distinct peaks at 0.5, 0.6, 0.7, 2.0, 2.5, 2.7, 6.9°**,** which suggested highly crystalline nature of RPG. However, PXRD patterns of optimized SNEDDS formulation and RPG-loaded self nanoemulsifying granules were characterized by diffuse spectra and reduction of characteristic drug peaks. These results recommended reduction of crystallinity in SNEDDS samples similar to that of DSC. The results of PXRD studies were in line with other reports on solid SNEDDS.[30,31]

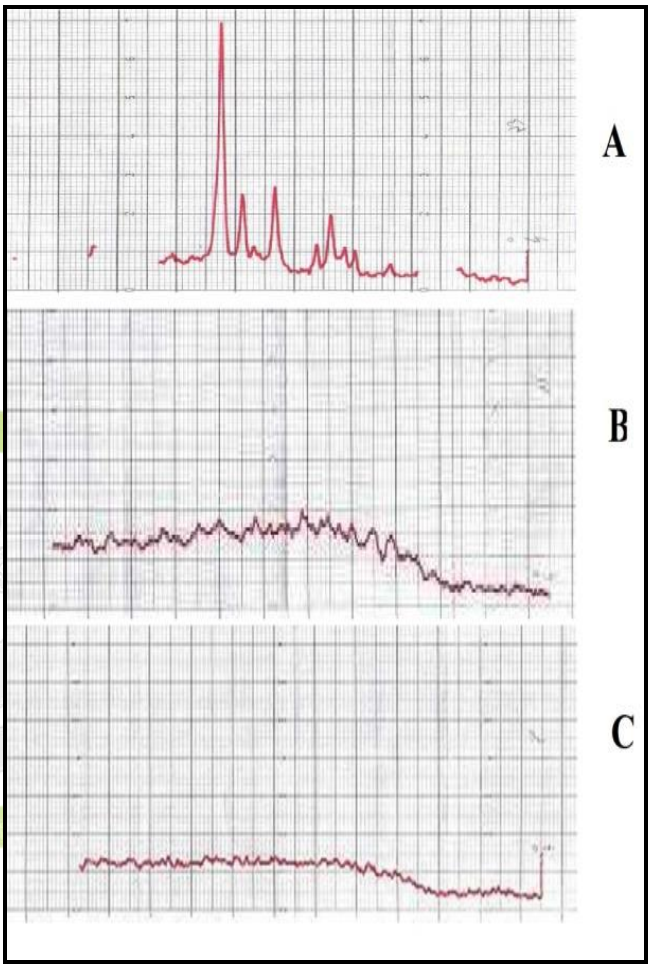

Figure 8: PXRD patterns of (A) RPG (B) optimized SNEDDS formulation and (C) RPG-loaded self nanoemulsifying granules

Accelerated Stability Studies

During accelerated stability studies (40°C/75%RH), different formulation parameters like SEF time, globule size, percent drug release in 30 min and disintegration time of prepared self-nanoemulsifying formulations were evaluated at specified time intervals (0, 1, 2, 3, and 6 months) are depicted in Table 7. Results showed that there was no significant change in these parameters with respect to temperature.

Table 7: Characterization of S-SNEDDS after Accelerated Stability Study at 40°C/75%RH

Sampling time (months)	SEF time (s)	percent drug release after 30 min	globule size in nanometer
0	42.34 ± 0.87	96.76 ± 1.32	98.45 ± 1.32
1	43.54 ± 0.45	98.56 ± 2.3	99.08 ± 1.86
2	41.34 ± 0.12	95.54 ± 1.65	102.34 ± 0.87
3	42.78 ± 0.35	98.87 ± 1.09	104.8 ± 2.1
6	42.93 ± 0.54	96.54 ± 0.95	103.5 ± 2.54

CONCLUSION

The present studies successfully developed a novel S-SNEDDS of RPG for successive utilization as a vehicle for oral drug delivery. The application of experimental design and response surface methodology helped in analyzing the effect of independent variables on responses viz. selfemulsification time, percentage drug release, and globule size, which acts as parameters for performance evaluation of SNEDDS. Among the porous carriers utilized Sipernat 22S showed better drug release property. In vitro dissolution studies showed that S-SNEDDS had a faster drug release rate than conventional marketed product and pure drug. The optimized S-SNEDDS formulation exhibited 2.5– 5-fold increase in dissolution rate as evident from in vitro dissolution studies. Further, the FT-IR, DSC and PXRD study showed no interaction of porous carriers used with developed self-emulsifying system. Thus, it can be concluded that the S-SNEDDS may be suitable formulation approach for bioavailability enhancement of RPG.

REFERENCES

1. Kawakami, K., Yoshikawa, T., Moroto, Y., Kanaoka, E., Takahashi, K. (2002). Microemulsion formulation for enhanced absorption of poorly soluble drugs. *Journal of Controlled Release*, 81(1-2), 65-74.
2. Tang, B., Cheng, G., Gu, J., Xu, C. (2008). Development of solid self-emulsifying drug delivery systems: preparation techniques and dosage forms. *Drug Discovery Today*, 13(13-14), 606-612.
3. Zerrouk, N., Chemtob, C., Arnaud, P., Toscani, S., Dugue J. (2001). In vitro and in vivo evaluation of carbamazepine-PEG 6000 solid dispersions. *International Journal of Pharmaceutics*, 225(1-2), 49-62.
4. Rouchotas, C., Cassidy, O., Rowley, G. (2000). Comparison of surface modification and solid dispersion techniques for drug dissolution. *International Journal of Pharmaceutics*, 195(1-2), 1-6.
5. Rajendrakumar, K., Madhusudan, S., Pralhad, T. (2005). Cyclodextrin complexes of valdecoxib: properties and anti-inflammatory activity in rat. *European Journal of Pharmaceutics and Biopharmaceutics*, 60(1), 39-46.
6. Hammad, M., Aller, B. (1998). Increasing drug solubility by means of bile saltâ€“phosphatidylcholine-based mixed micelles. *European Journal of Pharmaceutics and Biopharmaceutics*, 46(3), 361-367.
7. Rane, S., Anderson, B. (2008). What determines drug solubility in lipid vehicles: Is it predictable? *Advanced Drug Delivery Reviews*, 60(15), 1674-78.
8. Tuleu, C., Newton, M., Rose, J., Euler, D., Saklatvala, R. (2004). 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*, 93(6), 1495-02.
9. Hwang, R., Noack, R. (2011). Application of design of experiments to pharmaceutical formulation development. *International*

Journal of Experimental Design and Process Optimisation, 2(1), 58-64.

10. Singh, B., Kumar, R., Ahuja, N. (2005). Optimizing Drug Delivery Systems Using Systematic "Design of Experiments." Part I: Fundamental Aspects. *Critical Reviews in Therapeutic Drug Carrier System*, 22(1), 27-105.
11. Nazzal, S., Khan, M. (2002). Response surface methodology for the optimization of ubiquinone self-nanoemulsified drug delivery system. *AAPS PharmSciTech*, 3(1), 23-31.
12. Jannin, V., Musakhanian, J., Marchaud, D. (2008). Approaches for the development of solid and semi-solid lipid-based formulations. *Advanced Drug Delivery Reviews*, 60(6), 734-746.
13. Cole, E., Cada, D., Benameur, H. 2008. Challenges and opportunities in the encapsulation of liquid and semi-solid formulations into capsules for oral administration. *Advanced Drug Delivery Reviews*, 60(6), 747-756.
14. Ito, Y., Kusawake, T., Ishida, M., Tawa, R., Shibata, N., Takada, K. (2005). Oral solid gentamicin preparation using emulsifier and adsorbent. *Journal of Controlled Release*, 105(1-2), 23-31.
15. Boltri, L., Coceani, N., Curto, D., Dobetti, L., Esposito, P. (1997). Enhancement and Modification of Etoposide Release from Crospovidone Particles Loaded with Oil-Surfactant Blends. *Pharmaceutical Development and Technology*, 2(4), 373-381.
16. Marks, J. (2003). Clinical Diabetes and the Diabetes Epidemic. *Clinical Diabetes*, 21(1),2-3.
17. Office Guide to Diagnosis and Classification of Diabetes Mellitus and Other Categories of Glucose Intolerance. 1995. *Diabetes Care*, 3-4.
18. Stockman, J. (2011). Type 2 diabetes mellitus after gestational diabetes: a systematic review and meta-analysis. *Yearbook of Pediatrics*., 445-446
19. Dhole, S., Amnerkar, N., Khedekar, P. (2012). Comparison of UV spectrophotometry and high performance liquid chromatography methods for the determination of repaglinide in tablets. *Pharmaceutical Methods*, 3(2), 68-72.
20. Guo, F., Zhong, H., He, J., Xie, B., Liu, F. (2011). Self-microemulsifying drug delivery system for improved oral bioavailability of dipyridamole: Preparation and evaluation. *Archives of Pharmacal Research*, 34(7), 1113-23.
21. Singh, B., Khurana, L., Bandyopadhyay, S., Kapil, R., Katare, O. (2011). Development of optimized self-nano-emulsifying drug delivery systems (SNEDDS) of carvedilol with enhanced bioavailability potential. *Drug Delivery*, 18(8), 599-612.
22. Lawrence, M., Rees, G. (2012). Microemulsion-based media as novel drug delivery systems. *Advanced Drug Delivery Reviews*, 64, 175-193.
23. Pharmacopeia.cn. 2015. *USP Monographs: Repaglinide Tablets*. http://www.pharmacopeia.cn/v29240/usp29nf24s0_m73167.html
24. Kommuru, T., Gurley, B., Khan, M., Reddy, I. (2001). Self-emulsifying drug delivery systems (SEDDS) of coenzyme Q10: formulation development and bioavailability assessment. *International Journal of Pharmaceutics*, 212(2), 233-46.
25. Ahuja, G., Pathak, K. (2009). Porous carriers for controlled/modulated drug delivery. *Indian Journal of Pharmaceutical Sciences*, 71(6), 599-02.
26. Planinaiek, O., Kova, A., Bvrea, F. (2011). Carvedilol dissolution improvement by preparation of solid dispersions with porous silica. *International Journal of Pharmaceutics*, 406(1-2), 41-48.

27. Sharma, S., Sher, P., Badve, S., Pawar, A. (2005). Adsorption of meloxicam on porous calcium silicate: Characterization and tablet formulation. *AAPS PharmSciTech*, 6(4), E618-E625.

28. Yi, T., Wan, J., Xu, H., Yang, X. (2008). A new solid self-microemulsifying formulation prepared by spray-drying to improve the oral bioavailability of poorly water soluble drugs. *European Journal of Pharmaceutics and Biopharmaceutics*, 70(2), 439-44.

29. Kang, J., Oh, D., Oh, Y., Yong, Choi, H. (2012). Effects of solid carriers on the crystalline properties, dissolution and bioavailability of flurbiprofen in solid self-nanoemulsifying drug delivery system (solid SNEDDS). *European Journal of Pharmaceutics and Biopharmaceutics*, 80(2), 289-97.

30. Nekkanti, V., Karatgi, P., Prabhu, R., Pillai, R. (2009). Solid Self-Microemulsifying Formulation for Candesartan Cilexetil. *AAPS PharmSciTech*, 11(1), 9-17.

31. Kallakunta, V., Bandari, S., Jukanti, R., Veerareddy, P. (2012). Oral self emulsifying powder of lercanidipine hydrochloride: Formulation and evaluation. *Powder Technology*. 221, 375-82.

