Potential Investigation of Peceol for formulation of Ezetimibe self nano emulsifying Drug Delivery Systems

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ABSTRACT :

The present work was aimed at formulating and physicochemical characterization SNEDDS (self Nano emulsifying drug delivery system) of ezetimibe and evaluating its in vitro and in vivo potential. The solubility of both drugs was determined in excipient screening studies. Pseudoternary phase diagrams were used to evaluate the Nano emulsification existence area, composed of different surfactants, co surfactants, and oils at different surfactant to-cosurfactant (S/CoS) ratios, and the system exhibiting the largest percentage area of the self-Nano emulsifying region was selected and optimum oil ratio in the SNEDDS was selected by evaluating the clarity, precipitation and mean droplet size of the resultant Nanoemulsions. The release rate of ezetimibe was investigated using an in vitro dissolution test. In vitro dissolution studies showed that the SNEDDS composed of Peceol (20% wt/wt), Tween 80 (30% wt/wt), Ethanol (30% wt/wt), and ezetimibe (20% wt/wt) had higher initial dissolution rates for both drugs when compared with plain EZT. More importantly, EZT SNEDDS had a significantly increased dissolution profile in distilled water and pH 4.0 acetate buffer, implying enhanced bioavailability. The underlying mechanism of the loading capacity of EZT was elucidated by measurement of the zeta potential analysis. The results implied that EZT was located both in the Nanoemulsion core and the surfactant–cosurfactant layer. Comparative pharmacokinetic evaluation of EZT SNEDDS was investigated in terms of C max, tmax and AUC using rats. The SNEDDS formulation significantly increases pharmacokinetics parameter as compared with plain ezetimibe. The optimized formulation was then subjected to stability studies and was found to be stable over 6 months. Thus, the study confirmed that the SNEDDS formulation can be used as a possible alternative to traditional oral formulations of ezetimibe to improve its bioavailability

Keyword: Ezetimibe, Self nano emulsifying drug delivery systems (SNEDDS), Pseudoternary phase diagrams, Pharmacokinetic.

INTRODUCTION:

Lipid-based formulation approaches, particularly the self Nano emulsifying drug delivery system (SNEDDS), are well known for their potential as alternative strategies for delivery of hydrophobic drugs, which are associated with poor water solubility and low oral bioavailability [1-4]. Nanoemulsions are thermodynamically stable, isotropically clear dispersions of two immiscible liquids, such as oil and water, which are stabilized by amphiphile [5-6]. Pre-concentrates of Nanoemulsions which have transparent isotropic properties without water are known as self-Nano emulsifying drug delivery systems (SNEDDS) [6]. SNEDDS formulations are isotropic mixtures of an oil, a surfactant, a cosurfactant (or solubilizer), and a drug. The basic principle of this system is its ability to form one oil-in-water (o/w) Nanoemulsions under gentle agitation following dilution by aqueous phases (i.e., the digestive motility of the stomach and intestine provide the agitation required for self-emulsification in vivo in the lumen of the gut. This spontaneous formation of an emulsion in the gastrointestinal tract presents the drug in a solubilized form, and the small size of the formed droplet provides a large interfacial surface area for drug absorption.6 Apart from solubilization, the presence of lipid in the formulation further helps improve bioavailability by affecting the drug absorption [7]. Selection of a suitable self emulsifying formulation depends upon the assessment of (1) the solubility of the drug in various components, (2) the area of the self-emulsifying region as obtained in the phase diagram, and (3) the droplet size distribution of the resultant emulsion following self-emulsification.
Ezetimibe (EZT) is a lipid-lowering drug that inhibits intestinal uptake of dietary and biliary cholesterol

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without affecting the absorption of fat-soluble nutrients. However, EZT has a very low solubility and dissolution rate resulting in highly variable bioavailability, which is also in part due to extensive efflux by p-glycoprotein (P-Gp) [8]. When co-administered with statins, EZT provides significant incremental reductions in low density lipoprotein cholesterol and triglycerides and increases in high density lipoprotein cholesterol when compared with statin monotherapy. EZT is a practically insoluble and weakly basic compound with solubility 0.012 mg/mL, and p*K*a of 9.75 [8].

Application of SNEDDS technology to EZT is a promising strategy to improve their bioavailability. It has been reported that surfactants commonly used in SNEDDS can inhibit P-GP efflux of various drugs, including EZT [9-11]. Furthermore, oils used in SNEDDS can enhance lymphatic transport of drugs, bypassing hepatic first-pass metabolism [12]. These properties may be optimal for solubilizing EZT, ultimately increasing bioavailability of drug. In this study, a self-Nano emulsifying drug delivery system containing EZT was developed to enhance the therapeutic effects of drug by solubilization of EZT. The objective of the present study was to develop a SNEDDS containing EZT and enhance *in vitro* dissolution properties for enhanced bioavailability. The main objectives of the study were to develop and evaluate an optimal SNEDDS formulation containing EZT and to assess its Pharmacokinetics effect in terms of Cmax, Tmax and AUC.

MATERIALS

The following materials were obtained from the indicated sources and used without further purification. Ezetimibe was obtained as a gift sample from Sun Pharmaceuticals (Mumbai, India). The following materials were donated by Gattefosse (Mumbai, India) and were used as received: Peceol, Ethyl oleate, Oleic acid, Sunflower oil, Labrafac CM10 (C8 -C10 polyglycolized glycerides), Maisine 35-1 (glyceryl monolinoleate), Lauroglycol FCC (propylene glycol laurate), Labrafil 1944 CS (apricot kernel oil polyethylene glycol [PEG] 6 esters), and Labrafac PG (propylene glycol caprylate/caprate). Cremophor RH 40 (polyoxyl 40 hydrogenated castor oil), Cremophor EL (polyethoxylated castor oil), and Gelucire 44/14 (PEG-32 glyceryl laurate) and 50/13 (PEG-32 glyceryl palmistearate) were received from Colorcon Asia (Mumbai). Span 20 (sorbitan monolaurate), Tween 80 (polyoxyethylene sorbitan monooleate), and PEG 400 were bought from Merck (Mumbai, India). Deionized water was prepared by a Milli-Q purification system from Millipore (Molsheim, France). Acetonitrile and methanol used in the present study were of high performance liquid chromatography (HPLC) grade. All other chemicals were reagent grade. **Animals** Male Albino Wistar rats (weighing approximately 150-200 g) were used for the pharmacokinetics studies. The animals were maintained at a constant light (14L: 10D), temperature (24°C-25°C), and humidity (60%) and were supplied with food and water. The animal requirement was approved by the Institute Animal Ethics Committee (IAEC), and all experiments were conducted as per the norms of the Committee for the Purpose of Supervision of Experiments on Animals (CPCSEA, New Delhi), India.

METHODS

Solubility Studies

The solubility of EZT in various components (oils, surfactants, and co surfactants) excipients was determined. Each of the selected solvents was added to a screw-cap tube followed by excess quantities of EZT. The mixtures were capped and mixed for 5 min using a vortex mixer. The mixtures were then agitated at 150 rpm in a shaking water bath (REMI-, Mumbai, India) at 40°C for 72 h to reach equilibrium. After reaching equilibrium, each tube was centrifuged at 5000 rpm for 10 min (REMI-, Mumbai, India). The supernatants were filtered using a membrane filter (0.45 µm, 13 mm, Whatman, Mumbai, India) and the filtrates were diluted with methanol. The samples were then analyzed using a validated UV method [13-14]. The UV Visible Spectrophotometer (Agilent Corp., Germany) was utilized for analysis purpose using the detection wavelength 235 nm.

Primary screening of SNEDDS components (Oil, surfactant and co-surfactant)

Selection of oil was based upon solubility of drug in different oils. Selection of surfactant was depending upon the hydrophobic lipophilic balance (HLB) system; the surfactant in combination with co-surfactants was tried for screening a stable Nano emulsion system which could incorporate the optimum amount of internal phase [15-16]. The combination of surfactants and Co-surfactant which shows higher Nanoemulsion region was selected for further process.

Pseudoternary Phase Diagrams

Pseudoternary phase diagrams of oil, surfactant/ cosurfactant (S/CoS), and water were developed using the water titration method.

From the result of solubility studies and screening of excipients, Peceol, Tween 80, ethanol was selected as oily phase, surfactant and co-surfactant respectively [17]. A ratio of surfactant over co-surfactant (Km) i.e. S/Co was chosen and the corresponding mixture (S_{mix}) was made. At desired Km value (1:1, 2:1, 3:1,

4:1, 5:1) S_{mix} and oil were mixed at ratio of 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2 and 9:1 in pre-weighed ratio and diluted with water in a dropwise manner. For each phase diagram at a specific ratio of S_{mix} a transparent and homogenous mixture of oil and S/Co was formed by vortexing for 5 minutes. Then each mixture was titrated with water and visually observed for phase clarity and flowability. The amount of water added to the oil, surfactant, and cosurfactant mixture was recorded at the point of phase transition determined by visual inspection. The concentration of water at which turbidity-to-transparency and transparency-to-turbidity transitions occurred was derived from the weight measurements. These values were then used to determine the boundaries of the Nanoemulsion domain corresponding to the chosen value of oils, as well as the S/CoS mixing ratio [5, 13, 18]. To determine the effect of drug addition on the Nanoemulsion boundary, phase diagrams were also constructed in the presence of drug using drug-enriched oil as the hydrophobic component. Phase diagrams were then constructed using Chemix- School 9.0 software.

Preparation of SNEDDS Containing EZT

A series of SNEDDS formulations were prepared using Tween 80 and Ethanol as the S/CoS combination and Peceol as the oil (Table 1). In all the formulations, the level of EZT was kept constant. First, surfactant, and cosurfactant were accurately weighed in a glass vial and mixed by a magnetic stirrer. Then, the oil was added into the vial and continuously mixed for 5 min. EZT was slowly added to the resultant mixture followed by continuous stirring until a homogeneous, transparent mixture was obtained. The mixture was then stored in a glass vial in ambient temperature [5].

CHARACTERIZATION OF L-SNEDDS

Dispersibility tests or self emulsification efficiency

The efficiency of self-emulsification of oral Nano emulsion was assessed using a standard USP II dissolution apparatus. In brief 1 ml of each formulation was added to 250 mL of round bottom jar with a stainless steel dissolution paddle rotating at 50 rpm for gentle agitation in distilled water at 37 ± 0.5^{O}C. Precipitation/ Dispersibility were evaluated by visual inspection of the resultant emulsion after 24 hours [19-20]. The formulations were then categorized as clear (transparent or transparent with bluish tinge), non-clear (turbid), stable (no precipitation at the end of 24 hours), or unstable (showing precipitation within 24 hours) and graded as (A) Rapidly forming, slightly less clear emulsion, having a bluish white appearance; (B)Fine milky emulsion that formed within 2 minutes; (C) Dull, grayish white emulsion having slightly oily; (D) Appearance that is slow to emulsify (longer than 2 min); (E)Formulation, exhibiting either poor or minimal emulsification with large oil globules present on the surface

Globule Size Analysis

One hundred Nanoliters of each SNEDDS formulation was diluted to 250 mL in a beaker and gently mixed using a glass rod. The resultant emulsion was then subjected to particle size analysis (using Malvern Mastersizer (Zetasizer nano S90, Malvern Instruments Ltd., Malvern, U.K.) equipped with 2000 Hydro MU) with a particle size measurement range of 0.02 to 2000 µm. The mean droplet sizes and polydispersity index of the Nanoemulsions were analyzed using dynamic light scattering (DLS) at 25°C with a scattering angle of 90°. Particle size was calculated from the volume size distribution [21-22]. All studies were repeated in triplicate, with good agreement being found between measurements.

Zeta Potential

In addition, zeta potential of the same sample was analyzed using laser Doppler electrophoresis (Zetasizer 3000 HAS, Malvern Instruments Ltd.) at 25°C. The electrical potential in the plane of share of the charged Particle is called zeta potential. Zeta potential can be measured using method of electrophoresis [23-25]. Zeta potential predicting the stability of dispersion system it can be shown as resulting data.

Percent transmittance

As Nano emulsion is clear, isotropic in nature, its percent transmittance was determined with the help of UV-Visible Spectrophotometer (Agilent Corp, Germany). UV spectroscopy used to determine absorbance and transmittance from the liquid which is determinant of its quality [26]. 1 ml of Liquid SNEDDS formulation diluted up to 100 ml distilled water and observed for % transmittance and turbidity using water as blank.

In Vitro **Dissolution Studies**

The quantitative in vitro release test was performed in 900 mL of buffer pH 1.2 using dissolution apparatus XXVII (Electrolab, Mumbai, India). The paddles were rotated at 100 rpm. The SNEDDS formulations were put into hard gelatin capsules (0 sizes) and used for drug release studies; results were compared with those of plain EZT. During the release studies, a 5-mL sample of medium was taken out and subjected to drug analysis using UV Spectrophotometer. Aliquots of 5 mL were withdrawn and filtered using a 0.45μm filter at predetermined time intervals of 5, 10, 15, 30, and 60 min. The volume removed from each solution was replaced immediately with fresh dissolution medium. The drug concentration in the samples was de-

termined using the UV analysis method described in the solubility studies. For determination of the in vitro dissolution of plain EZT, the medium was changed to buffer pH 1.2 containing Tween 80 (equivalent to the amount used in the formulation) [8, 10, 12]. Dissolution studies were also performed in other media (buffer pH 4.5 and 7.2) to examine the effect of pH on drug release.

Thermodynamic Stability Studies

The SNEDDS formulations were put into empty hard gelatin capsules (size 0) and subjected to stability studies at 25°C/60% relative humidity (RH), 30°C/65% RH, and 40°C/75% RH. Samples were charged in stability chambers (Remi, Mumbai, India) with humidity and temperature control. They were withdrawn at specified intervals for analysis over a period of 6 months for intermediate and accelerated conditions [27-28]. Drug content of the capsules was analyzed using a previously developed and validated stability-indicating UV method.

Heating cooling cycle

The successive heating and cooling leads to the instability in the emulsion. So it was used as a tool to determine the stability of the emulsion. Liquid SNEDDS of Ezetimibe was diluted with deionized water (1:20) and subjected to six cycle of heating (upto 45°C for 48 hrs) and cooling (upto 4°C for 48 hrs). The sign of any phase separation was observed and stable formulation was subjected to centrifugation.

Centrifugation test

L-SNEDDS of Ezetimibe was diluted with deionized water (1:20) and 5 ml of L- SNEDDS was filled in a centrifuge tube. The tubes containing SNEDDS were subjected to centrifugation at 3500 rpm for 30 minutes and formulation was observed visually for phase separation.

Freeze Thawing

Freeze thawing was employed to evaluate the stability of formulations. The formulations were subjected to 3 to 4 freeze-thaw cycles, which included freezing at –4°C for 24 hours followed by thawing at 40°C for 24 hours. Centrifugation was performed at 3000 rpm for 5 minutes. The formulations were then observed for phase separation. Only formulations that were stable to phase separation were selected for further studies.

Drug content

The amount of Ezetimibe present in the liquid SNEDDS was determined usimg UV spectroscopic method. The 1 ml of L-SMEDD was dissolved in 5 ml of ethanol and volume was made upto 10 ml with distilled water [8]. The solution was filtered and from filtrate 1 ml was taken and diluted up to 100 ml with distilled water and analyzed at 235 nm using UV–visible spectrophotometer (Shimedazu corp, Japan).

Pharmacokinetics Studies

Male Albino Wistar rats (weighing approximately 150-200 g) were used for the pharmacokinetics studies. The animals were maintained at a constant light (14L: 10D), temperature (24°C-25°C), and humidity (60%) and were supplied with food and water before they were distributed by weight into experimental groups.

The rats fasted overnight and were then orally administered with 10 mg/kg for study. Control groups of rats were given the vehicle (plain saline), and experimental groups were given plain EZT (10 mg/kg body weight) or the SNEDDS formulation (equivalent to 10 mg/kg EZT). Without anesthesia and by restraining rats by hand, the oral dosing was performed by intubation using an 18-gauge feeding needle (the volume to be fed was 1.0 mL in all cases). Blood samples were drawn in Nanocentrifuge tubes at 0 hours, 24 hours, and 48 hours. Serum was separated by centrifugation at 10000g and used for Pharmacokinetics analysis [29-30]. The plasma was separated and stored at –20°C until drug analysis by HPLC (Agilent TC C18, Inertsil ODS-3V C18 Column, (4.6 mm (D) x 250 mm.). Pharmacokinetic parameters such as AUC, C_{max}, t_{max} were calculated from plasma profile curve. Statistical analysis of the collected data was performed using 1-way analysis of variance.

RESULT AND DISCUSSION

Solubility Studies

Solubility Studies One important consideration when formulating a self- emulsifying formulation is avoiding precipitation of the drug on dilution in the gut lumen in vivo. Therefore, the components used in the system should have high solubilization capacity for the drug, ensuring the solubilization of the drug in the resultant dispersion. As higher drug solubility in excipients enables higher loading capacity in the SNEDDS, the solubility of EZT in oils, surfactants and co-surfactants were determined as a screening test for development of the SNEDDS formulation. As the formulation incorporates the EZT, the main priority in this study was to find excipients with suitable solubility for drug to enhance drug loading capacity [31].

The oil not only solubilizes the required dose of drug but also facilitates the self emulsification of fraction of drug and transported via the intestinal lymphatic system. Oleic acid, Almond oil, Castor oil, Coconut oil, Sunflower oil were the oils selected for testing; Results from solubility studies are reported in Figure 1. As seen from the figure, Peceol (175±1.5 mg/ml) showed the highest solubilization capacity for Ezetimibe, followed by Maisine 35-1 (145±2.3 mg/ml) and Ethyl oleate (123±1.6 mg/ml).

Table 1: Developed Formulations of L- SMEDDS with Their Compositions

Batch Code	Drug (mg)	%Composition (w/w)			
		Peceol	Tween 80	Ethanol	Smix
A	1000	10	25	25	50
B	1000	15	27.5	27.5	55
C	1000	20	30	30	60

Table 2: Selected composition and Evaluation Parameters of SMEDDS formulations

Formulation	Drug (mg) /10g	% Composition (W/W)				Dispersion Time (Sec)	Clarity	Precipitation
		Oil	Tween 80	Ethanol	Smix			
A	1000	10	25	25	50	70 ±4	Clear	Unstable
B	1000	15	27.5	27.5	55	75±2	Clear	Unstable
C	1000	20	30	30	60	72±5	Clear	Unstable

Table 3: Thermodynamic stability testing of SMEDDS (Mean ±S.D. n=3)

Type of stability Study	Phase separation	Phase inversion	Precipitation	Creaming	Cracking	Viscosity (cPs)	Drug Content (%)
Heating cooling cycle (4°C and 45°C,48 hrs)	N	N	N	N	N	12.323 **±1.01**	98.78 ±1.19
Centrifugation test (3500 rpm, 30 min)	N	N	N	N	N	17.175 **±0.9**	99.11 ±1.19
Freeze thaw cycle (3-4 freeze thaw cycle, -21°C and +25°C, 48 hrs)	N	N	N		N	19.253 **±1.5**	99.44 ±1.16

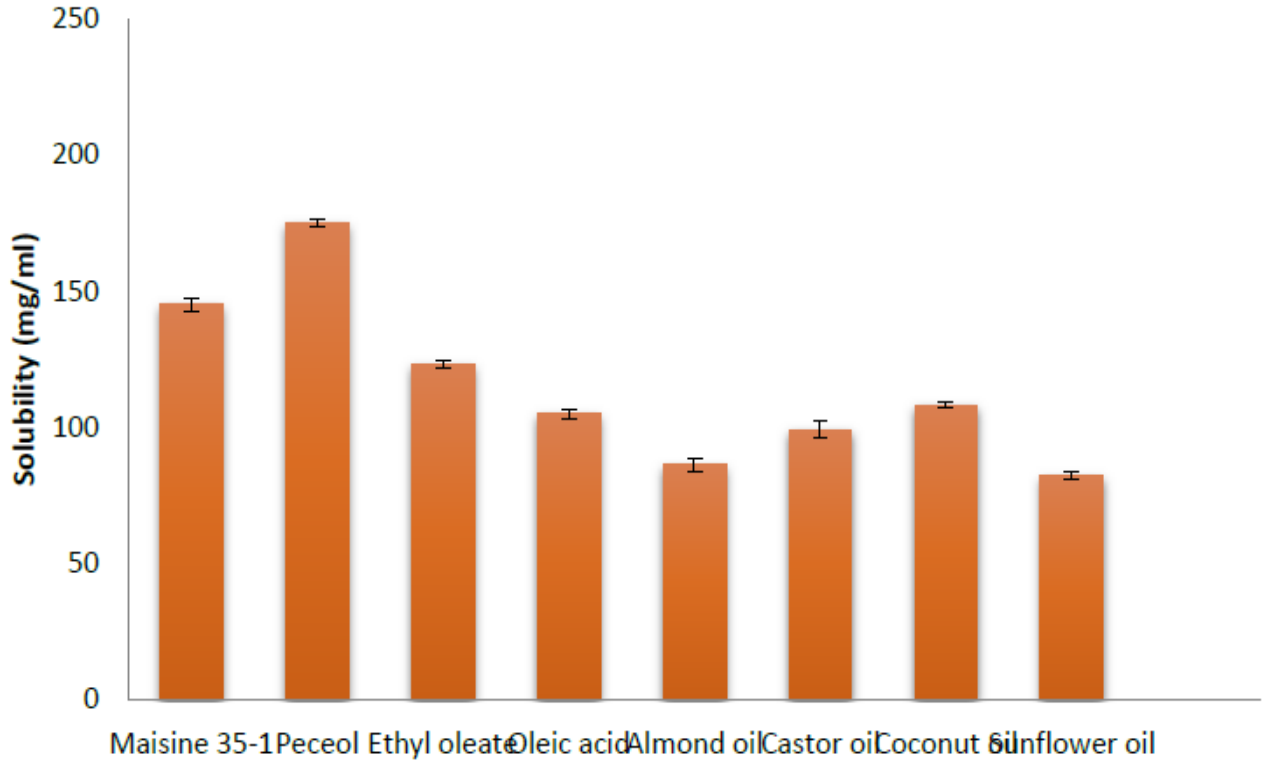

Figure 1: Solubility of Ezetimibe in various components (Mean ±S.D, n =3)

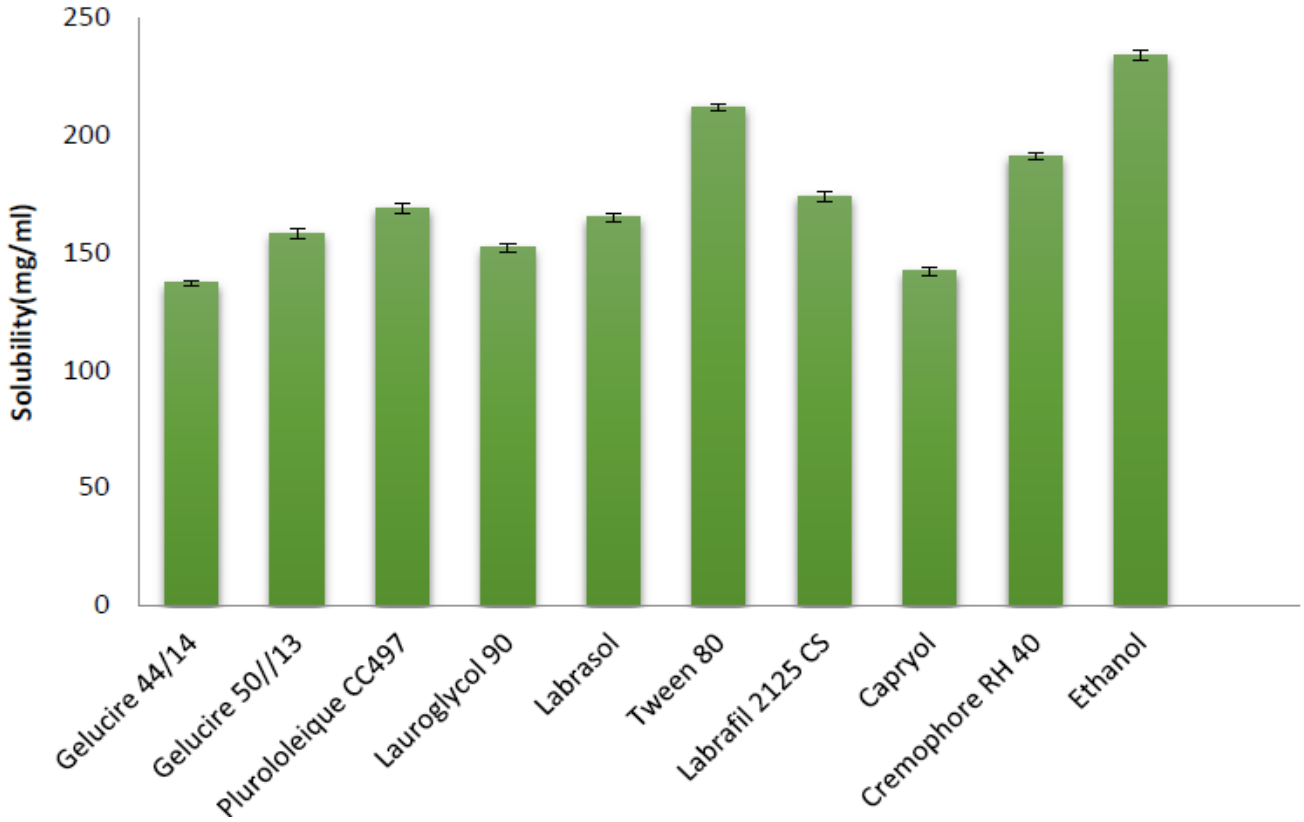

Figure 2: Solubility of Ezetimibe in various surfactant and co-surfactant (Mean ±S.D, n =3)

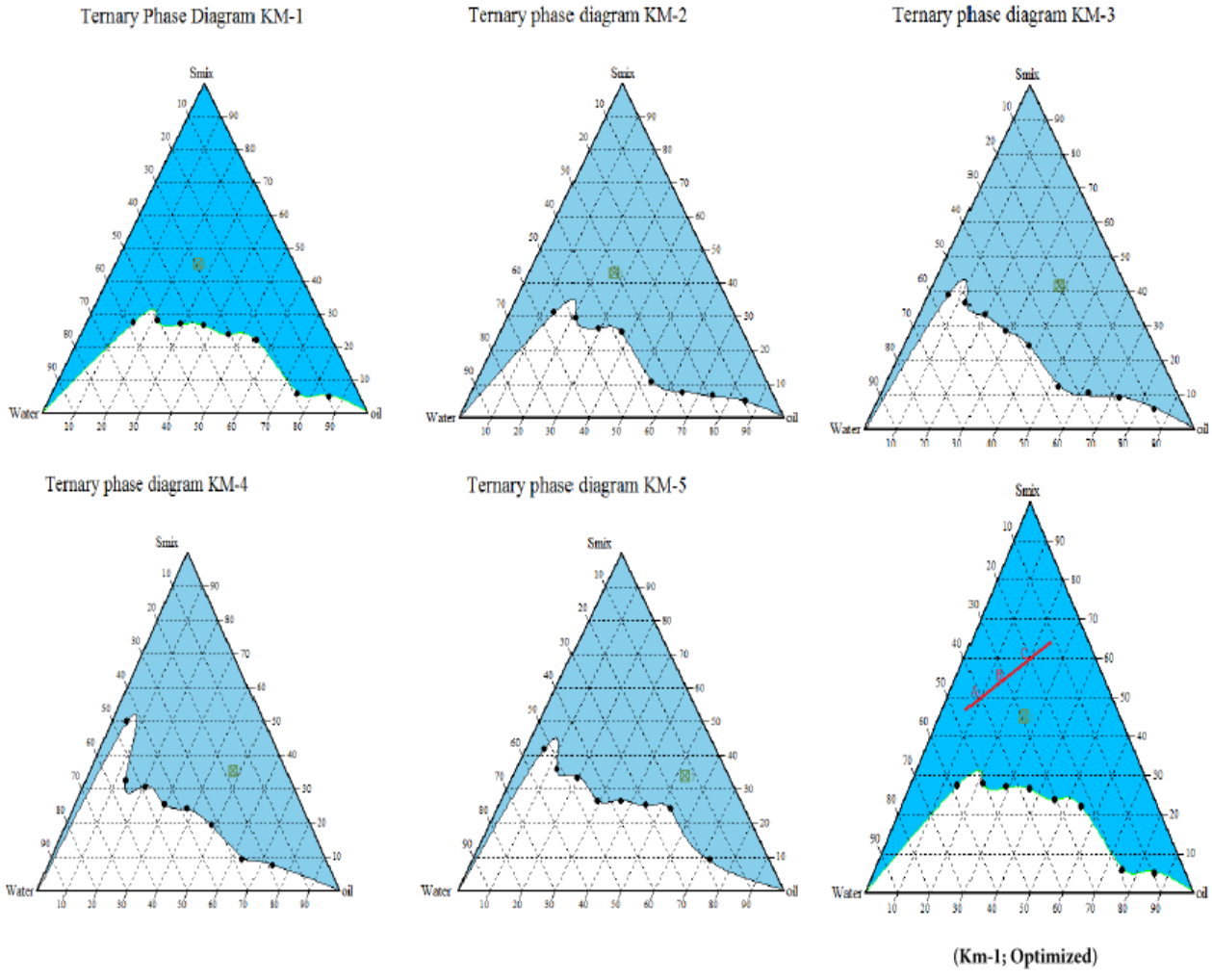

Figure3: Pseudo-ternary phase diagram of the Ezetimibe self nano emulsifying drug delivery systems (SNDDS) region with combination of co-surfactant (ethanol) and surfactant (Tween -80), i.e., S/Cos mix; (Km-1 is 1:1; Km-2 is2:1 ; Km-3 is 3:1; Km-4 is 4:1 and Km-5 is 5:1)

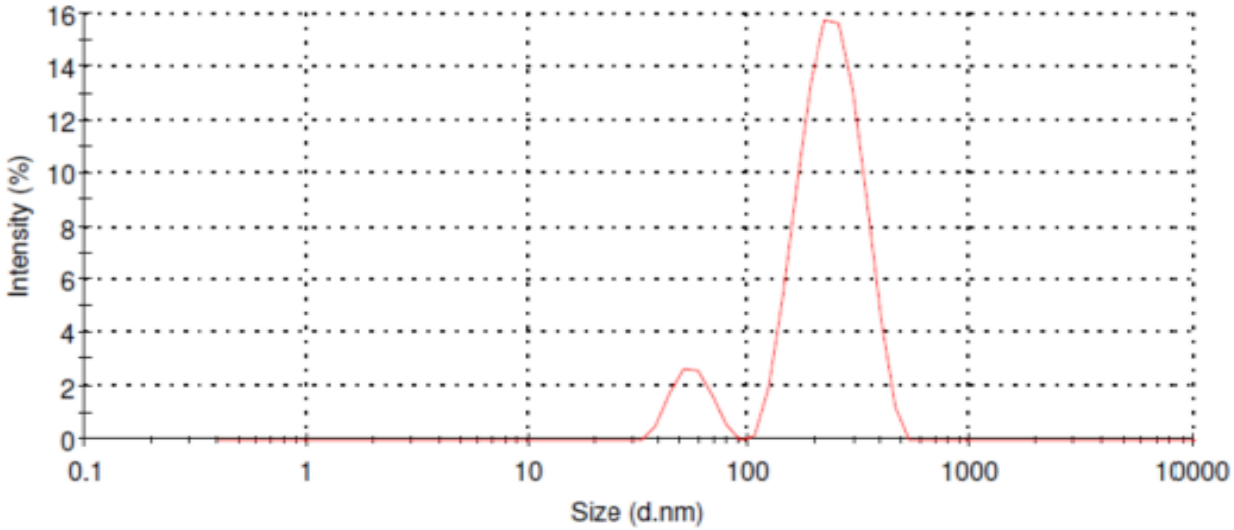

Figure 4: The Mean Droplet Size of SMEDDS (Batch -A) (Mean ±S.D., n =3)

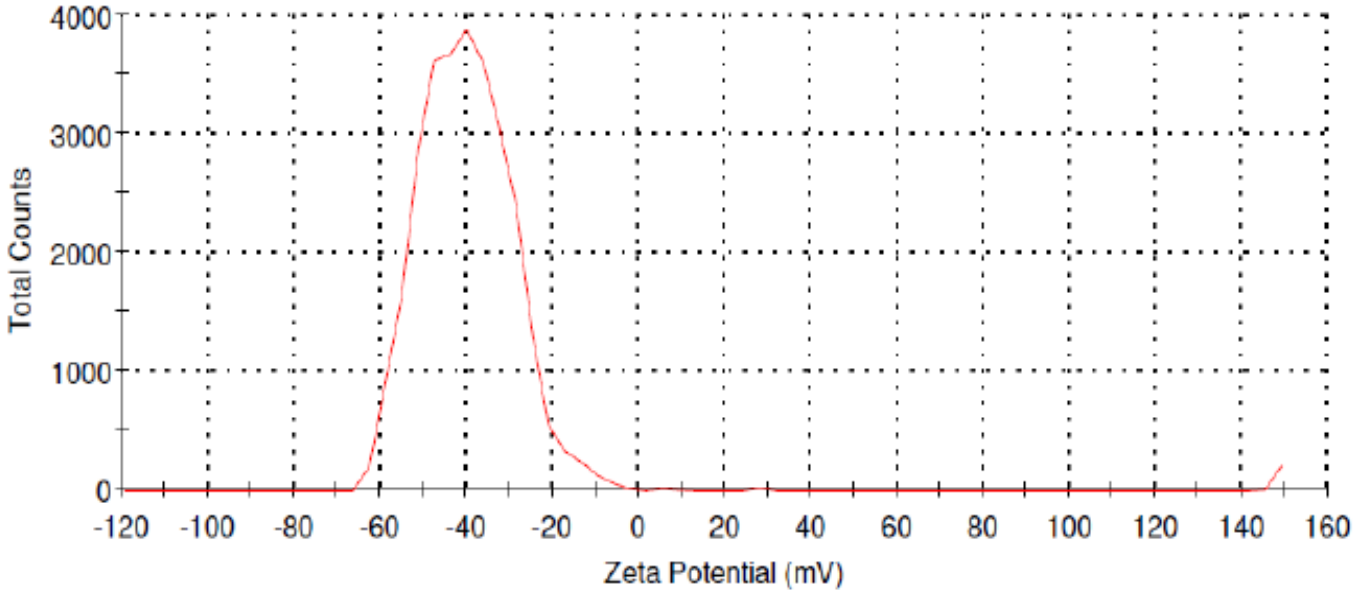

Figure 5: Zeta potential of optimized SMEDDS

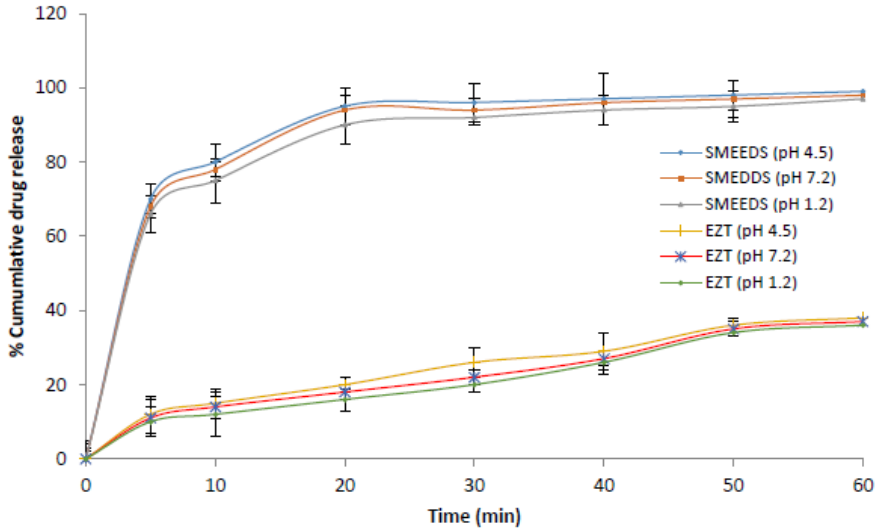

Figure 6: Comparative results of drug release from plain Ezetimibe and the SMEDDS formulation in different dissolution media; (Mean ±S.D. n =3) (EZT: plain Ezetimibe; SMEDDS, self-micro emulsifying drug delivery system).

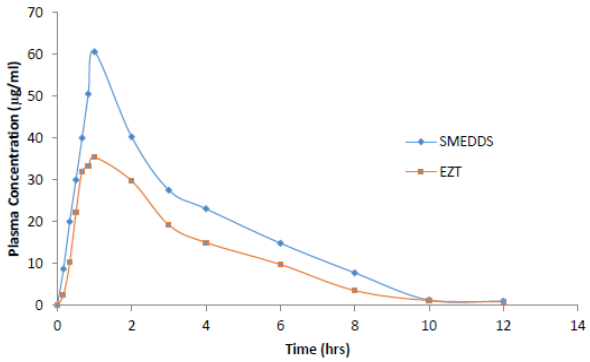

Figure 7: Plasma drug concentration vs. time curves for Ezetimibe SMEDDS

The Surfactant was also selected on basis of Ezetimibe solubility, ease and spontaneous formation of emulsion and increase the bioavailability by various mechanisms including improved drug dissolution, increased intestinal epithelial permeability, increased tight junction permeability. Gelucire 44/14, Gelucire 50//13, Plurololeique CC497, Lauroglycol 90, Labrasol, Tween 80, Labrafil 2125 CS, Capryol, Cremophore RH 40 were the surfactants selected; and Ethanol and PEG 600 were the cosurfactant chosen for further studies. Amongst all Tween 80 (212±1.4 mg/ml) and Ethanol (234±2.3 mg/ml) shows better solubility compare to others Thus, for further study Peceol as oil, Tween 80 and Ethanol as surfactant and cosurfactant, respectively was selected.

Primary screening of SNEDDS components (Oil, surfactant and co-surfactant)

The components Nano emulsion system was selected as oil with good solubilizing capacity of Ezetimibe as well as ease of emulsification. From solubility data, and self-emulsifying capacity Peceol shows 175 mg/ml and it selected as oil candidate because it has good solubilizing power for Ezetimibe amongst other oil investigated .The surfactant Tween 80 shows 212 mg/ml highest solubilizing capacity for drug than other surfactant. The surfactants generally suffer from major disadvantage that is toxicity and allergic reactions associated with long term use to decrease amount of surfactant co-surfactant are used. From many co-surfactant drug shows 234 mg/ml maximum solubility in ethanol. So for further studies peceol, tween 80 and ethanol are used as Oil, surfactant and co-surfactant respectively.

Pseudoternary Phase diagrams:

Self-Nano emulsifying systems form fine oil-water emulsions with only gentle agitation, upon their introduction into aqueous media. Surfactant and cosurfactant get preferentially adsorbed at the interface, reducing the interfacial energy as well as providing a mechanical barrier to coalescence. The decrease in the free energy required for the emulsion formation consequently improves the thermodynamic stability of the Nanoemulsion formulation. Therefore, the selection of oil and surfactant, and the mixing ratio of oil to S/CoS, play an important role in the formation of the Nanoemulsion. A series of SNEDDS were prepared and observed for their self-emulsifying properties. The percentage of self-Nano emulsifying regions was evaluated as a quantitative parameter of self-Nano emulsification efficiency.

In the present study Peceol was tested for phase behavior studies with Tween 80 and ethanol as the S/CoS mixture. Nine different potential combination of surfactant mixture to oil at different Km values (1, 2, 3, 4 and 5) were used for ternary plot study of Ezetimibe SNEDDS. The boundary layer of o/w Nano emulsion was determined in each phase diagram. The colored part of phase diagram shows Nanoemulsion region. As seen from the ternary plot (Figures 3).

The Nanoemulsion existence area increased as the S/CoS ratio increased. However, it was observed that increasing the surfactant ratio resulted in a loss of flowability. Thus, an S/CoS ratio between 3:1 and 4:1 was selected for the formulation study.

Ethanol is reported to be incompatible with hard gelatin capsules when used in high concentrations. Thus, while optimizing the S/CoS ratio, we tried to keep the concentration of ethanol as low as possible, as we had aim of putting the SNEDDS formulations into liquid-filled hard gelatin capsules.

Figure 3 shows phase diagrams in the presence of the drug. As seen from the figure, the inclusion of drug narrowed the Nanoemulsion existence area, because inclusion of the drug in the lipid phase led to expansion of the lipid phase and consequently a need for a higher S/CoS ratio for stabilization [32].

Therefore, considering the percentage area of Nano emulsifying region, system with S/CoS ratio of 5:1 has the best self-Nano emulsification efficiency. Tween 80 is a suitable surfactant for EZT because it inhibits P-gp efflux) Furthermore, Tween 80 inhibits both hepatic and intestinal CYP3A4 activity, which may have enhanced the bioavailability of EZT. Based on selected phase diagram three formulations were selected (A, B, C) for further studies.

Dispersibility tests or self emulsification efficiency

The self-emulsification of oral liquid Nano emulsion was observed using a standard USP II dissolution apparatus. The *in-vitro* performance of the formulations was visually assessed using the grading system. The results of self-emulsification and precipitation studies are given in Table 1. All the formulations have passed the dispersibility test with grade A that is it was rapidly forming, slightly less clear emulsion, having a slightly

bluish white appearance. It was seen that an increase in the proportion of Peceol in the composition resulted in decreasing self-emulsification time. The decrease in self-emulsification time can be assumed to be due to the relative decrease in surfactant cosurfactant, leading to decrease viscosity of the formulation. However, when the preconcentrate (SNEDDS) is dispersed in water, ethanol, being water-miscible, is anticipated to enter the water phase and redistribute mainly between the water phase and the emulsion-water interface, resulting in a loss of solvent capacity of the vehicle. Thus, the problem of precipitation was solved by increasing the surfactant proportion (S/CoS) in the system.

Globule Size Analysis

One of the most important physical properties of a Nanoemulsion system is mean droplet size. Nanoemulsion with small mean droplet size increases drug absorption and bioavailability, as well as stability. Droplet size is affected by the type and amount of surfactant, as well as other excipients and incorporated drugs. To further compare the suitability as SNEDDS, droplet sizes of Nanoemulsion of Km-1 are determined. The SNEDDS were diluted 100 times with water to mimic dilution by co-administered water. The average droplet size distribution of formulation Km-1 is determined. An increase in the ratio of the oil phase (Peceol) resulted in a proportional increase in particle size, because of the simultaneous decrease in the S/CoS proportion. Increasing the S/CoS ratio led to a decrease in mean droplet size. Km-1, with the highest proportion of surfactant (60% wt/wt Tween 80) at a fixed amount of oil (20% wt/wt), had the lowest mean particle diameter.

This could be attributed to an increased surfactant proportion. It's well known that the addition of surfactants to the Nanoemulsion systems causes the interfacial film to stabilize and condense, while the addition of cosurfactant causes the film to expand; thus, the relative proportion of surfactant to cosurfactant has varied effects on the droplet size [33].

It has been observed that liquid SNEDDS gives a normal distribution curve. The mean droplet sizes of SNEDDS preconcentrates were very low, and all were found to be in the nanometric range. In nano or Nanon range gives good transparency and increase surface area for partitioning of drug between oil and water. The sample EZ-A shows the smallest mean droplet size of 217.8 nm.

Zeta Potential

In Figure 5, the zeta potential of SNEDDS with the incorporated drugs was significantly negative when diluted with water. This may be due to dissociation of EZT which may be adsorbed or incorporated in the surfactant–cosurfactant layer. It has been reported that amphiphilic drugs can take part in surfactant–cosurfactant layer structure with acidic group facing the exterior water phase, changing the surface charge to more negative value. The effect of EZT on the surface charge of each droplet is negligible as EZT is a weakly basic compound and practically non-ionisable.

Zeta potential was measured by the Malvern (Malvern corp, Germany) zeta sizer. Particle-to-particle interaction is a crucial element in determining the characteristics of colloidal (solid particles) dispersed into liquid. One of the most important forces is electrokinetic repulsion. It was produced by the charge which is almost always found on the surfaces of particles in liquids. If their surface charge is relatively high, then adjacent colloids will repel each other and will tend to maintain their individuality [34-38]. The force of gravity is in significant on such small colloids and their rate of settling will be on the order of inches per hour or per day. As a result, highly charged colloids tend to remain discrete and in suspension.

On the other hand, a colloid with little or no charge has little resistance to the natural tendency for fine particles to gather together into aggregates. Small clumps will form and, in turn, aggregate into larger flocs which settle quickly or form an interconnected matrix. So the Nanoemulsion shows the zeta potential -11.7 having high potential and good stability. The significance of zeta potential is its value can be related to the stability of colloidal dispersions. The zeta potential indicates the degree of repulsion between adjacent, similarly charged particles in dispersion. From above result it concluded that liquid SNEDDS sample shows high potential and good stability of Nanoemulsion.

Percent transmittance

The liquid SNEDDS droplets spread easily in water and formed a fine transparent emulsion. It was concluded that the formed Nano emulsion was qualitatively good as droplets spread easily in water and formed a fine transparent Nanoemulsion. Percentage transmittance of liquid SNEDDS after dilution for 100 times with deionized water was 96.5%. Transmittance value of SNEDDS indicates that clear Nanoemulsion was formed. As the surfactant concentration increases the ease of emulsification also increases by getting increase in the transmittance. As the concentration of oil increases the transmittance as well as the ease of emulsification decreases. The transmittance of formulations was found less it may be due to least surfactant concentration this might be due to difference in CMC

concentration of surfactant.

In Vitro Dissolution Studies

In vitro release profiles of SNEDDS formulation (Batch A) was compared with plain EZT in various dissolution media including FDA-recommended dissolution conditions. It was found to be significantly higher as compared with that of plain EZT (Figure 6). This is further supported by the fact that the SNEDDS showed high dissolution rate even at non-sink conditions such as pH 4.5 acetate buffer or distilled water, whereas plain EZT showed less than 5% of total drug release. This indicates that the optimized SNEDDS may form Nanoemulsion rapidly at various media of different pH or surfactant concentration even at non-sink conditions, which accords with other previous studies.

It could be suggested that the SNEDDS formulation resulted in spontaneous formation of a Nanoemulsion with a small droplet size, which permitted a faster rate of drug release into the aqueous phase, much faster than that of plain EZT. Thus, this greater availability of dissolved EZT from the SNEDDS formulation could lead to higher absorption and higher oral bioavailability [39-40]. It was also seen that changes in the dissolution medium (buffer pH 1.2, 4.5, and 7.2) had no effect on the drug release from either plain EZT or the SNEDDS formulation (Figure 6).

This observation can be explained by the fact that EZT has no ionizable group and thus its solubility and dissolution is pH-independent. Although the actual bioavailability enhancement should be further evaluated, this result indicates that the SNEDDS may rapidly form small Nanoemulsion droplets and disperse in the digestive fluid regardless of various pH or surfactant concentrations, ready to be absorbed *in vivo*.

Thermodynamic Stability Studies:

Generally, SNEDDS formulations are put into hard gelatin capsules as the final dosage form. However, liquid-filled hard gelatin capsules are susceptible to leakage, and the entire system has a very limited shelf life owing to its liquid characteristics and the possibility of precipitation of the drug from the system. Thus, the developed formulation was subjected to stability studies to evaluate its stability and the integrity of the dosage form. Table 3 gives the results of the evaluation test conducted on stability samples. There were no changes in formulation after expose to Heating and cooling cycle, Centrifugation and freeze thaw cycles as discussed in Table 4. The formulation was found to be stable for 6 months at intermediate and accelerated conditions. There was no significant change in the drug content, drug release (t90%) of the resultant emulsion. It was also seen that the formulation was compatible with the hard gelatin capsule shells, as there was no sign of capsule shell deformation. There were also no significant changes in the appearance, disintegration time, or Nano emulsifying property. Furthermore, the formulation was found to show no phase separation, Phase inversion, drug precipitation, creaming or

cracking or capsule leaks. Thus, these studies confirmed the stability of the developed

formulation and its compatibility with hard gelatin capsules. Viscosity of SNEDDS was determined (Brookfield Rheometer, Brookfield) and observed that, viscosity after all stress testing formulations is in the range between 12-20 cPs. It was conclude that all liquid SNEDDS batches show less viscous clear solution. The drug content of all formulations was under the limit after all types of stability tests.

Pharmacokinetic Studies:

As shown in figure, The SNEDDS gives highest plasma concentration profile 60.6 µg/mL, along with plain EZT give 36.98 µg/mL of concentration. Similarly, SNEDDS and plain EZT gives T_{max} value 60 min. These results suggest that the absorption rate of SNEDDS is notably higher than that of plain EZT this might be due to increased in solubility of drug by Self-Nano emulsifying drug delivery system. The SNEDDS formulation resulted in complete dissolution of EZT, which could have increased absorption and thereby led to a higher plasma drug concentration (higher bioavailability). The low bioavailability of EZT is attributed to its poor aqueous solubility. The above difference in pharmacokinetics activity and the results from in vitro dissolution studies thus suggest that the SNEDDS formulation resulted in higher oral bioavailability owing to higher solubilization of EZT from the SNEDDS formulation as compared with plain EZT.

ONCLUSION

Formulation development was accomplished by selecting the excipient that showed the highest drug solubility for the less soluble drug. An optimized SNEDDS formulation consisting of Peceol (20% wt/wt), Tween 80 (30% wt/wt), Ethanol (30% wt/wt), and ezetimibe (20% wt/wt) was successfully developed with an increased dissolution rate, increased solubility, and, ultimately, increased bioavailability of a poorly water-soluble drug, ezetimibe. In addition, drawing phase diagrams and quantifying Nano emulsifying area for all possible combinations enabled rational selection of an optimal SNEDDS system. The large difference in loading capacity for EZT was explained by lower solubility of EZT in the oil phase and studying their molecular locations using zeta potential analysis,

which emphasizes the importance of rational choice of oil in enhancing the extent of solubilization. The *in vitro* dissolution tests showed rapid dissolution rate for both drugs regardless of type of dissolution media, indicating fast dispersibility of the SNEDDS and higher bioavailability. The developed formulation showed higher pharmacokinetic potential as compared with plain ezetimibe. Results from stability studies confirmed the stability of the developed formulation. Thus, our study confirmed that the SNEDDS formulation can be used as a possible alternative to traditional oral formulations of ezetimibe to improve its bioavailability.

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DECLARATION OF INTEREST

The authors report no conflict of interest. The authors alone are responsible for the content and writing of this paper.

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