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RESEARCH ARTICLE

Solubility Enhancement of Poorly Soluble Drug Ezetimibe by Developing Self Nano Emulsifying Drug Delivery System

Vishakha Vishwanath Doke[1]*, Nilesh M Khutle[2], Maya Sharma[3], Khemchand Gupta[4]

1 Research Scholar (Ph.D.), Faculty of Pharmacy, Pacific Academy of Higher Education and Research University, Rajasthan, Udaipur, India
2 Assistant Professor (Pharmaceutics), Dr. L. H. Hiranandani College of Pharmacy, Ulhasnagar, Maharashtra, India
3 Associate Professor, Pacific college of Pharmacy, Pacific Academy of Higher Education and Research University, Udaipur, Rajasthan, India
4 Professor and Principle, Venkateshwar Institute of pharmacy, Sai Tirupati University, Udaipur, Rajasthan, India

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* **Corresponding author**.

vishakhadoke17@gmail.com

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Abstract

Objectives: To enhance solubility, dissolution, and permeability of poorly water-soluble drug Ezetimibe (EZE) using a self-nano emulsifying drug delivery system (SNEDDS). **Methods:** Initially, the solubility of the EZE was determined in various oils and buffers. Surfactants and co-surfactants were screened based on the solubility of the drug in oil as per the emulsification efficacy test. Liquid SNEDDS was developed and characterized. Solid SNEDDS was developed and characterized using optimized liquid SNEDDS followed by the development of EZE-loaded Tablet SNEDDS. **Finding:** Liquid SNEDDS (L-SNEDDS) was formulated using Capmul MCM C8 EP, Cremophore RH 40, and Labrafil M 2125 CS as oil, surfactant, and co-surfactant respectively. Optimized L-SNEDDS formulation was found to be efficient with an average %T of 99.5%, drug content of 98.43%, flask inversion 0 numbers, and the average particle size of 36.7 nm, zeta potential of -57.5 mV, Polydispersibility index in of 0.119. Also, the in vitro release profile of drug from L-SNEDDS encapsulated in hard gelatin capsules was evaluated in different dissolution media viz. simulated gastric fluid and simulated intestinal fluid. For the drug, more than 95% cumulative release was observed within 30 min exclusive of the pH of the medium. The L-SNEDDS were adsorbed on a solid support and then mixed with tablet blends and compressed into tablets. Further, no adverse changes in globule size, shape, the zeta potential of SNEDDS, and dissolution profile were apparent on conversion to solid powder form and tablet form. **Novelty:** The developed liquid SNEDDS form of EZE showed enhanced solubility, dissolution, and permeability in comparison to pure drugs. Conversion of L-SNEDDS to P-SNEDDS would be a novel approach to overcome the limitations associated

with liquid dosage forms.

Keywords: Ezetimibe (EZE); Selfnano emulsifying drug delivery system (SNEDDS); liquid SNEDDS (L SNEDDS); powder SNEDDS (PSNEDDS); Tablet SNEDDS (TSNEDDS)

1 Introduction

Self-emulsifying drug delivery system (SEDDS) is an oral solid or liquid dosage form with a combination of oils, surfactants, and co-surfactants. After oral administration, it reaches the aqueous environment of the GIT and forms a fine nano-emulsion (o/w). GIT motility gives agitation to generate emulsion. Fine emulsion passes quickly from the stomach and helps in drug distribution all over GIT, hence reducing the irritation of the gut wall caused due prolonged contact with the drug. SEDDS shows enhancement in absorption and in-vitro dissolution rate of BCS II and IV drugs. This dosage form provides advantages like protection of drugs against the adverse atmosphere of the gut and selective targeting of drugs in GIT [(1)].Conventional SNEDDS are mostly formulated in a liquid form.

EZE is an azetidine derivative and pharmacologically it is categorized as an anticholesteremic agent that constrains intestinal sterol absorption. It is used to decrease total cholesterol and apolipoproteins B in the management of hyperlipidemias [(2)].According to BCS, EZE is categorized as a Class II drug (low soluble and high permeable). It has a very low bioavailability of 40% due to the extensive first-pass metabolism mainly in the liver. Moreover, it shows higher intra as well as inter-subject variation, deficiency in dose proportionality, and pH-dependent solubility [(3)]. EZE is an ideal drug candidate to be formulated in the form of SNEDDS thereby it will enhance the aqueous solubility, protect from first-pass metabolism and increase its absorption by lymphatic transport which could improve its bioavailability [(4)].

Different approaches have been utilized to enhance the solubility and bioavailability of the EZE by formulating different dosage forms. Yadav, P et al., developed liquid SNEDDS of EZE to improve the dissolution rate and found excellent results in terms of the solubility as well as dissolution rate enhancement [(5)]. Similarly, Ahmed R et al., developed combinatorial liquid SNEDDS containing atorvastatin and EZE to enhance the solubility and dissolution rate of both drugs [(6)]. Furthermore, Kazi Mohsin et al., also developed liquid SNEDDS for talinolol and improved solubility, dissolution, and bioavailability [(7)]. The extensive literature search demonstrated the potential of SNEDDS for solubility, dissolution, and bioavailability enhancement. But most of those SNEDDS are available in liquid emulsion form. Despite the advantages of liquid SNEDDS, they have several limitations, including drug/component precipitation when stored, interactions between the filler and the capsule shell, and formulation stability during storage. To address these issues, the most common technique is to convert liquid SNEDDS formulations into solid SNEDDS formulations. The conversion of liquid SNEDDSs to solid SNEDDSs is thought to result in lower production costs, improved formulation stability, the convenience of handling, precise dosing, and improved patient compliance. S-SNEDDS are formulated by the liquid loading techniques. They may have the combined advantages of solid dosage form and L-SNEDDS (i.e improved solubility and bioavailability) [(8)].

So far, only a limited amount of research work has been done on the development of directly compressible tables containing solid EZE SNEDDS. We have performed this research work to manufacture L-SNEDDS with particle sizes in the nanoscale range, converted them into P-SNEDDS by liquid loading technique, and then into T-SNEDDS by direct compression method, taking into account all of the potential benefits of solid SNEDDS. The important consideration while making solid-SMEDDS of EZE was to have the dual benefit of its Self-emulsifying properties which spontaneously

produce fine microemulsion with small globule size to improve solubility, dissolution, and bioavailability enhance the dissolution and bioavailability of EZE and have the benefit of the solid system which enhances the patient compliance and improves the stability of formulations.

2 Materials and methods

2.1 Saturation Solubility of EZE[9]

EZE equivalent to its dose (10 mg) was added to each vial containing pre-warmed 1g of oil, it was vortexes using a cyclo mixer for 5-10min and heated on a water bath at 40-50°C. The mixture was visually observed for the extent of the solubility of EZE in particular oils. The oils exhibiting the solubility for the first aliquot of EZE (10mg) in such oils the next aliquots of EZE (10 mg) were further added following the same procedure. The process was repeated again and again till the oils were completely saturated with the drug. Adding EZE was stopped after precipitation of the drug due to saturation of oils. The total amount of drug added was noted and the approximate solubility of EZE in each vehicle was determined. Oil showing the maximum capability of solubilizing EZE was selected for further study.

2.1.1 Determination of solubility of EZE in different buffer solutions (pH 6.8 and pH 7.0) [6]

1g of each buffer solution was added to two different vials, and an additional quantity of EZE was added until the buffer solutions get saturated (based on the visual observations). After the addition of the first aliquot, these solutions were vortexed on a cyclo-mixer and heated up to 40-50°C using a water bath. Mixtures were kept on continuous shaking for 24 hours in an incubator at 37°C $\pm$ 2°C after that allowed to stand still up to 24 hours at R.T. for settling of undissolved drug EZE which was then filtered through0.45μm membrane filter. The level of EZE was then quantified using UV-spectrometric analysis.

2.2 Surfactant and co-surfactant selection[10]

In brief, 300 mg of each surfactant and Capmul MCM C8 EP was taken in a glass vial and gently heated up to 40-50°C using a water bath followed by vortexing on cyclomixer for 3-4min to homogenize the components and again heated gently at 40-50°C on a water bath. 50 mg mixture then diluted to 50.00 mL inside volumetric flask using double distilled water. The completion of the formulation was monitored via observing the total inversion of inversion necessary to produce clear plus even emulsion. After 2 hours of storage of these flasks at room temperature, the resultant formulations were visually observed for the comparative turbid nature and phase separation if any. Then these nano-emulsions were evaluated for % transmittance (%T) at 638.2 nm by UV spectrophotometer. Also, emulsions formed must be checked to see the size of the globule, polydispersity index (PDI), and zeta potential. Surfactants and co-surfactants at a ratio of 2:1 (w/w) were mixed properly. This surfactant mixture was added to Capmul MCM C8 EP in a 1:1(w/w) ratio to evaluate the comparative efficiency of the co-surfactant to increase the emulsification capacity of the selected surfactant and check the ease of emulsion formation by a reduction in several flask inversions if any by addition of co-surfactant/s. A similar procedure was followed as that of surfactant.

2.3 Formulation and optimization of L-SEDDS

Formulation of trial batches was done for each of the above-mentioned systems. Nine different formulations as per Table 1 the above systems were prepared (Changing the concentration of Oil: Surfactant and Surfactant: Co-surfactant ratio) and evaluated for Freeze-thaw cycles, Flask inversion, %T, Particle size, PI, and Zeta potential. The formulations were prepared in the following steps[5]. EZE was added into a prewarmed vial having the required amount of oil. This oil and EZE mixture was heated up to 40 to 50°C using a water bath trailed by vortexing for 5-10 min for ensuring the complete and uniform solubilization of drug EZE into the oily phase i.e. Capmul MCM C8 EP. Required quantities of Cremophore RH 40 and Labrafil M2125 CS for the system were added and the vials and heated up to 40 to 50°C using a water bath trailed by homogenization on cyclomixer up to 10-15 min to confirm uniformity. The mixtures (containing 10mg EZE) were filled in hard gelatin capsules of size "2" using a micropipette.

2.4 Evaluation of optimized formulation of EZE loaded L-SNEDDS

2.4.1 Stability to freeze-thaw cycles[11]

Entire 9 batches of L-SNEDDS were kept at room temperature for up to 24 hrs then 24 hours at 5°C for refrigeration, thus completing one such freeze-thaw cycle. Likewise, three alternate freeze-thaw cycles were done and examined for phase separation or drug precipitation. The batches which can survive freeze-thaw cycles were exposed to flask inversion, zeta

Table 1. Formula for optimization trials

Formula	C1	C2	C3	C4	C5	C6	C7	C8	C9
	Components (in mg) per unit								
Ezetimibe	10	10	10	10	10	10	10	10	10
Capmul MCM C8 EP	120	120	120	120	120	120	120	120	120
Cremophore RH 40	60	80	90	90	120	135	120	160	180
Labrafil M 2125 CS	60	40	30	90	60	45	120	80	60
Mass filled per capsule (mg)	250	250	250	310	310	310	370	370	370

potential, %T, globule size, and polydispersity index analysis.

2.4.2 Drug content, Globule size, PI, and zeta potential

L-SNEDDS (containing 10.0 mg EZE) was kept in a volumetric flask and a 10 ml volume was made by adding methanol. The system was sonicated for 20 min for complete extraction of the EZE. The EZE concentration was determined by a standard calibration curve in methanol at 233 nm. A sufficient quantity of EZE loaded L-SNEDDS, was dispersed in distilled water, simulated gastric fluid (SGF), and simulated intestinal fluid (SIF) and sonicated properly. The mean globule size, PI, and zeta potential of the nanoemulsions were determined by Particle Size Analyzer.

2.4.3 Robustness to dilution[12]

EZE Formulation was diluted with different pH media to correlate in vivo conditions where the formulation come across dilution. This test was performed by diluting L-SNEDDS of EZE by 100, 1000, and 10000 folds with 0.1 N HCl and pH 6.8 phosphate buffer. The diluted formulations were kept for 2 hours at room temperature and observed for globule size, the appearance of nanoemulsion, PI, %T, and zeta potential.

2.4.4 In vitro dissolution and Ex-vivo permeability studies study

The dissolution of pure EZE and EZE L-SNEDDS was carried out in USP type I dissolution apparatus

operating at 50 rpm at 37°±0.5°C in 0.1N HCl at pH 1.2 (SGF) and phosphate buffer pH 6.8 (SIF) L-SNEDDS of Ezetimibe equivalent to 10 mg of EZE were filled in HGC size "2" and placed in dissolution test apparatus. In the study, 5 ml of aliquots must take out from dissolution media at pre-determined time laps.e.0, 5, 10, 15, 20, 25, 30, 45, and 60 min, and sink condition was maintained by replacing with buffer. Aliquots were filtered through Whatman filter paper and analyzed using a UV spectrophotometer.

Non-everted chicken intestinal sacs were used to perform an ex-vivo permeability study of EZE L-SNEDDS. The intestinal sac was placed in cold pH 7.2, KRBS. The solution was aerated electrical aerator. A long sac was prepared (approximately 5-6 cm) using cotton thread and tying of the two ends of the sac. 5 mL of L-SNEDD of EZE and plain drug solution were put in the two different sacs as a reference to compare. The sacs were kept in two different beakers having 100.00 mL KRBS and with supply air, at 37±0.5°C, at 50rpm. Aliquots were taken out at a particular time point with a calibrated syringe. Replacement is done using fresh KRBS. The permeability study was performed for 60 minutes. The quantity of EZE from L-SNEDDS permeated through the intestinal sac was analyzed by assessing the area at 233nm by UV-visible spectrophotometry[13].

2.5 Formulation of solid SNEDDS (S-SNEDDS

Two adsorption techniques were used namely using mortar and pestle and using a glass rod. L-SNEDDS was added dropwise onto the bed of a fixed amount of Neusilin US2 and mixed uniformly until the free-flowing properties of powder were retained. Different ratios of L-SNEDDS were taken with the adsorbent and were analyzed for free-flowing properties. The resultant P-SNEDDS were stored in desiccators till evaluation. Flow properties of the mixture were studied. The weight of L-SNEDDS adsorbed by the adsorbing agent was recorded[14]. The optimized formulation of L-SNEDDS was added drop by drop on 1g of NeusilinUS2 in a broad porcelain dish. After each addition of the liquid drop of L-SNEDDS, the mix was properly homogenized

with a glass rod for uniform distribution of formulation. The process of the addition of L-SNEDDS was stopped when the optimized adsorbent ratio was achieved.

2.5.1 Evaluation of P-SNEDDS

2.5.1.1 Powder flow properties[15]. The angle of repose, Carr's index (CI), Hausner's ratio (HR), bulk density (BD), and tapped density (TD) were determined.

2.5.1.2 Reconstitution Properties of P-SNEDDS. P-SNEDDS was reconstituted with pH 1.2 of SGF and pH 6.8 of SIF. Reconstituted P-SNEDDS were checked for the size of the globule, zeta potential, %T analysis, and Polydispersity index.

2.5.1.3 Drug content. Accurately weight P-SNEDDS equivalent to 10mg of EZE was put in a 10mL volumetric flask and the volume is made up to 10mL by methanol; EZE was extracted in methanol by shaking the flask manually, followed by sonication in a bath sonicator for 10-15min. The resultant dispersion was stored at room temperature for 15-20mins to settle down the large particles of the solid carrier. The supernatant was carefully removed and filtered using Whatmann filter paper. Thus, an obtained sample was further diluted with methanol and EZE content was analyzed by measuring at 233 nm.

2.5.1.4 Differential Scanning Calorimetry thermogram. Thermal property of EZE loaded P-SNEDDS powder investigated using SII NANOTECHNOLOGY SEIKO EX STAR DSC 6220 (Measurement and standard analysis software) [12].

2.5.1.5 X-Ray Diffraction Analysis. Powder X-ray diffraction patterns were recorded for pure drug EZE and optimized P-SNEDDS at room temperature on a Bruker X-ray diffractometer, a 0.2 step size.

2.5.1.6 Morphology of EZE and Neusilin US2 by Scanning Electron Microscopy. The macroscopic structure of plain powder, Neusilin US2 was investigated by JEOL JSM-7600F FEG-SEM (Field emission gun-scanning electron microscope), operating at 0.1 to 30 kV.

2.5.1.7 Morphological analysis and comparison of P-SNEDDS with the drug by Cryo Field Emission Gun Scanning Electron Microscopy. The outer macroscopic structure of P-SNEDDS was investigated by Cryo Field Emission Gun Scanning Electron Microscope.

2.5.1.8 In vitro dissolution study. Studies of plain Ezetimibe powder and Ezetimibe loaded P-SNEDDS (containing 10 mg EZE) filled in hard gelatin capsules shells were carried out similarly to that of L-SNEDDS

2.6 Formulation EZE loaded Tablet SNEDDS (T-SNEDDS

Microcrystalline cellulose (MCC-Avicel PH 102) was used as the directly compressible diluent, while magnesium stearate and talc were added as glidant and lubricant respectively. Crospovidone was used as super disintegrant. The commonly used super disintegrating agent Crospovidone at four different concentrations namely 2%, 2.5%, 3%, and 3.5% w/w were compared for their ability to disintegrate EZE-loaded T-SNEDDS with maintaining the hardness of the tablet. Based on the disintegration study, Crospovidone (3%w/w) was selected as a disintegrating agent. P-SNEDDS was blended with crospovidone and MCC after, by addition of magnesium stearate (1% w/w) and talc (1% w/w), the obtained blend was then mixed thoroughly. The resultant powder mixture was compressed to form a tablet by using a single punch tablet machine using a 10 mm circular punch. Sufficient pressure was applied to keep the hardness of 30-50N.

2.6.1 Evaluation of EZE loaded T-SNEDDS

2.6.1.1 Physical properties of tablet[16]. The prepared T-SNEDDS was evaluated for hardness, thickness, weight variation, friability, and disintegration time.

2.6.1.2 Drug content. Ten tablets were taken randomly and powdered, the tablet powder equivalent to 10 mg of EZE was accurately weighed and transferred to a 10mL volumetric flask and followed the same procedure as that of P-SNEDDS.

2.6.1.3 In-vitro dissolution study. In-vitro release studies of marketed Ezetimibe tablet (10 mg) and Ezetimibe loaded T-SNEDDS (containing 10 mg of EZE) were carried out in a similar way as that of P-SNEDDS.

3 RESULT AND DISCUSSION

3.1 Saturation solubility studies

EZE has been classified as a BCS Class II drug depending on its solubility determined under various pH conditions and its permeability through Caco-2 monolayers. EZE solubility in pH 6.8 was found to be 0.011 ± 0.2mg/mL and that in distilled water (pH 7.0) was found to be 0.00846± 0.5mg/mL. It was observed that out of 18 oils screened, 1 oil showed maximum drug solubilization capacity over all other oily phases. As shown in Figure 1**A** Capmul MCM C8 EP and Clove Oil showed a high EZE solubility capacity. Precipitation of drug was observed in clove oil after some time. Capmul MCM C8 EP is glyceryl monocaprylate. It is a glycerin mono and diester of caprylic acid (97%) and capric acid (3%). The study conducted by Patel et al. also showed the highest solubility of Gliclazide in Capmul MCM C8 so used as oil for the development of SNEDDS. According to them, drug solubility is higher in medium-chain triglycerides (MCT) than in long-chain triglycerides (LCT) because MCT has a higher ester content per gram[17]. Our results are also consistent with already published research work. Thus, Capmul MCM C8 EP was chosen as the oil phase for the SNEDDS formulation of the drug EZE.

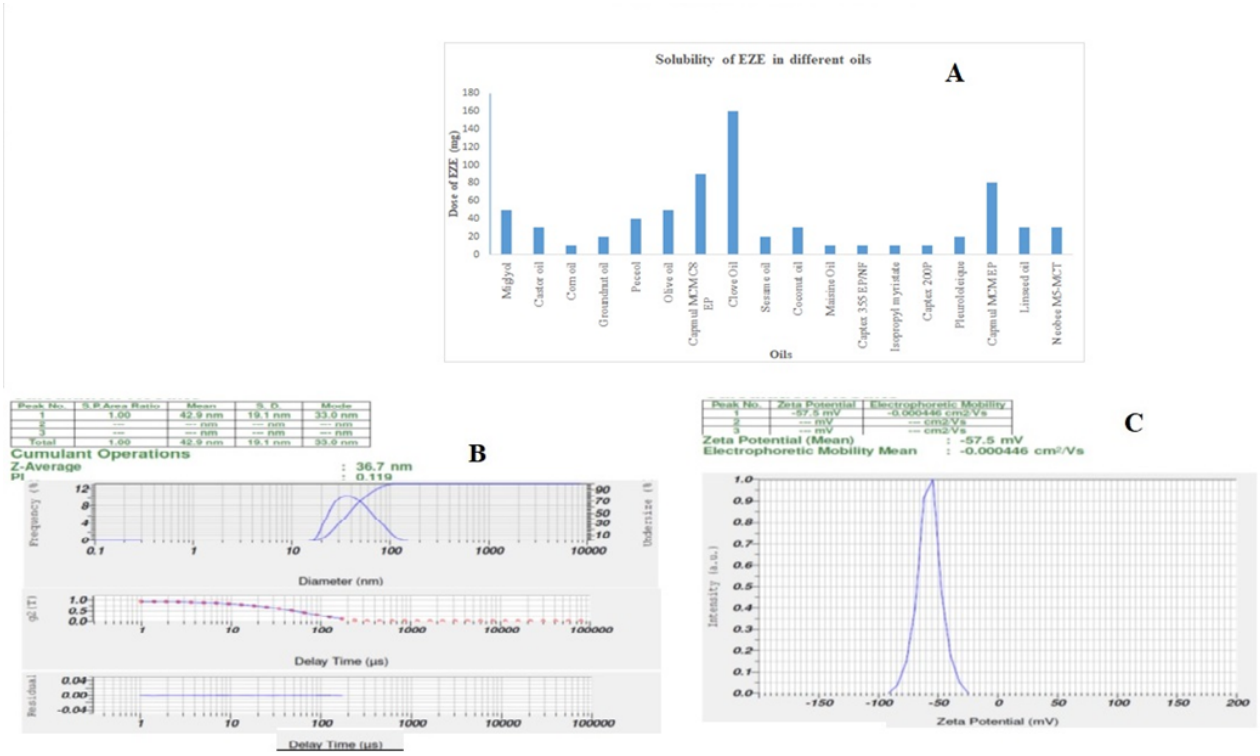

Fig 1. A: Solubilityof EZE in different oils; B: Globulesize and PI and zeta potential of optimized formulation in distilled water; C:Zeta potential of optimized formulation in distilled water

3.2 Surfactant and co-surfactant selection

The surfactants were compared for ease of emulsification for the selected oily phase i.e. Capmul MCM C8 EP. As per**Table 2**, amongst the surfactants tried Cremophore RH 40 was found to be better in emulsification ability with an average %T of 99.6, requiring 3 flask inversion for ease of emulsification, the average particle size of 35 nm, the zeta potential of -58 mV, and PI was 0.125. The appearance of the formed nanoemulsion was observed to be transparent. Non-ionic surfactants, such as Cremophore RH 40, are widely accepted for oral consumption since they are less toxic than ionic surfactants. Furthermore, Cremophore RH 40 has a bioactive effect since it inhibits the p-gp and CYP enzymes137. EZE is substantially processed by CYP enzymes, lowering its oral bioavailability[18]. Based on these reports and the results of the emulsification efficiency study, it was determined that Cr-RH 40 surfactant should be studied further to produce EZE SMEDDS, which could improve EZE oral bioavailability.

It has been already proven fact that when co-surfactants are added to the formulation containing surfactants it leads to lower the interfacial tension, decreases the bending stress, and improves the dispersibility as well as absorption of the drug from formulations [10]. Co-surfactants were added to increase the spontaneity of self-emulsification efficiency of selected surfactants Cremophore RH 40 to emulsify selected oily phase Capmul MCM C8 EP. Out of 17 co-surfactants evaluated, 9 co-surfactants **(Table 2)** were found to increase the spontaneity of self-emulsification more efficiently, out of which, Labrafil M 2125 CS was selected as a co-surfactant.

Table 2. Emulsification efficiency of surfactants for Capmul MCM C8 EP

Sr.No.	Surfactant/ Co-surfactant	% Transmittance[b] (at 638.2 nm)	No. of flask inversions	Globule size(nm)	P.I	ZetaPotential (mV)	Appearance
Surfactants							
1	Tween 80	72.9 ± 0.06	5	158.8	0.364	-69.7	Colloidal
2	Cremophore RH 40	99.6 ± 0.03	3	35.0	0.125	-58	Transparent
3	Kolliphor HS15	78.5 ± 0.15	3	67.3	0.168	-62.1	Slight bluish
4	Tween 20	13.8 ± 2.78	7	7537.3	1.698	-	Turbid
5	Span 20	38 ± 4.90	10	3974.7	0.874	-	Turbid
6	Kolliphor EL	75.3 ± 0.021	5	31.1	0.120	-71.9	Transparent
7	Poloxamer P407	38.2 ± 2.17	15	4924.8	1.037	-	Turbid
8	Poloxamer P188	50.2 ± 5.72	15	4874.4	8.736	-	Turbid
9	Gelucire 50/13	88.69 ± 0.65	5	111.3	0.201	-61.2	Slight bluish
Co-surfactants							
1	Labrafil M 2125 CS	99.5 ± 0.09	0	62.0	0.170	-75.0	Transparent with light blue ting
2	Capmul GMO50	76.8 ± 2.19	0	109.7	0.360	-65.3	Bluish white
3	Caprol PGE 860	86.5 ± 0.04	0	56.8	0.258	-63.5	Transparent light blue tinge
4	Lauroglycol FCC	89.5 ± 0.078	0	89.1	0.149	-55.0	Bluish white
5	Transcutol HP	64.31 ± 0.32	0	143.2	0.292	-60.1	Whitish
6	Labrafac lipophile WL 1349	59.77 ± 3.18	0	1964.4	0.182	-60.2	Turbid
7	PEG 600	82.93 ± 0.28	0	383.1	4.513	-65.2	Bluish white
8	Labrafil M 2130 CS	93.23 ± 0.034	0	82.0	0.268	-55.0	Bluish white
9	Transcutol P	87.885 ± 0.056	0	92.6	0.276	-61.7	Bluish white

b: Values are stated as mean (n=3)

3.3 Evaluation of optimized formulation of EZE loaded L-SNEDDS

Capmul MCM C8 EP, Cremophore RH 40, and Labrafil M 2125 CS as oil, surfactant, and co-surfactant respectively. The systems were evaluated for spontaneity of self-emulsification and ease of emulsification of systems by gentle agitation. Out of 9 formulations tried, 4 formulations showed stability in the freeze-thaw cycle. 5 formulations showed precipitation indicating instability. Out of which C2 was found to be efficient to produce fine transparent nanoemulsion which retained similar properties of the spontaneity of self-emulsification, globule size, P.I., and zeta potential even after the freeze-thaw cycle.

3.3.1 Globule size, zeta potential, PI, and drug content:

In SNEDDS, the size of the globules plays a very vital role in self-emulsification efficiency because it greatly impacts the rate and extent of the drug release from formulations as well as absorption. Furthermore, the relatively small globular size of the droplets helps to improve absorption and bioavailability [18]. In the present study, the globule size of the emulsion was found to be within the acceptable range for optimized formulation (C2) i.e., <100 nm (Figure 1 B) which might be helpful for the permeation of the drug delivery system through various biological membranes. Furthermore, the nonionic surfactant used in this study can also enhance the penetration of the EZE through epithelial lining by enhancing solubility and dissolution of the

drug as well as by reducing the interfacial tension between the two phases [17]. The results of globule size, zeta potential, and PI are presented in Table 3.

EZE L-SMEDDS was found to have a polydispersity index of 0.119 (Figure 1B). The findings of this study demonstrated that the optimized EZE L-SMEDDS produced fine microemulsions with a small mean size and a narrow particle size distribution. Dispersion with a high zeta potential (negative or positive) is usually electrically stabilized since it prevents aggregation owing to electric repulsion (Figure 1C). When the zeta potential is low, the attraction takes precedence over repulsion, resulting in coagulates or flocculates. This study used a nonionic surfactant that reduced zeta potential values. Furthermore, the optimized batch remained stable throughout the study period, suggesting that steric contributions from nonionic surfactants may promote stability in such systems. Our results are more promising than those reported by Patel et al., [17]. When the surface charge (Zeta potential) on oil globules is positively charged, emulsions become electrostatically attracted to the mucosal cell surface, resulting in increased drug bioavailability.

Drug content of optimized L-SNEDDS (C2) was observed in the acceptable range i.e., 98.43±1.54%. Results suggested that EZE is nicely dissolved and uniformly distributed in a self-emulsifying system. The higher drug content was observed due to the higher solubility of the EZE in oil, surfactant, and co-surfactant system resulting in the uniform distribution and solubilization of the drug in a self-emulsifying system.

Table 3. Results of all formulation trials

Formulations	Stability to freeze thaw cycle	% Transmittance at 638.2nm		Flask Inversions (FI)	Globule size (nm)		P.I		Zeta potential (mV)	
		Before freeze thaw	After freeze thaw		Before Freeze-thaw	After freeze thaw	Before Freeze-thaw	After freeze thaw	Before Freeze-thaw	After freeze thaw
C1	Stable	92.7	91.4	0	75.0	37.3	0.145	0.340	-3.8	-4.5
C2	Stable	99.5	99.3	0	54.3	46.2	0.222	0.180	-1.4	-1.4
C3	Unstable	89.6	-	0	44.8	-	0.145	-	-1.4	-
C4	Unstable	85.54	-	0	79.0	-	0.296	-	-4.8	-
C5	Unstable	93.58	-	0	60.1	-	0.365	-	-0.5	-
C6	Stable	84.02	85.35	0	337.2	298.7	0.275	0.436	-12.0	8.0
C7	Unstable	97.16	-	0	51.8	-	0.119	-	1.0	-
C8	Stable	94.5	93.8	0	435.1	307.0	0.131	0.247	0.4	8.0
C9	Unstable	99.24	-	0	38.3	-	0.359	-	-5.5	-

3.3.2 Robustness to dilution

Formulation C2 was found robust to all dilutions as globule size remained nearly constant below 100nm on dilution with different pH solutions. Even after 24 hours, no drug precipitation was detected with the steady increase in dilution and change in dilution media. Drug precipitation is more likely to have occurred at higher dilutions in vivo, which might have a major impact on drug absorption and performance. To verify that a consistent emulsion is created from SNEDDS, robustness to dilution was tested. The findings showed that the optimized formulation was dilution robust and would sustain its efficacy in vivo. Details are provided in Table 4 **.**

Table 4. Globule size, zeta potential, PI, and %Transmittance (%T) after dilution

Dilutions	pH 1.2				pH 6.8			
	Average %T	Globule Size (nm)	PI	Zeta Potential (mV)	Average %T	Globule Size (nm)	PI	Zeta Potential (mV)
100	98.04 ±0.04	75.7 ±5.01	0.381 ± 0.02	-1.8 ± 0.4	93.39 ± 0.03	36.7± 0.25	0.019 ±0.04	-5.8 ±0.26
1000	97.54 ± 0.002	54.0 ±0.35	0.243 ± 0.06	-4.8 ±1.62	98.97 ± 0.04	53.4± 0.43	0.142 ±0.03	-3.8 ± 0.40
10000	99.45 ±0.002	49.7 ±3.25	0.184 ±0.14	-2.0 ±0.14	99.84 ±0.01	55.4± 4.47	0.232 ±0.07	-5.2 ±0.34

d: Values are stated as mean (n=3)

3.3.3 In vitro dissolution and Ex-vivo permeability study

The comparative cumulative drug release of L-SNEDDS and plain drugs is shown in Figure 2 A. EZE L-SNEDDS showed faster in vitro drug release as compared to plain EZE at pH 1.2 and pH 6.8. C2 showed nearly 94% release in SIF at 20 min and about 98% drug release was observed in SGF at 15 min, It showed the release of EZE from C2 was not dependent on the pH of dissolution media. Plain EZE showed a poor rate of dissolution (less than 20% and less than 85% in SIF and SGF release at the end of 60 min respectively) in both the dissolution media compared to C2. The rate and extent of EZE released from C2 were excellent suggesting that L-SNEDDS may improve the oral bioavailability of EZE. EZE belongs to the BCS class II having lower solubility and higher permeability. Its pharmaceutical application is limited due to low solubility, dissolution, and bioavailability. In L-SMEDDS, the EZE is present in a dissolved form which produces the very fine globules and gets dispersed immediately in all pH conditions. Similar observations were reported by Yadav et al[5]. Because the rate and amount of EZE release from C2 were excellent, L-SMEDDS may increase EZE oral bioavailability.

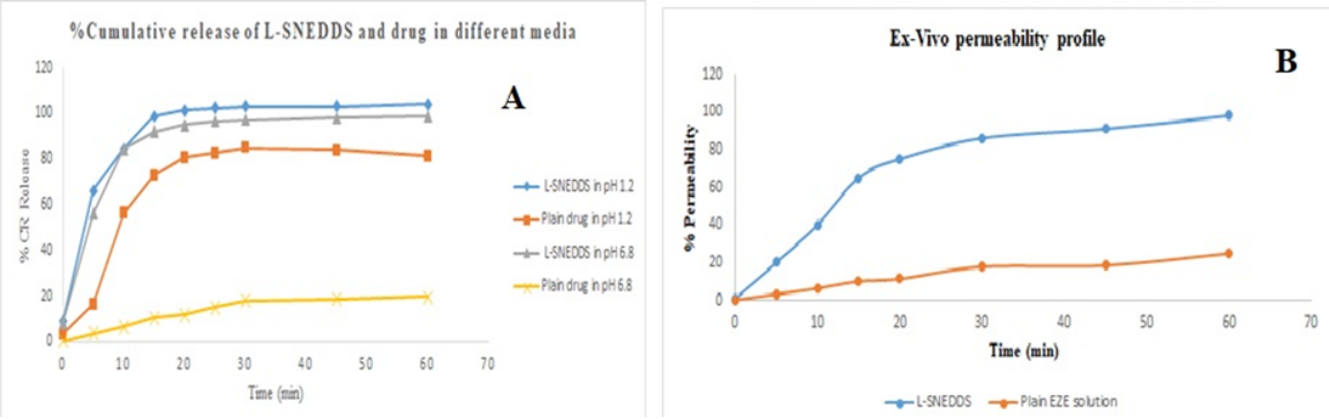

Fig 2. In vitro dissolution study of L-SNEDDS and plaindrug in pH 1.2 and 6.8; B: Ex-vivo permeability profile by everted sactechnique

L-SNEDDS produced fine, clear, and transparent nanoemulsion in Krebs-Ringer buffer solution, and % T was found to be 98.12%. A significant increase in permeability of EZE was observed from L-SNEDDS as compared to plain drugs. From Figure 2B, it was observed that 25% of EZE was transported through the intestinal lumen from EZE solution and 98% of EZE was transported through the intestinal lumen from nano emulsion produced from L-SNEDDS formulation. Such improvement in permeability was observed due to uniformly dispersed nano-size globules of EZE which increases surface area and improve the drug release.

3.4 Evaluation of P-SNEDDS

Neusilin US2 is a synthetic magnesium aluminometasilicate that comes in the form of an amorphous fine powder or granules, has a large specific surface area (300 m^2/g) and high oil adsorption capacity, is extremely porous, heat resistant, and has a long shelf life. This multipurpose excipient is frequently used to improve tablet, powder, granule, and capsule quality. The Neusilin US2 may also adsorb up to 2.2mL/g of lipid formulations including Cyclosporine[19]. Stirring with glass rod method was selected as an adsorbent technique for the ratio of NeusilinUS2: L-SNEDDS (125:250) was 1:2 as it gives free-flowing powder with a final weight of 375 mg/unit.

3.4.1 Powder flow properties, reconstitution properties, and drug content of P-SNEDDS

Despite adsorbing almost double the amount of L-SMEDDS compared to MCC and Aerosil 200P, Nesulin US2 (NU2) has a similar flow property to MCC and Aerosil 200P. For all three adsorbing agents used to manufacture P-SMEDDS, Carr's index was determined to be "Good" (in the range of 12.50 to 13.33) and the Hausner ratio was found to be below 1.20. These results of the flow properties suggested that Neusilin US2 (NU2), despite adsorbing double the quantity of L-SNEDDS possesses the excellent flow property. By assessing globule size, P.I., and zeta potential of reconstituted P-SMEDDS solution, the effect of solidification of L-SMEDDS formulation by adsorbing on Neusilin US2 by physical mixing was examined. The globule size was found less than 40 nm in both SGF and SIF reconstituted formulation. Also, PI of 0.346 and 0.171 was observed in SGF and SIF reconstituted formulation. Zeta potential values (5.8 in SGF and -1.2 in SIF) confirmed the stability in both reconstitution media. Drug content of P-SNEDDS was observed 102.6± 4.87%. The results indicated that the L-SNEDDS was uniformly adsorbed

and distributed over the adsorbing agent; Neusilin US2 resulting in higher drug content.

3.4.2 DSC thermogram

The DSC thermograms of plain EZE and P-SNEDDS formulation are shown in Figure 3A and B. A very sharp endothermic peak was observed at 160.87°C for pure EZE in crystalline form. The absence of noticeable EZE peaks in the P-SNEDDS formulation implies a shift in EZES melting behavior and crystallization inhibition following lipid surfactant solubilization and physical mixing with a solid carrier.

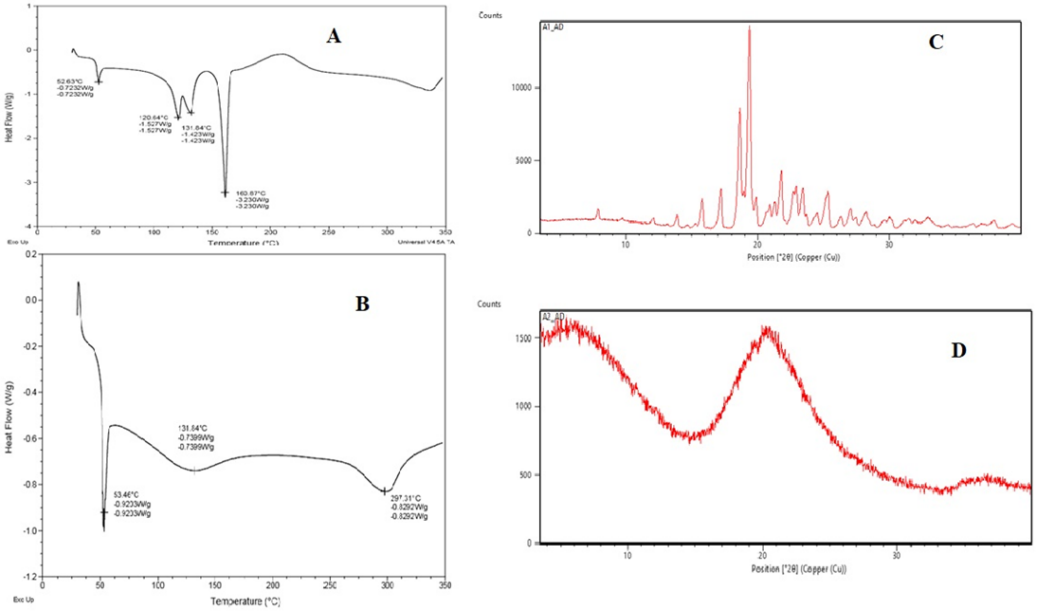

Fig 3. A: DSC of EZE; B: DSC of P-SNEDDS of EZE; C: X-ray diffractogram of EZE; D: X-ray diffractogram of P-SNEDDS

3.4.3 X-Ray Diffraction Analysis

The X-ray diffractograms of plain EZE and EZE P-SNEDDS were presented in Figure 3 C and D. It is evident from the obtained results that, EZE had sharp peaks at the diffraction angles of 2θ 3.5114°, 7.8769°, 9.4877°, 12.0393°, 13.8491°, 15.7306°, 17.1449°,20.6525°, 21.7417°, 22.9173°, 25.1393°, 26.9912°, 31.0900°, 37.0721°, 39.9764° showing a typical crystalline pattern. Less intense peaks of P-SNEEDS were observed at diffraction angles of 2θ 20.311°, and 35.354° thus demonstrating the crystalline nature of EZE has changed to an amorphous form. Like the DSC results, in the P-SNEDDS formulations prepared with Neusilin US2, EZE was observed in an amorphous state.

3.4.4 Surface Morphology study of EZE and Neusilin US2 by Scanning Electron Microscopy

Photo image obtained by SEM analysis is presented in Figure 4 A and B. EZE appeared to be made up of rod shape crystalline structures of nearly 1 μm size. Neusilin US2 looks like spherical porous particles of size 50μm. Micrographs of P-SNEDDS showed L-SNEDDS adsorbed on the surface of Neusilin US2 are shown in Figure 4C. The formulation process was carried out using adsorption, in the field of vision partially enclosed Neusilin US2 was observed. The crystalline nature of EZE was not observed in P-SNEDDS micrographs showing that the drug is in dissolved form in the P-SNEDDS.

3.4.5 In vitro dissolution study

No significant difference was observed in the rate and extent of drug release from P-SMEDDS of EZE as compared to L-SMEDDS of EZE. More than 85% release was observed from P-SMEDDS irrespective of dissolution media. In comparison to plain EZE powder, P-SMEDDS showed a high rate and extent of drug release (Figure 5 A and B). The study suggested that in vitro dissolution profile of L-SMEDDS was not changed even after converting it to P-SMEDDS. After dispersing in dissolution media, Neusilin US2 rapidly releases the L-SMEDDS in dissolution media, which further spontaneously dispersed and produce fine microemulsion and maintained the EZE in solubilized form.

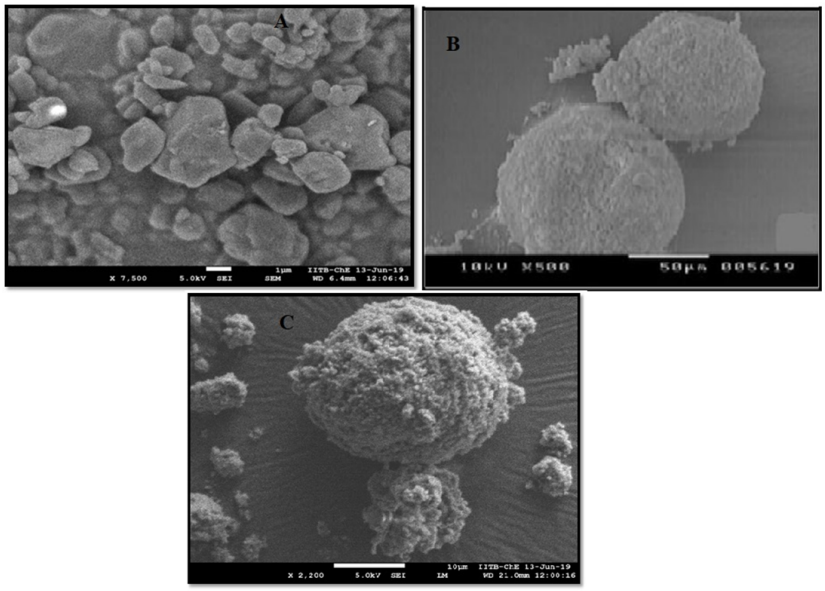

Fig 4. A: Morphology of EZE; B:Morphology of Neusilin US2; C: Morphology of P-SNEDDS

3.5 Development of EZE loaded Tablet SNEDDS (T-SNEDDS and characterization)

Initially, the EZE P-SNEDDS was blended with directly compressible diluents; MCC (Avicel PH 102), magnesium stearate, and talc, to which the super disintegrant crospovidone was added (2 to 3.5 %), and the compressed tablet of this mixture was evaluated for disintegration time. Based on disintegration time crospovidone (3%) was selected as the optimized super disintegrating agent to produce sufficiently hard (30-50 N), non-friable (< 1%), rapidly disintegrating (within 1min) T-SNEDDS of EZE (C2). Table 5 depicts the results of parameters evaluated for T-SMEDDS of EZE. The average weight of tablets did not deviate more than 5% w/w and none of the tablets deviated by 10% w/w of average weight, indicating that tablets passed the weight variation test. The produced tablets were found to be hard enough (35-45 N). The average disintegration time of 6 tablets was found to be 15 to 43 sec showing the ability of crospovidone as super disintegrants in spontaneity to disperse the tablet in a dissolution medium. T-SMEDDS of EZE passes the friability test (<1%). The all-physical parameters were found to be within an acceptable range.

Table 5. Evaluation of T-SMEDSS

Sr. No	Parameters	Observation	Inference
1	Drug content (%)	104.23 ± 0.03%	Within the acceptable limit
2	Weight variation	495 mg-505 mg	Passes the test
3	Thickness	4.2 ± 0.06mm	Within the acceptable limit
4	Hardness	35-45 N	Within the acceptable limit
5	Disintegration time		
6	In 6.8 pH	15 sec	Passes the test
7	In 1.2 pH	43 sec	
8	Friability	0.6048%	Passes the test

The drug dissolution from T-SMEDDS was found similar to L-SMEDDS and P-SMEDDS, indicating that the self-emulsifying properties of P-SMEDDS were unaffected following compression to produce a tablet. In comparison with a marketed tablet, it was observed that marketed tablet preparation of EZE releases 85.89%, and 62.47 % of EZE was released in 0.1 N HCl, and pH 6.8 phosphate buffer (Figure 5 C and D). On the other hand, at 60 min complete drug was released from T- SMEDDS irrespective of dissolution media. These results indicate that the marketed tablet formulation of EZE is slower than the self-emulsifying tablet formulation of EZE.

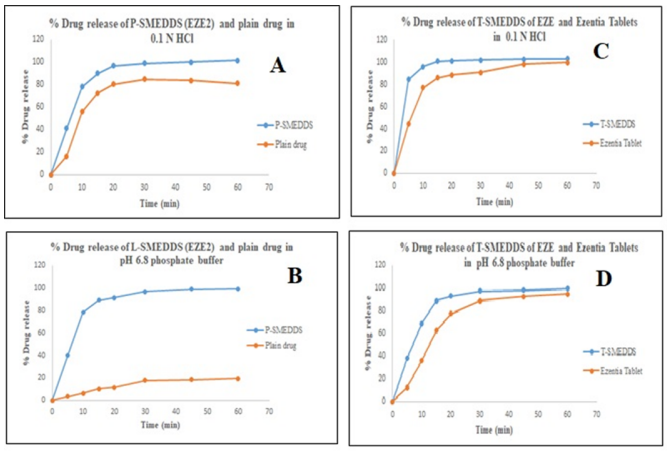

Fig 5. A:In-vitro dissolution of P-SMEDDS (EZE2) and plain drug in 0.1 N HCl; B: In-vitro dissolution of P-SMEDDS (EZE2) and plain drug in pH 6.8 phosphate buffer; C: In-vitro dissolution of T-SMEDDS of EZE and Ezentia Tablets in 0.1 N HCl; D: In-vitro dissolution of T-SMEDDS of EZE and Ezentia Tablets in pH 6.8 phosphate buffer

4 CONCLUSION

The present study demonstrates the successful conversion of liquid SNEDDS to solid tablets with enhanced solubility and bioavailability. In the present study, the globule size of the emulsion was found to be within an acceptable range i.e., <100 nm with a PDI of 0.119. Zeta potential study revealed the stability of the liquid SNEDDS. Drug content was observed in the acceptable range of 98.43±1.54%. C2, optimized formulation showed nearly 94% release in SIF at 20 min, and about 98% drug release was observed in SGF at 15 min. Improvement in permeability was observed due to uniformly dispersed nano-size globules of EZE which increases surface area and improve the drug release. Drug content of P-SNEDDS developed with NeusilinUS2: L-SNEDDS (1:2) was found to be 102.6± 4.87%. Analytical characterization demonstrated no drug excipient compatibility with amorphous characteristics of the EZE. The crystalline nature of EZE was not observed in P-SNEDDS micrographs showing that the drug is in dissolved form in the P-SNEDDS. In comparison to plain EZE powder, P-SMEDDS showed a high rate and extent of drug release. The developed tablets from P-SNEDDS with Crospovidone showed excellent drug release of L-SMEDDS and P-SMEDDS without affecting self-emulsifying properties. Conversion of L-SNEDDS into P-SNEDDS was found to be a potential approach to overcome the limitations of incompatibility with hard gelatin capsules and to improve patient compliance.

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