ENHANCEMENT OF SOLUBILITY AND ORAL BIOAVAILABILITY OF EZETIMIBE BY A NOVEL SOLID SELF NANO EMULSIFYING DRUG DELIVERY SYSTEM (SNEDDS)

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ABSTRACT

Objectives: To develop a stable formulation for self emulsifying drug delivery systems (SEDDS) in order to enhance the solubility, rate and extent of drug release and oral bioavailability of the poorly soluble drug, ezetimibe.

Experimental work done: A solubility of drug was determined in various oils, surfactants and co-surfactants by using spectrophotometric method. Pseudo-ternary phase diagrams were constructed to study the phase behavior and the most efficient self emulsifying regions when the system was diluted with water. Prepared solid SEDDS were filled in hard gelatin capsule and *in vitro* release was performed using a USP Type-II apparatus.

Results and discussion: Ezetimibe shows highest solubility in Capmul PG8 (oil), Tween 80 and Cremophore EL (surfactant), and propylene glycol (co-surfactant) so these vehicles were selected for further study. A microemulsion region was found to be increase in pseudo-ternary phase diagrams when surfactant concentration increased as compared to co-surfactant when diluted with water, and the final formulation with good self-emulsifying ability as well as a high solubilization capacity was established. The optimized formulation F5 showed highest release rate (97.3 %) among all the solid SEDDS formulations and marketed tablet (Ezetimibe USP 10 mg). The mean particle size of the resultant emulsion was about 16.46 nm and the ζ-potential of the solid SEDDS in distilled water was -19.9 mV, determined by Zetasizer Nano S 90 (Malvern Instruments, U.K).

Conclusions: In this study, a new ezetimibe solid SEDDS containing oil phase (Capmul PG 8) of 11.11%, Tween 80:Cremophor EL (1:1) of 59.26%, and propylene glycol of 29.63 % was developed from data of evaluation of drug solubility in various solvents performed and detailed study of pseudo-ternary phase behavior. The resultant emulsion was negative charged, with a small mean size and a narrow particle size distribution. The release rate of ezetimibe from solid SEDDS was faster than from conventional tablets.

Keywords: Ezetimibe, Self-nanoemulsifying drug delivery systems (SNEDDS), Hyperlipidemic, Zeta potential.

INTRODUCTION

Ezetimibe is a first member of new class (BCS class-II) of cholesterol absorption inhibitors indicated for use as a monotherapy or in combination with statins for the treatment of primary hypercholesterolemia. It prevents the transport of dietary and biliary cholesterol across the intestinal wall without affecting the absorption of fat-soluble vitamins, triglycerides and bile acids. The absolute bioavailability of ezetimibe has not been determined and bioavailability is variable in different person (34-60 %) and it is virtually insoluble in aqueous media. As a result pharmacokinetic studies in humans have been limited to the investigation of oral dosages [1, 2].

Approximately 40% of new drug candidates have poor water solubility and the oral delivery of such drugs is frequently associated with low bioavailability, high intra- and inter-subject variability, and a lack of dose proportionality. To overcome these problems, various formulation strategies are exploited including the use of surfactants, lipids, permeation enhancers, micronisation, salt formation, cyclodextrins complex, nanoparticles and solid dispersions [3]. Recently, much attention has been paid to lipid-based formulations with particular emphasis on self nano emulsifying drug delivery systems (SNEDDS) to improve the oral bioavailability of lipophilic drugs. SNEDDS or self-emulsifying oil formulations (SEOF) are defined as isotropic mixtures of natural or synthetic oils, solid or liquid surfactants or alternatively, one or more hydrophilic solvents and co-solvents/ surfactants [4, 5].Upon mild agitation followed by dilution in aqueous media, such as gastrointestinal (GI) fluids, these systems can form fine oil-in-water (o/w) emulsions or nano-emulsions or Self micro-emulsifying drug delivery system (SMEDDS).

MATERIAL AND METHOD

Material

Ezetimibe was gifted by Cadila Pharmaceutical Limited. Captex 355, Campmu PG-8, Captex 200 and Capmul MCM were gifted by Abitec Corporation, USA. Labrafac lipophile 1349, Cremophore El and Cremophore RH 40 were gifted by BASF Mumbai, India. Transcutol P was gifted by Gattefosse India Pvt. Ltd. Arachis oil, Oleic acid, Soybean oil, Ethyl oleate and Isopropyl myristate were purchased form National Chemicals, Baroda. Propylene Glycol, Tween 80, Tween 40, Span 20 and Span 80 were purchased from Loba Chemicals Pvt. Ltd., Mumbai. PEG-6000 was purchased from Chiti-chem Corporation, Baroda.

Method

Solubility study

The solubility of ezetimibe in various oils, surfactants, and co-surfactants was determined as follows: Five ml of each selected vehicle was added to test-tubes containing an excess of ezetimibe. After capping the tubes, the mixtures were shaken at 25°C for 72 hrs to achieve solubility equilibrium. Then, each tube was centrifuged at 4,000 rpm/min for 10 min and the supernatant was passed through a membrane filter (0.45 μm) to remove the undissolved drug. Solubility of ezetimibe was determined by analyzing the filtrate spectrophotometrically (UV 1601, Shimadzu Corporation, Japan) after dilution with methanol at 234 nm. Appropriately diluted solutions of oil, surfactant and co-surfactant in methanol were taken as blank and the procedure was repeated thrice [6].

Construction of ternary phase diagram

One of the most important characteristics of SNEDDS is the change that occurs when the system is diluted (since it will be diluted by body fluids after administration), which may cause drug precipitation due to the loss of solvent capacity [7]. Therefore, the phase behavior of each SNEDDS needs to be carefully studied using the phase diagram as a guide. On the basis of the solubility studies of drug, Capmul PG8 and campmul MCM were selected as oil phase. Tween80 and Cremophore EL were used as surfactants; and propylene glycol and Transcutol P were used as co-surfactants. Distilled water was used as an aqueous phase for the preparation nano-emulsion. Oil, surfactant and co-surfactant were grouped in different combination for phase studies [8].

Capmul PG8, Tween80: Cremophore EL (1:1) and propylene glycol as oil, surfactant, and co-surfactant were found as a best combination among all combinations. Surfactants and co-surfactant (smix) were mixed in different ratios (1:1, 2:1 and 3:1) to produce three formulations of the system. These smix were chosen in increasing concentration of surfactant with respect to co-surfactant for detailed study of phase diagram for the formulation of nano-emulsion. Mixture of the oil and smix were prepared at ratios (w/w) of 10:0, 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7; 2:8, 1:9 and 0:10 in vials. To the resultant mixture, distilled water was added drop wise and observed for transparency and easily flowable O/W nano-emulsion. The physical state of nano-emulsion was marked on a pseudo-ternary phase diagram with one axis representing aqueous phase,

the other representing oil and third representing a mixture of surfactant and co-surfactant (smix) at fixed weight ratios.

Preparation of SNEDDS of ezetimibe

A series of SNEDDS formulations were prepared using oil, Surfactant and Co-surfactant as shown in Table 1. In all the formulations, the level of ezetimibe was kept constant (i.e. 10 mg). The amount of SNEDDS should be such that it should solubilize the drug (single dose) completely. The ezetimibe (10 mg) was added in the mixture. Then the components were mixed by gentle stirring and vortex mixing, and heated at 37°C. The mixture was stored at room temperature until used. So the prepared SNEDDS was the concentrate of oil, surfactant, co-surfactant and drug [9].

Table 1: Composition of different batches containing Capmul PG8, Tween 80: Cremophore EL (1:1) and Propylene glycol as oil, surfactant and co-surfactant.

Batch No.	Smix ratio	Drug (mg)	% composition		
			Oil	Surfactant	Co-surfactant
F1	1:1	10	10	45	45
F2	1:1	10	11.11	44.44	44.44
F3	1:1	10	12.5	43.75	43.75
F4	2:1	10	10	60	30
F5	2:1	10	11.11	59.26	29.63
F6	2:1	10	12.5	58.34	29.16
F7	3:1	10	10	67.5	22.5
F8	3:1	10	11.11	66.66	22.23
F9	3:1	10	12.5	65.2	21.87

Preparation of solid SNEDDS

All prepared SNEDDS pre-concentrate batches and solidifying agent PEG-6000 (25%) were mixed in water bath at a temperature 65-75 °C. Allow it to solidify at room temperature. Solid SNEDDS equivalent to 10mg of ezetimibe were weighed and filled in hard gelatin capsule. The capsules were capped and stored in moisture proof container until used [10].

Characterization

Characterization of SNEDDS pre-concentrate

0.5 ml of SNEDDS pre-concentrate was diluted with 250 ml distilled water. This nano-emulsion solution considered for the further assessment of various in vitro parameters

Appearance

Appearance of nine batches (F1-F9) was tested against white and black background and turbidity was checked. The test was carried out as described in the United state Pharmacopoeia [11].

Refractive index

Refractive index measures clarity of prepared system. Abbe's refractometer (1310 E-20 Atago, Japan) was used to determine R.I of nine batches (F1-F9), distilled water was considered as standard to compare the values obtained samples [12].

Conductance

Type of emulsions (o/w or w/o) can be determined by measure of conductance. The electro conductivity of the resultant system was measured an electro conductometer (CM 200 conductivity meter, welltronix, India) equipped with in built magnetic stirrer. This was done by using conductivity cell (with a cell constant of 1.0) consisting of two platinum plates separated by desired distance and having liquid between the platinum plate acting as conductor. The electrode was calibrated before using it for sample determination, using 0.1 N KCl having conductivity 0015.39 ms/cm at 35 °C. For the conductivity measurements, the microemusions test samples were prepared with a 0.01N aqueous solution of sodium chloride instead of distilled water. The measurements were carried out in triplicate [13].

Transmittance Measurements

The percentage transmittance of preconcentrate (F1-F9) were measured at 650 nm using Ultra Violate spectrophometer (UV-1601,

Shimadzu Corporation, Japan) keeping distilled water as a blank. Each measurement was carried out in triplicate. [14].

Characterization of solid SNEDDS

Visual observation

To assess emulsification properties each formulation (F_1 to F_9) was introduced into 100 ml of distilled water in a glass beaker at room temperature and the content was gently stirred manually. The tendency to form a transparent or clear emulsion was judged as good and bad when the formation was poor or milky in appearance. The prepared nanoemulsions were studied for this and observed for 24 hrs any sign of drug precipitation [15].

Robustness to dilution

SNEDDS were diluted to 10, 100, 1000 times with various dissolution media viz. water, 0.1 N HCl, pH 1.2 buffer, pH 4.5 buffer and pH 6.8 buffer. The diluted nano-emulsions were stored for 12 hr and observed for any sign of phase separation or drug precipitation [16].

Drug content

Assay of weighed amount of formulation was carried out to determine the drug content. The weighed samples were dissolved in 10 ml methanol and vortex for mixing. The solutions were filtered, using whatman filter paper. The content was estimated spectrophotometrically (UV–1601, Shimadzu Corporation, Japan) at 234 nm.

Uniformity of weight for capsules

Weigh an intact capsule. Open it without losing any part of the shell and remove the contents as completely as possible. For hard gelatin capsules, wash the shell with a suitable solvent and keep aside until the odour of the solvent is not perceptible. Weigh the shell. The difference between the weightings gives the weight of the contents. Repeat the procedure with another 19 capsules. Not more than two of the individual weights deviate from the average weight by more than the percentage shown in the table 2 and none deviates by more than twice that percentage. [17]

Table 2: Limit for Uniformity of weight for capsules

Average weight of capsule content	Percentage deviation
Less than 300 mg	10%
300 mg more	7.5%

In vitro dissolution rate studies

For *in vitro* dissolution study of all the formulation (F1 to F9) containing 10 mg of ezetimibe, filled in hard gelatin capsules shell was done using USP dissolution Apparatus- type II (scientific USP standard DA-60). The dissolution vessels contained 500 ml 0.05M of acetate buffer solution with pH 4.5 with SLS 0.45 % w/v maintained at 37 ± 0.5° ^{0}C and the paddle speed was set at 50 rpm. 5 ml aliquots were withdrawn at of 5, 10, 15, and 20 min intervals. Next, volumes so withdrawn were replenished with 5 ml of fresh media maintained in the reservoir pot at the same temperature. All the samples were analyzed for ezetimibe content by spectrophotometric method at 234 nm using UV visible double beam spectrophotometer (UV-1601, Shimadzu Corporation, Japan).

Comparison with marketed product

The prepared promising batche of S-SNEDDS (F5) were compared with marketed product (Ezetimibe tablet 10 mg USP), with respect to drug release. [18]

Particle size distribution (PSD) and ζ-potential analysis

Formulations (F1 to F9) were diluted with 250 ml of distilled water in a 250 ml volumetric flask. The volumetric flask was inverted twice to ensure complete dispersion of the formulation. After ensuring complete dispersion of the formulation the droplet size of resultant nano-emulsion was determined by photon correlation spectroscopy that analyze the fluctuation in light scattering due to the Brownian motion of the droplets as function of time using a Zetasizer Nano S 90 (Malvern Instruments, U.K). Light scattering was monitored at 25°C at 90° angle. **[19, 20]**

Transmission electron microscopy

One drop of nanoemulsion of promising Batch was placed on carbon coated copper grid (3 mm) and was allowed to dry. 1% phosphotungstic acid solution was used as negative stain to create more contrast between oil globule and water continuous phase.

Sample was allowed to air dry for 2 min. Sample was loaded in TEM using horizontal sample holder. Images were taken using appropriate magnification up to 1600. As high as 300 KV acceleration voltages allow the Tecani 20 to reach a point resolution of 0.2 nm, line resolution of 0.10 nm and limit of 0.14 nm. **[21]**

Differential Scanning Calorimetry Studies

DSC is very useful in investigation of thermal properties of S-SNEDDS providing both qualitative and quantitative information about the physical state of drug inside S-SNEDDS, The DSC thermograms of pure drug (Ezetimibe) and some selected samples of solid S-SNEDDS were recorded on a DSC (Perkin Elmer Instruments, Pyris-1DSC). The samples were weighed and hermetically sealed in aluminum pans. Thermal analysis system instrument pyris-1 DSC with intercooler, a refrigerated cooling system, was used. Indium standard was used to calibrate the DSC temperature and enthalpy scale. The system was purged with nitrogen gas at a flow rate of 80mL/min. Initially a Sample was held at 0 ^{0}C for min and after the heating was performed from 0 ^{0}C-250 ^{0}C at a rate of 5 ^{0}C/min. **[22, 23]**

RESULTS AND DISCUSSION

Solubility study

Solubility of ezetimibe was determined in ten different oils to find the oil showing highest solubility of ezetimibe. The results of solubility of ezetimibe in oils various are as shown in Figure-1. The solubility of ezetimibe was observed in capmul PG8 (87±1.55 mg/ml), next it was followed by capmul MCM hence these were chosen as oil phase for preparation of SNEDDS**.** The results of solubility of Ezetimibe in various surfactants and co-surfactants are as shown in Figure-2. The solubility of Ezetimibe was observed in cremophore EL (142.74 ±4.47), tween 80 (361 ±2.59) transcutol P (517 ±6.049 mg/ml) and propylene glycol (51.99 mg/ml) and hence these were chosen as different surfactants and co-surfactants combinations for the preparation of SNEDDS.
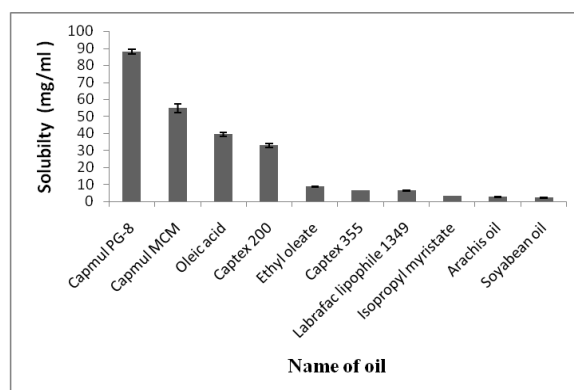
Fig. 1: It shows Solubility studies of ezetimibe in various oils.

Data expressed as mean ± S.D. (n = 3)
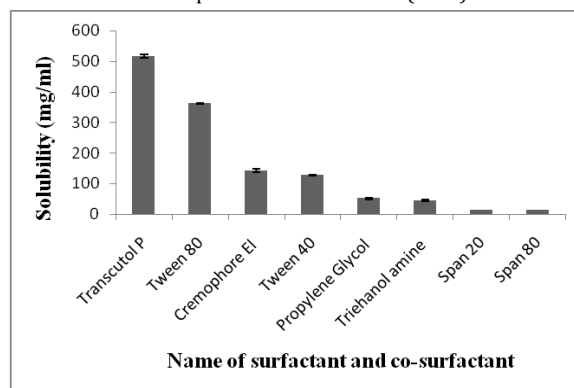
**Fig. 2: It shows Solubility studies of ezetimibe in various surfactant and co-surfactant.**

Data expressed as mean ± S.D. (n = 3)

Construction of pseudo ternary phase diagram:

Oil, surfactant and co-surfactant were grouped in different combination for phase studies. Pseudo-ternary phase diagrams were constructed separately for each group so that SNEDDS regions could be identified. Different combinations of oil, surfactant and co-surfactant were tried e.g. i) Capmul PG8, Cremophore EL, Transcutol P ii) Capmul MCM, Cremophore EL, Propylene Glycol iii) Capmul

PG8, Tween 80, Transcutol P iv) Capmul PG8, Cremophore EL, Propylene Glycol v) Capmul PG8, Tween 80, Propylene Glycol etc.

From these, first two combinations do not give satisfactory results and last combinations were not dilutable with water and nanoemulsion region was less so these combinations were not selected for further study. The combination of Capmul PG 8, Tween80: Cremophore EL (1:1), propylene glycol was selected for formulation.
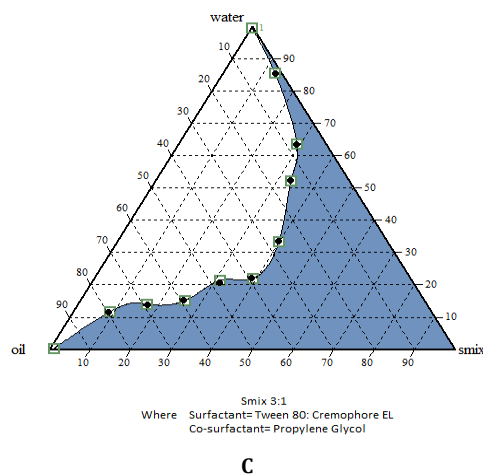

A

B

C
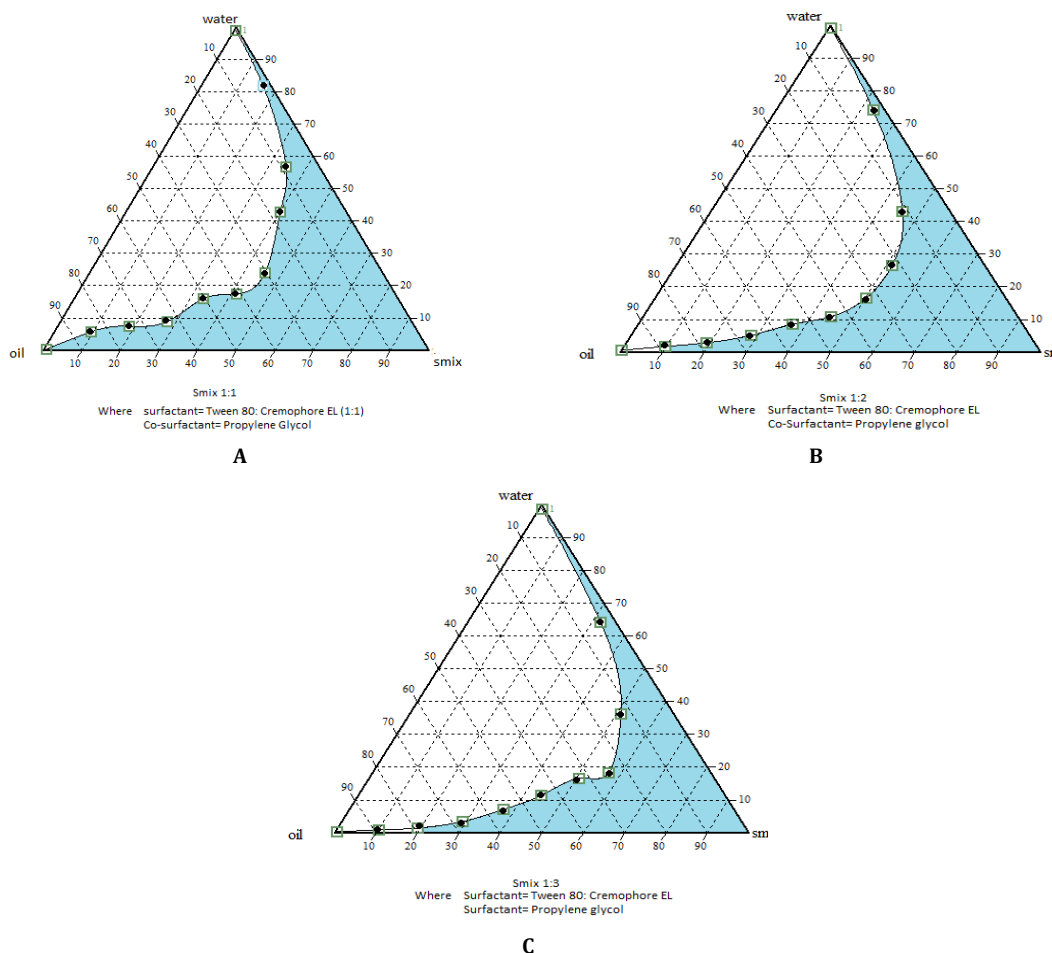
Fig. 3: A, B and C: It shows Pseudo-ternary phase diagrams of SNEDDS containing Capmul PG8, Tween 80: Cremophore EL (1:1), Propylene glycol with smix ratio 1:1, 1:2, 1:3 respectively

Figure-3 represents pseudo-ternary phase diagrams of Capmul PG8, Tween 80: Cremophore EL (1:1), Propylene glycol combination for smix ratios 1:1, 1:2, 1:3 respectively. Pseudo-ternary diagrams

represent nanoemulsion region in light blue color. As a ratio of co-surfactant to surfactant increased the nanoemulsifying region was decreased.
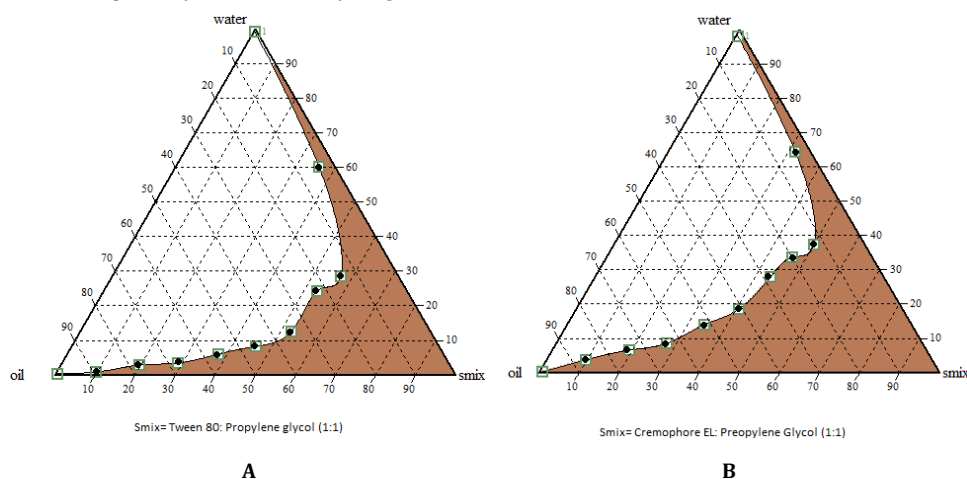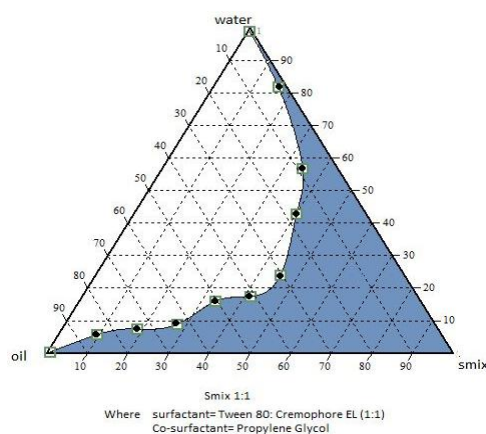

A

B

Smix 3:1
Where   Surfactant= Tween 80: Cremophore EL
Co-surfactant= Propylene Glycol

C
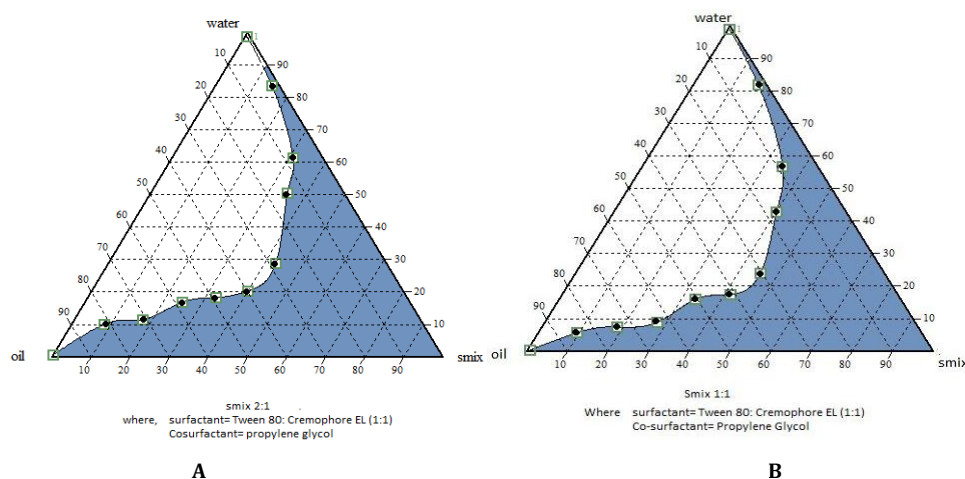
Fig. 4: A, B and C: It shows Pseudo-ternary phase diagrams of SNEDDS containing Capmul PG8, Tween 80: Cremophore EL (1:1), Propylene glycol with smix ratio 1:1, 2:1, 3:1 respectively

Figure-4 shows pseudo-ternary phase diagrams of Capmul PG8, Tween 80: Cremophore EL (1:1), Propylene glycol combination for smix ratios 1:1, 2:1, and 3:1 respectively. Pseudo-ternary diagrams

represents nanoemulsion region in blue color. As the ratio of surfactant to co-surfactant increased the nano-emulsifying region was increased.

Smix= Tween 80: Propylene glycol (1:1)

A

Smix= Cremophore EL: Preopylene Glycol (1:1)

B

Fig. 5: It shows Pseudo ternary phase diagrams of SNEDDS containing A. Capmul PG8, Tween 80: Propylene glycol (1:1) and B. Capmul PG8, Cremphore EL (1:1).

Smix 1:1
Where   surfactant= Tween 80: Cremophore EL (1:1)
Co-surfactant= Propylene Glycol

Fig. 6: It shows Pseudo ternary phase diagram of SNEDDS containing capmul PG8, Tween 80: Cremophore EL (1:1), propylene glycol with smix ratio 1:1.

Figure-5 A and B represent pseudo-ternary phase diagrams of capmul PG8, tween80: Propylene glycol (1:1) and Capmul PG8, Cremphore EL, Propylene glycol (1:1) respectively. Pseudo-ternary diagrams represents nanoemulsion region in brown color while Figure-7 represents nanoemulsion region in blue color. The

nanoemulsion region was decreased in case of use of single surfactant while the nanoemulsion region was increased comparatively by use of combination of surfactants which gave clear, transparent and stable nanoemulsion region at fixed weight ratios.

Characterization of SNEDDS pre-concentrate

Appearance

All nine formulations (as such solid or in diluted form) were observed for 24 hr for clarity, phase separation and precipitation of drug. The formulation were categorized as clear (transparent or transparent with bluish tinge), non-clear (turbid), stable (no precipitation at end of 24 hr) and unstable (showing precipitation within 24 hrs) [24, 25]. All the batches found to be clear after 24 hr except batch F2 and F3 become turbid after 24 hr regardless to surfactant concentration all the batches were found to be stable showing no phase separation.

Table 3: Assessment of physical compatibility by Visual observation of prepared batches

S. No.	Formulation	After 24 hours		
		Phase separation	Precipitation	Clarity
1.	F1	✓	✓	✓
2.	F2	✓	✓	×
3.	F3	✓	✓	×
4.	F4	✓	✓	✓
5.	F5	✓	✓	✓
6.	F6	✓	✓	✓
7.	F7	✓	✓	✓
8.	F8	✓	✓	✓
9.	F9	✓	✓	✓

*Where, √-Passed and ×-Failed

Refractive index

Table 4 shows results of refractive index of nine different batches (F1-F9) at 33 ^{0}C which were compared with refractive index of distilled water at 33 ^{0}C. Refractive index of distilled water at 33 ^{0}C is 1.3318±0.745 nD. Batch F2 and F3 shows some deviation from standard value. Other batches were considered clear as water.

Table 4: Refractive index of SNEDDS containing ezetimibe

Batch No.	Refractive index at 33 ^{0}C (nD)*
F1	1.3317±0.014
F2	1.3328±0.361
F3	1.3330±0.523
F4	1.3316±0.0365
F5	1.3317±0.425
F6	1.3319±0.0361
F7	1.3320±0.075
F8	1.3316±0.036
F9	1.3320±0.549

* Values are mean ± SD; standard deviation (n=3)

Conductance

Presence of o/w nano-emulsion formulation was confirmed by measuring conductivity. The increase in conductivity of water is likely caused by a transition from an oil continuous nano-emulsion system to water continuous nanoemulsion system. [26] As all SNEDDS are water continuous nano-emulsion, some decrease in conductance was due to presence of oil droplets shows resistance to conductance and so decrease in conductivity was observed. F5 batch shows highest conductivity as compared to all other system shown in Table 5.

Percentage Transmittance Measurements

The clarity of nano-emulsions was checked by transparency, measured in terms of transmittance (%T). SNEDDS forms o/w nano-emulsion since water is external phase. Formulation has % transmittance value greater than 99%, These results indicate the high clarity of nano-emulsion. In case of other systems %T values were about 96% suggesting less clarity of nano-emulsions. This may be due to greater particle size of the formulation. Due to higher particle size, oil globules may reduce the transparency of nano-emulsion and thereby values of %T. The results of %T are as shown in Table 6. Formulation F2 & F3 shows lower transmittance as compared to other formulations.

Table 5: Electroconductivity of SNEDDS containing ezetimibe

Batch No.	Conductance (µs/cm)			
	I	II	III	Mean ±SD*
F1	95.7	95.2	95.4	95.43±0.25
F2	94.6	94.8	95	94.80±072
F3	93.4	93.6	93.4	93.46±0.11
F4	97.5	97.3	97.9	97.56±0.30
F5	98.9	98.5	97.9	98.43±0.50
F6	96.8	96.5	97	96.76±0.25
F7	95.4	95.2	95.3	95.30±0.1
F8	94.8	94.3	94.2	94.43±0.32
F9	93.9	94.2	94.1	94.06±0.15

* Standard deviation (n=3)

Table 6: % Transmittance of SNEDDS containing ezetimibe

Batch No.	% Transmittance			
	I	II	III	Mean± SD*
F1	98.54	96.1	96.45	97.03±1.31
F2	96.25	96.02	96.45	96.24±0.21
F3	96.45	96.85	95.9	96.4±0.47
F4	98.59	99.36	99.54	99.16±0.50
F5	99.31	99.21	99.01	99.17±0.15
F6	98.35	98.25	99.75	98.78±0.83
F7	97.25	97.13	97.01	97.13±0.12
F8	98.19	98.32	99.65	98.72±0.80
F9	96.52	96.2	95.92	96.21±0.30

* Standard deviation (n=3)

Characterization of solid SNEDDS

Visual observation

All nine formulations were observed for 24 hr for clarity, phase separation and precipitation of drug. The formulation were categorized as clear (transparent or transparent with bluish tinge), non-clear (turbid), stable (no precipitation at end of 24 hr) and unstable (showing precipitation within 24 hrs). All the batches found to be clear and stable. Batch F4 and F5 were transparent with slight bluish tinge. Regardless to surfactant concentration all the batches were found to be stable showing no phase separation.

Robustness to dilution

Diluted S-SNEDDS did not show any precipitation or phase separation on storage in various dilution media. This suggests that all media were robust to dilution.

Drug content

The drug content of ezetimibe S-SNEDDS formulation was measured using UV spectroscopic method. The % drug content was determined by considering 10 mg of ezetimibe as a 100%. All are in specified limit (98-102 % w/w) of drug content.

Table 7: Drug content (% w/w) of S-SNEDDS containing ezetimibe

Batch No.	Drug content (% w/w)			
	I	II	III	Mean ± SD*
F1	98.2	98.5	98.5	98.4±0.17
F2	99.2	99.7	99.4	99.43±0.25
F3	98.5	98.4	98.6	98.5±0.1
F4	99.4	99.3	98.9	99.2±0.26
F5	98.7	98.4	98.1	98.4±0.3
F6	99.1	99.5	99.3	99.3±0.2
F7	98.3	98.6	98.7	98.53±0.20
F8	97.9	98.2	98.6	98.23±0.35
F9	99.4	99.0	99.1	99.16±0.20

* Standard deviation (n=3)

Uniformity of weight for capsules

Uniformity of weight was determined for all formulations. The value of average weight of capsules range from 622.5-627.6 mg. the weight variation was observed within acceptable limit i.e. less than ±7.5% capsules as per IP 2007.

In vitro dissolution rate studies

In-vitro dissolution of formulations is a valuable tool to predict behavior of a particular formulation with respect to drug transport across membrane various physicochemical parameters pertaining to formulations such as flux, partition coefficient and diffusion coefficient can be derived using *in vitro* evaluation techniques. The formulation F5 showed highest release rate among all the S-SNEDDS formulations i.e. 97.3% as shown in Figure-7. Thus, it was taken as the optimized S-SNEDDS formulation.
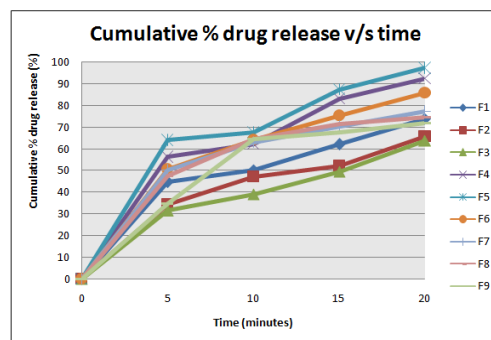
Fig. 7: It shows dissolution profile of S-SNEDDS containing ezetimibe

Comparison with marketed product

The prepared promising batch F5 of S-SEEDS was compared with marketed product and plain drug (Ezetimibe 10 mg) with respect to drug dissolution profile. Cumulative % drug dissolution data are shown in table 5.15.
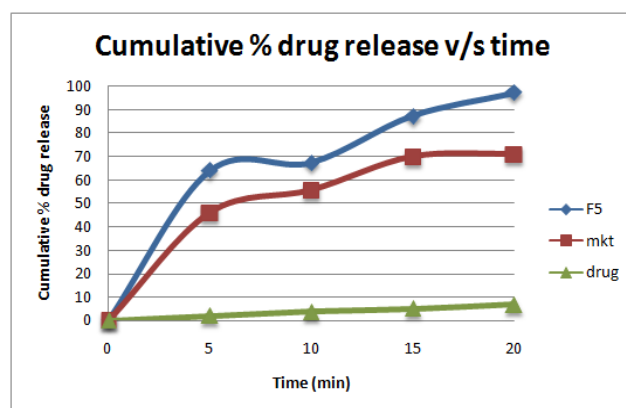
Table 8: Comparative dissolution profile of promising batch with marketed product and plain drug.

Time(Min)	Cumulative % drug release*		
	F5	mkt	Drug
0	0	0	0
5	64.1±0.352	46.05±0.15	2.1±0.156
10	67.6±1.231	55.67±0.345	3.7±0.954
15	87.45±0.365	69.92±0.245	5.1±1.235
20	97.3±1.354	71.12±0.326	6.9±1.123

*Values are mean ± SD; standard deviation (n=3)

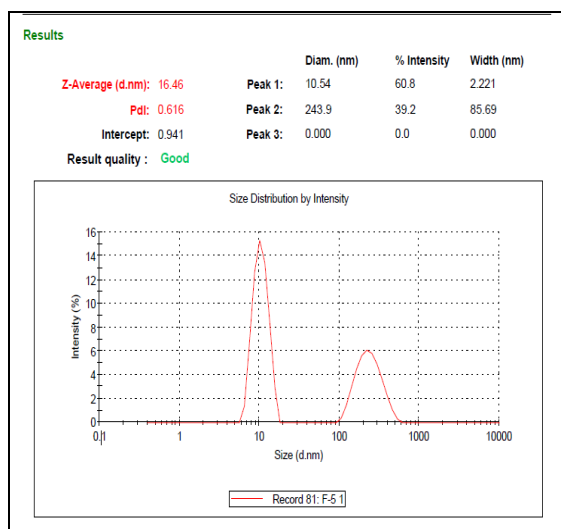Where, F5=Optimized batch; mkt= Marketed tablet of Ezetimibe USP 10 mg

Drug = Plain drug

Fig. 9: Dissolution profile of optimized bath (F5) with marketed tablet and plain drug

Particle size distribution (PSD) and ζ-potential analysis

The particle size and zeta potential of optimized batch (F5) was found to be 16.46 nm (Figure-11) and 19.9 mV (Figure 12) respectively in water.


Fig. 11: Particle size distribution of optimized batch (F5) in distilled water
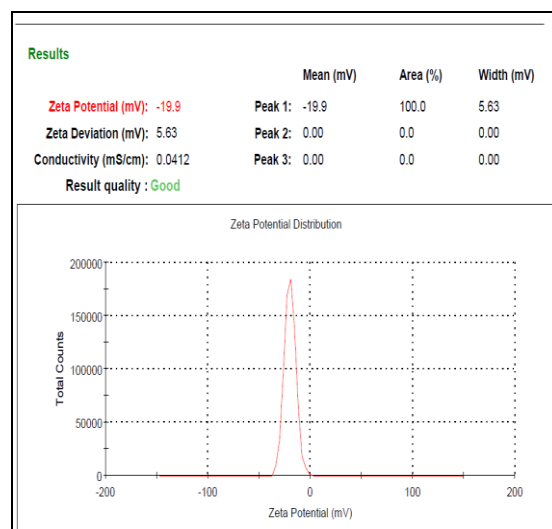
Fig. 12: Zeta-potential distribution of optimized batch (F5) in distilled water

Transmission electron microscopy

As shown in Figure-13 TEM image of F5 shows spherical globules of self nano-emulsion which are less than 100 nm. Globules were seen as uniform in size and spherical in shape indicate the good state of nano-emulsion.
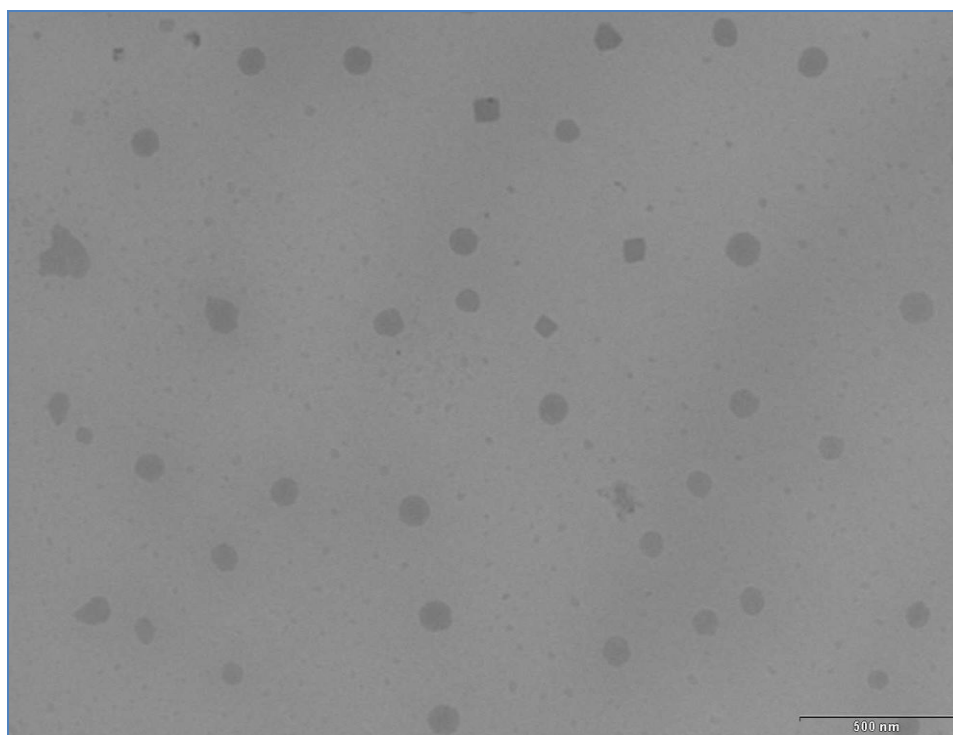
Fig. 13: It shows Transmision elctron microscoy of ezetimibe S-SNEDDS

Differential Scanning Calorimetry Studies

DSC is very useful in investigation of thermal properties of S-SNEDDS providing both qualitative and quantitative information about the physical state of drug inside S-SNEDDS. The DSC thermograms of pure drug (Ezetimibe) and promising batch F5 of S-SNEDDS were on a DSC (Perkin Elmer Instruments, Pyris-1DSC). There no detectable endoderm if the drug is present in a molecular dispersion or solid solution in the S-SNEDDS. The DSC thermogram of pure drug (Ezetimibe), drug loaded S-SNEDDS batch F5 are shown in Figure-14.

Ezetimibe shows a sharp peak at 164.659 °C due to melting point, but in case of ezetimibe S-SNEDDS no characteristic peak was observed at 164.659 °C, suggesting that ezetimibe is molecularly dispersed in the matrix.
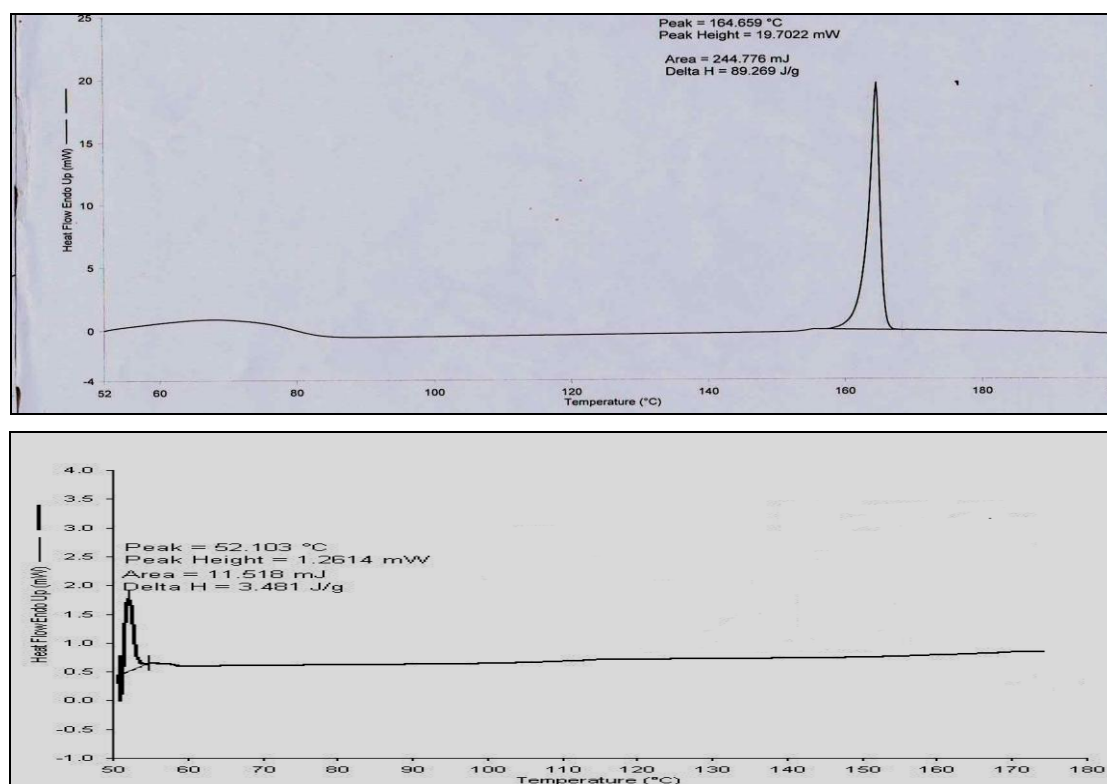
Fig. 14: DSC thermogram of plain drug and optimized batch (F5) batch

CONCLUSION

In this study, the solid SNEDDS of Ezetimibe was prepared using PEG 4000 as a solid carrier. The formulation (F5) was found to be the optimized formulation on the base of results of pseudo ternary phase diagram, in vitro drug release, droplet size zeta potential and other parameters. DSC measurements suggested that Ezetimibe in the solid SNEDDS may be in the molecular dispersion state. In vitro dissolution test showed that the solid SNEDDS had a faster in vitro release rate than the powder and marketed tablet formulation. The present study clearly indicated that the usefulness of Solid SNEDDS in the improvement of dissolution rate and there by oral bioavailability of poorly water soluble drugs like Ezetimibe without incompatibility between the ingredients.

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