Acta Scientific Pharmaceutical Sciences (ISSN: 2581-5423)

Volume 5 Issue 8 August 2021

Research Article

Application of Quality by Design Approach to Develop Novel Optimized Self-emulsifying Drug Delivery System of Ezetimibe for Treatment of Poorly Water-soluble Antilipidemic Drug to Enhance its Bioavailability by Using D-optimal Mixture Design

Mukesh S Patel[1]*, Alpesh D Patel[1] and Mayur Patel[2]

[1]Department of Pharmaceutics, Shri B.M. Shah College of Pharmaceutical Education and Research, Gujarat, India

[2]Research Scholar, Gujarat Technological University, Ahmedabad, Gujarat, India

***Corresponding Author:** Mukesh S Patel, Department of Pharmaceutics, Shri B.M. Shah College of Pharmaceutical Education and Research, Gujarat, India.

Received: June 28, 2021

Published: July 14, 2021

Abstract

The purpose of the research aimed to develop and optimize a novel dosage form of the self-emulsifying drug delivery systems of the Ezetimibe using quality by design (QbD) to enhancing its solubility. We performed solubility and emulsification tests to select a suitable initial risk assessment facilitated the selection of Capmul PG-8 (oil), Kolliphor RH40 (surfactant), Transcutol P (co-surfactant) as Critical Material Attributes (CQA) for the formulation of SEDDS. A d-optimal mixture design use to optimize the concentration of components used in the SEDDS formulation to achieve excellent physicochemical characteristics. Sixteen distinct formulations were prepared and assessed to check the model fit. It earmarked the patient-centric quality target product profile and CQAs. QbD resulted in a powerful and economical technique for improving the bioavailability of the medication, as confirmed by the portrayal investigations of the optimized batch. It saw a great arrangement among anticipated and exploratory qualities for mean droplet size, drug content, emulsification time, and percentage of medication delivered in 20 minutes. Optimized SEDDS formulation showed a smaller droplet size of 56.25 nm, PDI 0.230, and zeta potential -13.6 mV. To sum up, we successfully used QbD oriented development of novel Ezetimibe-loaded SEDDS formulation using the d-optimal mixture design to improve the oral absorption of poorly water-soluble drugs.

Keywords: Ezetimibe; SEDDS; QbD; d-Optimal Mixture Design; Risk Estimation Matrix

Abbreviations

SEDDS: Self-emulsifying Drug Delivery Systems; QbD: Quality by Design (QbD); CQAs: Critical Quality Attributes; CPPs: Critical Process Parameters; CMAs: Critical Material Attributes; HLB: Hydrophilic Lipophilic Balance; ANOVA: Analysis of Variance; QTPP: Quality Target Product Profile

Introduction

The oral route is the most accessible and most convenient route for non-invasive administration. However, the solubility of the orally administered drug is significant challenges of the pharmaceutical industry as nearly 35-40% of newly launched drugs possess low aqueous solubility, which leads to their poor dissolution and low bioavailability resulting in high intra and inter-subject variability and lack of dose proportionality [1]. Solubility is the significant interaction for the vast majority of the drugs to solubilize in an offered solvent to give a homogenous arrangement. The more noteworthy the solvency of the drug, the more prominent will be the total dissolution showing a wanted pharmacological response [2].

Many new methods enhance the solubility of inadequately soluble drugs like Solid dispersion, Complexation, Particle size reduc-

tion, co-solvency, etc. Among these, Self-Emulsifying Drug Delivery System (SEDDS) is gaining popularity for improving the solubility of lipophilic drugs [3]. SEDDS characterized as isotropic combinations of at least one hydrophilic solvent and cosolvent/surfactants that can form fine oil-in-water (o/w) microemulsions upon mild fomentation by dilution in aqueous media, for example, G.I. fluids. Later on, the formed emulsion absorbs through the lymphatic pathway. Therefore, it would improve the oral bioavailability of drugs in SEDDS to bypass the liver's first-pass effect. Thus, SEDDS has become a vital strategy to increase the oral bioavailability of poorly water-soluble drugs. Moreover, there is no fluid phase in SEDDS; it is a homogeneous framework, thus being appropriate for huge scope creation and steadier than traditional emulsion [4,5].

SEDDS has proven to be promising in facilitating the oral bioavailability of poorly water-soluble drugs. Self-emulsifying formulations spread promptly in the G.I. path, the motility of the stomach and the intestine provide necessary agitation for self-emulsification. SEDDS commonly produce emulsions with the droplet size somewhere in the range of 100 to 300 nm [6,7]. In comparison, SEDDS form clear microemulsions with a droplet size of 50 nm. Compared with emulsions, which are delicate and metastable scattered forms, SEDDS are physically steady formulations that are not difficult to make. Consequently, for a lipophilic drug that displays dissolution rate-restricted absorption, these systems may offer an improvement in the rate and degree of absorption and create a more reproducible blood time profile [8].

The pharmaceutical business is highly controlled, and the authoritative regulatory bodies control it. The regulatory agencies emphasize the quality of pharmaceutical products. However, it can design quality, and most of the quality deficiency occurs when the process was designed and developed. The quality expert Joseph Moses Juran introduced Quality by Design and its application in product development. The principles of QbD have been used in every industry to improve the quality of the product and process. In addition, all-around controlled and reproducible outcomes secure to accomplish the necessary therapeutic objectives of the formulation by the exact strategy called quality by design [3,8-10].

In the present study, Ezetimibe-loaded SEDDS formulations were designed and optimized by a Design of Experiments approach called D-optimal mixture design. It is a mathematical technique used to pick the framework's parts (exploratory inputs), which will apply the most unimportant impact on the product inconstancy. The Mixture Design created using the JMP® trial Software 16 version (SAS Institute, Cary, NC, USA) [11]. The Critical Material Attributes (CMAs) decided for the examination were oil (Capmul PG-8), surfactant (Kolliphor RH 40), and co-surfactant (Transcutol P), and the Critical Process Parameters (CPPs) were conditions like blending speed, sort of stirrer, and the mixing temperature. In addition, CQAs chose to enhance globule size, emulsification time, drug content, and *in vitro* drug discharge study [9,12].

Materials and Methods

Ezetimibe was acquired as a blessing sample from Lupin Ltd, Ahmedabad. Labrasol, Transcutol CG, Transcutol P, MCT, Labrafac P, Capryol 90, Labrafac PG, Capmul PG 8 N.F., and Labrafil M 2125 were acquired Gattefosse, France. Capex 200 P, MCT, Capmul MCM, and Captex 355 was received from Abitech corporation, U.S. Acryl EL 135, Acrysol K 160, Acrysol K 140, and K150 were purchased from Corel chemical Pvt Ltd Ahmedabad, India. Sunflower oil, Seasame oil, Canola oil, and Olive oil were procured from Kiran oil Ltd, Ahmedabad. Flaxseed oil/ Linseed oil, Soyabean oil, and Coconut oil were acquired from Kush protein Ltd, Anand. Sefsol 218 (Monocaprylic Ester) was obtained from Nikko Chemical Co. Ltd, Japan. Kolliphor RH40 was supplied from BASF, Germany. Tween 80, Span 80, Tween 20, and Polyethylene glycol was purchased from finer chemical limited, Ahmedabad, India, and All the materials and reagents utilized for the research project were of the analytical grade. Deionized water was used throughout the study.

Defining quality target product profile (QTPP) and critical quality attributes (CQAs)

QTPP approach establishes a planning system for item improvement, and it begins with "plan as a primary concern" to ensure product efficacy and safety. The QTPP is a product sketch that precise the characteristics expected during the development of the product to respond to the therapeutic objective of the medication. At last, the quality objective item profile frames the root for the drug [13]. Ultimately quality target product profile forms the core for the development of the product. Through the QbD approach, the product development advocates defining the QTPP, and it is one of the prerequisites to deliver therapeutic benefits as per the label claim. The QTPP for liquid SEDDS was determined based on the patient-centric (emulsification time, globule size, drug content, drug release) and product-centric (PDI, zeta potential) drug product quality attributes. The CQAs identified from QTPP were interlinked to give the desired quality, safety, and efficacy to the product, showing noticeable changes when QTPP is altered [6,9,13]. The QTPP and CQAs were listed in table 1 and 2, correspondingly.

QTPP elements	Target	Justification
Dosage type	Lipid-based formulation	Enhancement of Bioavailability of poorly water-soluble drugs
Dosage form	Capsule	Ease of administration and Formulated as hard gelatin capsules for improved patient consistency, compactness, and assembling ease
Dosage strength	100mg	Required to show the therapeutic action at the target site
Route of administration	Oral	Most convenient route for patients
Packaging	Alu-Alu Blister	To secure against the degradation of medication and lipids within sight of atmospheric air, acts as a permeation and photo barrier and gives a prolonged shelf life
Product Quality Attributes	Physical attributes Droplet size Transmittance PDI and Zeta potential Emulsification efficiency Drug content Drug release	The formulation must meet the compendial quality standards
Stability	According to the states of ICH Q1B Long term stability study.	To assess the depredatory pattern of the Drug and Excipients used in the formulation.

Table 1: Quality target product profile of Ezetimibe -SEDDS.

Quality attributes of drug	Product Target	Is this a CQA?	Justification
Physical attributes	Suitable to patient	No	It did not straightforwardly identify with the related safety and efficacy of the product; hence they were considered non-critical.
Color, Odor, and Appearance			
Droplet size (nm)	<100 nm	Yes*	Smaller and consistent globule size is essential for the stability and bioavailability of the formulation
Transmittance (%)	>95%	Yes*	Transparency of the product ensures the minimization of the droplet size
Poly dispersibility index	Dimension less value (0.1-1)	Yes	The value close to zero indicates homogeneity in the droplet size and ensures the physical stability of the product.
Zeta potential (mEv)	Negative value	Yes*	The magnitude of the Zeta potential value indicates the stability of the dispersion system.
Emulsification efficiency(s)	>20 s	Yes*	Spontaneous emulsification ensures the fast release of drug and influences the size of dispersed particles
Drug content	As per the target dose	Yes*	Ensures safety and efficacy of the product
In vitro drug release (%)	>80% in the target time or 100%	Yes*	Dissolution will have a direct impact on bioavailability

Table 2: Critical quality attributes (CQA) of SEDDS system with justification.

SEMDDS: Self-emulsifying drug delivery systems **CQAs considered as critical.

Risk assessment

The dosage form development under the QbD framework involves evaluating material and process attributes that have a more vital influence on the quality of the drug. Risk assessment is a combined effort to identify and assess the factors that may impact the product's CQAs. The risk assessment tools help identify and mitigate the risks and prioritize the risk as high, medium, or low based on the impact level. Table 3 represents the risk estimation matrix for Ezetimibe SEDDS. The two qualitative tools used in the present study were the Fishbone diagram and the Risk Estimation Matrix table. The Fishbone diagram (Figure 1) constructed using JMP® Software 16 version (Academic license from SAS, SAS Institute, Cary, NC, USA). The Fishbone diagram depicts the cause and sub-causes affecting the CQAs, and the risk assessment matrix helps categorize the risk [8,9,13,14].

CMA /CPP CQAs	API Particle size	Oil	Surfactant	Cosurfactant	Stirring speed	Stirring time	Stirring temperature
Drug content	Low	High	High	High	Low	Low	Low
Globule size	Low	High	High	High	Medium	Medium	Medium
Zeta potential	Low	High	High	High	Low	Low	Low
Emulsification Time	Low	High	High	High	Medium	Medium	Medium
PDI	Low	High	High	Medium	Medium	Medium	Medium
% Transmittance	Low	High	High	High	Medium	Medium	Medium
Drug release in 20 minute	Low	High	High	High	Low	Low	Low
	High-Risk Factor			Medium Risk Factor		Low-risk factor	

Table 3: Risk Estimated Matrix.

Figure 1: Fishbone diagram depicting causes and sub causes affecting Ezetimibe -SEDDS quality attributes.

CMAs/CPPs.	API Particle size	Is it critical?	Justification
API particle size	Drug content	No	The drug's particle size does not likely impact the drug product CQAs, as the SEDDS are homogenous systems containing the drug molecularly dispersed in the self-emulsifying form. Thus, the material attribute particle size possesses a low risk.
	Globule size	No	
	Zeta potential	No	
	Emulsification Time	No	
	PDI	No	
	% Transmittance	No	
	Drug release	No	
Type of lipid	Drug content	No	The type of lipid employed for formulating SEDDS have relatively less consequential little on the stated CQAs. The majority of lipids employed in the formulation of SEDDS are mixtures of mono/triglycerides with chain lengths varying between C8 and C10. The type of lipid used does not significantly differ in their change length, lipophilicity, HLB, etc. Therefore, there is a lower threat connected with the sort of lipid.
	Globule size	No	
	Zeta potential	No	
	Emulsification Time	No	
	PDI	No	
	% Transmittance	No	
	Drug release	No	
Type of surfactant and co-surfactant	Drug content	No	In SEDDS formulations, primarily the water-soluble surfactants and co-surfactants are used. Although the surfactant/co-surfactant type has a gentle to direct effect on the chose quality attributes of the medication product, these held as low-risk parameters in the formulation.
	Globule size	No	
	Zeta potential	No	
	Emulsification Time	No	
	PDI	No	
	% Transmittance	No	
	Drug release	No	
Amount of lipid, surfactant, and co-surfactant	Drug content	No	It measures lipid, surfactant, and co-surfactant, which is answerable for solubilization of the medication, globule size, and emulsification time, dissolution execution of the SEDDS definitions. Change in the measure of lipid, surfactant, and co-surfactant can influence most medication product CQAs. Consequently, these outlines as high-threat boundaries.
	Globule size	No	
	Zeta potential	No	
	Emulsification Time	No	
	PDI	No	
	% Transmittance	No	
	Drug release	No	
Type of stirrer. stirring speed and stirring time T	Drug content	No	The SEDDS formulations are isotropic mixtures, where the drug is present in the solubilized state in the lipidic and emulsifying excipients. The solubility of the drug majorly attributes to the amount of lipid, surfactant, and co-surfactant. The process parameters like type of stirrer used, stirring speed, and stirring time used to mix the drug with excipients have little influence on the listed CQAs. Thus, the associated risk rates as relatively low.

Table 4: Justification of risk allotment and identification of Critical Material Attributes and Critical Process Parameters.

Composition and limits of experimental domain			
Critical Material Attributes (Factor)	**Role**	**Values**	
		Low	**High**
Capmul PG-8	Mixture	0.15	0.2
Kolliphor RH 40	Mixture	0.3	0.5
Transcutol P	Mixture	0.3	0.5
Responses in mixture design.			
Responses	Goal	Lower Limit	Upper Limit
Drug release in 20 minutes (%)	Maximize	80	100
% Drug Content	Maximize	95	100
Emulsification time (seconds)	Minimize	20	60
Droplet size (nm)	Minimize	50	100

Table 5: Mixture design components and response.

Formulation Code	Capmul PG-8	Kolliphor RH 40	Transcutol P	Formulation Code	Capmul PG-8	Kolliphor RH 40	Transcutol P
F1	0.2	0.5	0.3	F9	0.185	0.5	0.315
F2	0.15	0.35	0.5	F10	0.2	0.44	0.36
F3	0.15	0.5	0.35	F11	0.15	0.425	0.425
F4	0.165	0.404	0.431	F12	0.166623	0.333377	0.5
F5	0.165	0.5	0.335	F13	0.2	0.36	0.44
F6	0.185618	0.314382	0.5	F14	0.185	0.462	0.353
F7	0.2	0.3	0.5	F15	0.19	0.367	0.443
F8	0.2	0.5	0.3	F16	0.2	0.3	0.5

Table 6: Composition of SEDDS as per the d-Optimal Mixture Design.

Preformulation study

Selection of oil

We selected oils based on the solubility of Ezetimibe, % transparency, and ease of emulsification in various modified oils, surfactants, and Co-surfactants. Take 2 ml of each element in screw cap vials with the known amount (100 mg) of the drug. A vortex mixture was utilized for the solubilization of the drug. Sealed vials were put on an isothermal mechanical shaker at 40 ± 2°C for 48 hours. After balance, each test tube was centrifuged at 3000 rpm for 20 min using a centrifuge. The supernatant was separated through a membrane filter by utilizing a 0.45-µm filter paper. The separate solution was appropriately diluted with methanol and examined in U.V. Spectrometers at 315 nm. The amount of solubilized drug was determined using a standard equation. The results of solubility study data are depicted in figure 3A.

Selection of surfactant

We selected surfactants based on the solubility of the drug, % transparency, and ease of emulsification. Solubility of the drug in surfactant was evaluated in a comparative way to portray in the above segment of oil [7,15]. It can screen the emulsification capacity of various surfactants in the oil phase. Briefly, take 0.5 ml of surfactant and 0.5 ml of oil mixed and heated at 50 °C for homogenization of components. Each 0.1 ml mixture was diluted with the 50 ml distilled water in a glass stopper conical flask. The ease of emulsification was determined by the number of flask inversion required for the homogeneous emulsion. A resulting emulsion was examined visually for the relative turbidity. An emulsion was allowed to stand for 2 hrs. And their transmittance was observed at 650 nm through a UV-1800 double beam spectrophotometer utilizing double distilled water as blank. Desired HLB value to form

o/w SEDDS must be in the range of 12- 18, and the surfactant was mainly selected based on this. Naked eyes further examined emulsification for any turbidity and phase separation. The emulsification ability of different surfactants exhibits figure 3B.

Selection of cosurfactant

We made a selection of cosurfactant based on the solubility of the drug, % transparency, and ease of emulsification. First, the solubility of the drug in cosurfactant was examined as per mentioned procedure in the above selection of oil. Then, a mixture of co-surfactant, surfactant, and selected oil was prepared and analyzed as described in the above section of surfactants [15,16]. Emulsification ability appears in figure 3C.

Drug excipient compatibility study using FTIR 8400S spectrometer

The FTIR instruments dictated the recognition of pure drugs. Ezetimibe mixed thoroughly with potassium bromide, an infrared transparent matrix, 1:10 (Drug: KBr) proportion. The KBr discs were set up by compressing the Ezetimibe with KBr. Spectra were obtained at a resolution from 4000 cm^{-1}- 400 cm^{-1}.

A FTIR-8400S spectrometer outfitted with attenuated total reflectance adornment use to get the infrared spectra of the drug in the isotropic combination of excipients, Analysis of pure drug, physical mixture of the medication with the other excipients. The formulation is lyophilized utilizing a freeze dryer, and FTIR studies have carried out diffuse reflectance spectroscopy FTIR with KBr disc. Every one of the samples dried using a vacuum before getting any spectra to eliminate the influence of residual moisture. For each range spectrum, eight scans were obtained at a resolution of 4 cm^{-1} from a frequency range of 4000 - 400 cm^{-1} and demonstrated in figure 2.

Figure 2: A). FTIR spectra of Ezetimibe. B). FTIR spectra of Ezetimibe loaded SEDDS formulation.

Figure 3A: Schematic diagram of Ezetimibe in different oils.

Figure 3B: Schematic diagram of Ezetimibe solubility in different surfactant.

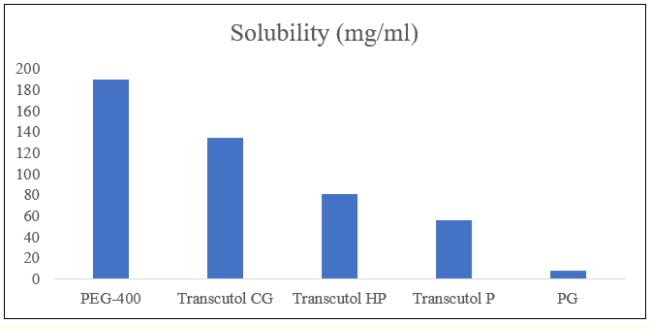

Figure 3C: Schematic diagram of Ezetimibe solubility in various co-surfactants.

Figure 3: Schematic diagram of Ezetimibe solubility in different oils, surfactants, and Co-surfactants.

Preparation of SEDDS (Without Ezetimibe)

The formulation consists of different ratios of oil (Capmul PG-8), surfactant (Kolliphor RH40), and cosurfactant (Transcutol P). First, the combination was combined by vortexing until a transparent solution was acquired. Then, the formulations were equilibrated at the surrounding temperature for 24 hrs. Finally, the formulations were examined for signs of turbidity or phase separation before self-emulsification.

Construction of phase diagrams

We constructed pseudo-ternary phase diagrams to obtain the appropriate components and their concentration ranges, resulting in a large existence area of the microemulsion. The microemulsion region was initially delineated by its isotropic nature and low viscosity to optimize the oil, surfactant, and co-surfactant phase concentration; different batches of varying concentrations were prepared and titrated with distilled water to appear turbidity. A two-dimensional ternary phase diagram can be set up by keeping the composition of one component fixed and varying the other two or using a constant ratio of surfactant to co-surfactant. Here, the ternary phase diagram was set up by utilizing a consistent surfactant ratio to co-surfactant [7,8,15,16].

Pseudo-ternary phase diagram for SEDDS

Pseudo ternary phase diagrams have been built utilizing the chosen component, demonstrating the ingredients' amount. That can bring results in an enormous area of the microemulsion. These plots were constructed with the use of oil (Capmul PG-8), surfactant (Kolliphor RH40), co-surfactant (Transcutol P), and water with the help of water titration technique at room temperature. The surfactant and co-surfactants were combined in various proportions like 1:1, 1:2, and 2:1 accordingly. The oil and Smix were mixed, which varied from 9:1 to 1:9 parts. We created a ternary phase diagram with the help of an aqueous titration strategy. Every mixture was titrated with distilled water and stirred at room temperature to acquire equilibrium. Then, the mixture was visually evaluated for transparency. The ternary diagrams prepare using oil, surfactant, and co-surfactant; it considered the point showing the transparent and isotropic mixtures to be withinside the microemulsion area [17,18]. Pseudo ternary plots were constructed using Ternary Plot.com. Results of the phase diagram are stated in figure 4A-4C.

Figure 4: Pseudo-Ternary Phase diagram. A) Surfactant (Kolliphor RH40)/co-surfactant (Transcutol p) ratio (1:1) and oil (Capmul PG-8) B). Surfactant (Kolliphor RH40)/Co-surfactant (Transcutol P) ratio (2:1) and Oil (Capmul PG-8)) C). Surfactant (Kolliphor RH40)/Co-surfactant (Transcutol P) ratio (1:2) and Oil (Capmul PG-8) D). Surfactant (Kolliphor RH40)/co-surfactant (Transcutol p) ratio (1:1) and oil (Capmul PG-8) E). Surfactant (Kolliphor RH40)/co-surfactant (Transcutol p) ratio (2:1) and oil (Capmul PG-8) F). Surfactant (Kolliphor RH40)/Co-surfactant (Transcutol P) ratio (1:2) and Oil (Capmul PG-8).

Formulation of SEDDS (With Ezetimibe)

The formulations were frame-up by dissolving 25 mg of Ezetimibe in oil, surfactant, and co-surfactant at close temperatures. The final mixture was vortexed for 24 hrs to get a clear solution. Then all the mixtures are stored at room temperature for further use. Finally, the formulations were inspected for turbidity or phase separation indications before self-emulsification and particle size determination. Table 7 shows all different SEDDS formulations of Ezetimibe. Different ratios of oil and Smix were used to study various evaluation parameters of the optimized formulation.

Formulation Code	Visual Assessment	Transmittance (%) n- = 3	% Drug content	Emulsification Time (sec) n = 3	Droplet Size	PDI	Zeta Potential
F1	I	98.06 ± 0.14	98.3	25 ± 1	78.25	0.222	-12.7
F2	I	99.8 ± 0.10	99.98	21 ± 1	56.25	0.230	-13.6
F3	I	99.1 ± 0.10	99.6	22 ± 2	75.35	0.381	-11.1
F4	I	98.3 ± 0.19	99.8	24 ± 1	81.24	0.300	-3.8
F5	I	99.5 ± 0.32	99.68	29 ± 1	59.25	0.171	-12.41
F6	I	98.42 ± 0.26	99.5	25 ± 2	57.54	0.252	-12.8
F7	I	97.72 ± 0.71	98.7	28 ± 1	75.23	0.290	-2.29 mv
F8	I	98.03 ± 0.70	98.87	24 ± 1	56.32	0.230	-7.46 mv
F9	I	98.53 ± 0.83	99.69	23 ± 2	65.25	0.310	-6.86 mv
F10	I	99.5 ± 0.72	99.3	25 ± 1	65.68	0.170	-9.74 mv
F11	I	98.72 ± 0.71	98.6	27 ± 1	57.54	0.280	-1.29 mv
F12	I	97.93 ± 0.55	99.03	26 ± 1	58.25	0.173	-0.144 mv
F13	I	97.19 ± 0.90	98.87	30 ± 1	78.84	0.300	-8.70 mv
F14	I	98.03 ± 0.70	98.5	26 ± 2	58.98	0.210	-7.86 mv
F15	I	97.23 ± 0.83	98.25	30 ± 1	89.58	0.290	-6.26 mv
F16	I	97.8 ± 0.72	98.4	29 ± 1	78.25	0.180	-7.74 mv

Table 7: Evaluation of SEDDS F1 to F16 Formulation of d-optimal mixture design.

Effect of ezetimibe in phase diagram

They examine the impact of Ezetimibe on the self-Microemulsifying performance of SEDDS; 10 mg of Ezetimibe was added to 1 ml of a limit plan of SEDDS and checked for the formation of a clear solution. The effect of Ezetimibe on the phase diagram demonstrates in figure 4D-4F.

Evaluation of SMDDS formulations

Self-emulsification time

USP dissolution apparatus II use to evaluate the self-emulsification time of SEDDS. Then add one ml of every formulation in the 900 ml of distilled water at 37 ± 0.5℃. Next, mild fomentation was given by a standard stainless steel dissolution paddle rotating at 50 pm. Emulsification time checked visually. Emulsification must complete within 1 minute [1,19]. The results of self-emulsification time exhibit in table 7.

Visual assessment

Take one ml of SEDDS diluted with 500 ml of purified water at 37 ± 0.5℃. A stainless steel dissolution paddle rotating at 50 rpm leads to gentle agitation. The time is taken (seconds) by every formulation to shape a clear homogenous framework noted in three sets. In light of the item's last debut, the emulsified batches were graded per the accompanying evaluating framework. Grade I: Quickly forming microemulsion which is explicit or slightly bluish in appearance within less than 1 minute. Grade II: Quickly forming, little less clear emulsion having a bluish-white appearance within 2 minutes. Grade III: milky white emulsion within 3 minutes. Grade IV: Tedious, grayish-white emulsion with an oily appearance that is slow to emulsification for more than 3 minutes. Grade V: Tedious, grayish-white emulsion with an oily appearance that is slow to emulsification for more than 3 minutes [20]. Result display in table 7.

Transmission test

The transmission test of optimized SEDDS formulations checked using a U.V. spectrophotometer (UV 1800, Shimadzu). First, the sample's percentage transmittance was measured at 650 nm, with distilled water as the blank, and for each piece, triplicate assays were performed. Then calculate the percentage transmittance in 100 µL of the SEDDS formulation was diluted with 100ml of distilled water [3,21]. Results of the transmission test are shown in table 7.

Drug content

The Ezetimibe loaded- SEDDS formulation was dissolved in methanol. The solutions were filtered utilizing Whatman filter paper. The methanolic solution was estimated for the Ezetimibe content with the help of a U.V. spectrometer at 232 nm using a standard curve. Results of % drug content display in table 7.

Robustness to dilution

This estimation was utilized to investigate the impact of dilution on SEDDS, with a particular ultimate objective to follow physiological dilution procedure after oral administration. The formulations were diluted in different proportions (1:50, 1:100, and 1:1000) with various diluents (distilled water, 0.1 N HCL, and acetate buffer (pH4.5)). The resultant emulsions were examined for any physical changes after storage for 24 hrs [8]. Results of robustness to dilution are shown in table 8.

Formulation Code	2-4°C			Room Temperature			Centrifugation Stability	Robustness to dilution	
	PS	F	PR	PS	F	PR	Phase separation	Distilled water	0.1N HCL
F1	X	X	X	X	X	X	X	√	√
F2	X	X	X	X	X	X	X	√	√
F3	X	X	X	X	X	X	X	√	√
F4	X	X	X	X	X	X	X	√	√
F5	X	X	X	X	X	X	X	√	√
F6	X	X	X	X	X	X	X	√	√
F7	X	X	X	X	X	X	X	√	√
F8	X	X	X	X	X	X	X	√	√
F9	X	X	X	X	X	X	X	√	√
F10	X	X	X	X	X	X	X	√	√
F11	X	X	X	X	X	X	X	√	√
F12	X	X	X	X	X	X	X	√	√
F13	X	X	X	X	X	X	X	√	√
F14	X	X	X	X	X	X	X	√	√
F15	X	X	X	X	X	X	X	√	√
F16	X	X	X	X	X	X	X	√	√

Table 8: Temperature stability study, Centrifugation Stability study and Robustness to dilution of F1 to F16 Formulations.

Footnote: X =Not seen √= seen P= Phase separation F= Flocculation PR=Precipitation

Where √ is stable preparation

Droplet size determination and polydispersibility index (PDI)

Take one ml of SEDDS formulation and dilute with 100 ml of water in a volumetric flask. Then the volumetric flask was inverted twice to ensure complete dispersion of the formulation. After confirming the total distribution of formulation, the droplet size of the resultant microemulsion was estimated using photon correlation spectroscopy that analyzes the fluctuation in light scattering due to the Brownian motion of the globule as a purpose of time utilizing a Zetasizer Nano Series. Light scattering was examined at 25°C at 90°C angles [22-25]. Results show in table 7.

Zeta potential determination

The stability of the emulsion is straightforwardly recognized with the actual surface charge. One ml of SEDDS was diluted 100

times with distilled water in a beaker with continuous stirring on a magnetic stirrer. The Zeta-potential of the SEDDS formulations was determined using a Malvern Zetasizer [26,27]. Results present in table 7.

Optimization of Ezetimibe-loaded SEDDS formulations using d-optimal mixture design

The d-optimal mixture design was used to optimize the composition of the SEDDS formulation. The experiment was designed using the three components as independent variables [3]. Based on the solubility study and pseudo-ternary phase diagram, concentrations of Capmul PG-8 (oil; X1), Kolliphor RH 40 (surfactant; X2), and Transcutol P (cosurfactant; X3) were set within ranges of 15%–20%, 30%–50%, and 30%–50%, correspondingly. For any test, the focuses of X1, X2, and X3 added up to 100%. Percentage drug content (Y1), Percentage Drug Release in 20 minutes (Y2), Emulsification time in second (Y3), and Mean droplet size (Y4) were evaluated to determine the optimal SEDDS formulation with excellent physiochemical characteristics. We used JMP@ Trail 16 Version (SAS, New Jersey, USA) to develop and evaluate the trial plan. The base design allowed 16 trials to fit the best model and estimate experimental error responses (Y1Y2, Y3, and Y4) shown in table 5 and 6. The CPP was not calculated in the plan thought due to their insignificant impact on the reactions as shown by the Risk Estimated Matrix (REM). The different batches (Table 6) got according to the plan are exposed to the portrayal. Coherent validation of different risk(s) for each of the material attributes and process factors resultant to the individual CQAs display in table 4.

Transmission electron microscopy (TEM)

The morphology of the emulsion drop for the optimized Ezetimibe-loaded SEDDS formulation was noticed utilizing a TEM (JEM 1010, JEOL Ltd, Tokyo, Japan) with a speed increase voltage of 80 kV. The enhanced SEDDS formulation was diluted with water (1:1,000). One drop of the example was straightforwardly stored on a copper network and dried at 25°C.

In vitro dissolution studies

In-vitro release investigations of SEDDS were carried out utilizing USP dissolution apparatus type-II with rotating paddle at 50 rpm and maintained at 37±0.5°C temperature in dissolution media pH 1.2 buffers (900 ml) to examine drug discharge from SEDDS. The Ezetimibe loaded-SEDDS formulation and pure drug were filled in a hard gelatin capsule and introduced into the dissolution medium. At predetermined time intervals of 5 min to 1 hr, 10 ml of the samples were drawn and filtered through 0.45 µm Whatman filter paper. The fresh dissolution medium was simultaneously replaced in the apparatus to keep the steady volume. The filtered solution was analyzed using a U.V. spectrophotometer at 232 nm to check the amount of drug release in dissolution media. All estimations were done in triplicate [28,29]. The graph was plotted against drug release v/s time to investigate formulations and pure drugs, as demonstrated in figure 7.

Thermodynamic stability study

All SEDDS formulations of Ezetimibe were disclosed to the heating-cooling cycle and centrifugation to investigate thermodynamic stability:

- **Heating cooling cycle:** The SEDDS formulation is put in a refrigerator temperature at 2 to 4°C and room temperature with storage at each temperature less than 48 hrs. Each one of those arrangements, which are steady at this temperature, was further investigated through centrifugation.
- **Centrifugation:** The formulations, which were cleared the heating-cooling cycle, were centrifuged at 3500 rpm for 30 min. Table 8 demonstrate the results of the thermodynamics stability study.
- **Freeze-thaw cycle:** we use three freeze-thaw cycles at -21°C and +25°C with storage at every temperature for not less than 48 hours for the formulations.

Result and Discussion

Risk assessment

The dosage form development under the QbD framework involves evaluating material and process attributes, significantly influencing product quality. Through the fishbone chart (Figure 1), the potential variables influencing the product CQAs were recognized. Regarding SEDDS readiness, the material ascribes like oil, surfactant, and cosurfactant/cosolvent significantly contribute to product responses than the process attributes because the preparation method is simple. Hence, we use the operation characteristics involved in the SEDDS preparation like stirring time, temperature, and stirring speed as the least preferred because of their minimal contribution towards the product inconstancy. Thus, the

threat related to measuring boundaries is assessed as low (Table 3). The practical headway of SEDDS formulations depends upon the proper decision of excipients with their general degree in the definition formulation [9,13-15].

Preformulation study

Screening of vehicles: (Oil, Surfactant, Co- surfactants)

Solubility study was performed to identify suitable oil phase, surfactant, and co-surfactant to develop ezetimibe SEDDS. Maximum solubilizing capacities of components (oil, surfactant, and co-surfactant) are necessary to achieved optimum drug loading [30,31].

Selection of oil

The oil is one of the critical excipients within the SEDDS formulation due to the fact it could solubilize commercialized amounts of the lipophilic drug or facilitate self-emulsification. Moreover, mainly because it can enhance the segment of lipophilic drug transported through the intestinal lymphatic system. So, that absorption from the G.I. tract relaying on the molecular nature of the triglyceride. Therefore, long and medium-chain triglycerides oils with different degrees of saturation have been used to formulate a self-micro emulsifying drug delivery system.

The solubility study described in the section the solubility of Ezetimibe in different oil show in figure 3A, Screening of appropriate oil is the primary requirement of SEDDS development. Therefore, solubility studies were aimed to identify suitable oil having maximal solubilizing potential for the development of SEDDS. Capmul PG-8 was found to solubilize the maximum amount of Ezetimibe (99.73 mg/ml) among the selected oil. However, the other oils exhibited different solubilizing capacities, reported in table 7 and figure 3. Hence, (Capmul PG-8) was preferred for further study.

Selection of surfactants

The surfactant concerned with the SEDDS formulation has a high HLB value around 8.6 to 16.7 and hydrophilicity of surfactant, which cause instant development of oil in water droplet and quick dispersion of formulation aqueous media like gastrointestinal fluid. The drug dispersed within the SEDDS formulation would remain solubilized for a long time at the absorption site, thus preventing precipitation of drug compounds inside G.I. lumen surfactant were absorbed in the interface because of its amphiphilic nature, and they can dissolve a high amount of lipophilic drug in the oily phase.

According to the solubility method described in the selection of surfactant, the solubility of Ezetimibe in different surfactants represents in figure 3B. Within the solubility data of Ezetimibe in various surfactants, the highest solubility of the drug was found in Kolliphor RH40 (57.21mg/ml) and Acrysol K160 (52.21mg/ml). The oily phase Capmul PG-8 showed the highest emulsification performance with Kolliphor RH40 for the homogenous emulsion formulation, and additionally, Acrysol K160 produced better transparency in the formulation. On the other hand, Capmul PG-8 showed poor emulsification properties with other surfactants. Therefore, Kolliphor RH 40 surfactant was selected for SEDDS formulation. A proper amount of low and high HLB value surfactants were required to form the steady microemulsion in preparing a self-emulsified formulation. Therefore, Kolliphor RH 40 with an average HLB value of 15 and Transcutol P with an HLB value of 4 were utilized. Campus PG-8 is entrapping in the surfactant with more HLB value, enhancing the emulsification process upon dilution with an aqueous medium. In addition, these excipients provide better stability to the emulsion.

Selection of co-surfactants

The co-surfactant use to diminish the oil-water interface fluidizes the interfacial film's hydrocarbon area and licenses the spontaneous development of microemulsion. Therefore, the selection of a cosurfactant is critical to forming microemulsion formation and solubilization in microemulsion. According to the solubility method described in the selection of the co-surfactant, the solubility of Ezetimibe in different co-surfactant describe in figure 3C. Among the solubility information of Ezetimibe in distinctive co-surfactant, most of the drug dissolved in Transcutol P (135.2mg/ml), which indicates 99.40% transparency. Accordingly, Transcutol P chose as a cosurfactant in the SEDDS formulation. Furthermore, the investigation recognizes the ability of various co-surfactants to enhance the microemulsion of selected surfactants. Therefore, Increase the spontaneity of the microemulsion formulation increased by increasing the co-surfactant.

Oil, surfactant, and co-surfactant are constructing excipients of the self-Microemulsifying drug delivery system. The drug needs to be soluble in all three excipients in the mixture. Therefore, the

solubility of the drug needs to be suitable for the choice of oil, surfactant, and co-surfactant. Moreover, the solubility of the drug is likewise crucial to dictate the dose of SEDDS. Hence, SEDDS need to comprise oil, surfactant, and co-surfactant that accommodate the amount of the drug. Another element, which might be affected due to solubility, is the partitioning effect. If the drug isn't always appropriate in the mixture, it will diffuse towards the water when developing microemulsion. Each of those facts, choice of excipients is a crucial factor for successful formulation. The solubility of oil data (Figure 3A) indicates that Ezetimibe has better solubility in synthetic oil than vegetable oil.so; Capmul PG-8 was selected as the oil phase. Kolliphor RH40 acts as a surfactant because of its extreme HLB value. Therefore it showed good solubility of Ezetimibe in the oil phase. The third element of SEDDS, i.e., the Co-surfactant (Transcutol P), helps surfactant stabilize the system.

Drug-excipient compatibility study

FT-IR of formulation and the drug were carried out to investigate any drug-excipient interactions using KBr disc along with scanning range of 4000-400 cm^{-1} and resolution 1 cm^{-1}. The I.R. spectrum of the drug was in contrast with the standard I.R. spectra of Ezetimibe. The FTIR spectra of ezetimibe confirmed peaks at 3411.84-3235.30 cm^{-1} (O-H, Alcohol/Phenol), 3085.75-3015.34 cm^{-1} (C-H, Aromatic), 1684.28 cm^{-1} (N- C=O Amide), and 1097.31 cm^{-1} (C-F bending). These peaks might be considered as ideal peaks of Ezetimibe and had been now no longer affected and prominently noticed in I.R. spectra of Ezetimibe together with oil, surfactant, and co-surfactant. So, from the comparison, it was concluded that the drug and excipients were suitable for each other. The FTIR spectra of pure Ezetimibe drug and Ezetimibe formulation's overlapping spectra were proven in figure 2A and 2B. Comparison of vibration Frequency of FTIR spectra of Ezetimibe and Ezetimibe formulation indicates there might be no important distinction in characteristics peak at a wavenumber of the drug within the presence of excipients. It indicates that formulations became well suited with excipients.

Pseudo-ternary phase diagram

The self-micro emulsifying system produces oil in water emulsion with gentle agitation into the aqueous medium. A surfactant or co-surfactant preferentially adsorbed on the interface, lowering the interfacial energy and offering a mechanical barrier to coalescence. The reductions of the free energy required for the microemulsion formulation concurrently improve the microemulsion formulation's thermodynamic balance. Therefore, the choice of oil, surfactant, co-surfactant, and the combination ratio of oils to Smix play a vital role in the microemulsion formulation.

Based on the results of preliminary studies, a ternary phase diagram was constructed to study the relationship between the phase behavior and composition of SEDDS. It also helps to determine the concentration range of components for the formulation of a microemulsion. It had used a combination of surfactant (Smix) with high and low HLB values in the present work. Campus PG-8 has less HLB value, and Kolliphor RH 40 having a high HLB value. A combination of low and high HLB surfactant leads to more rapid dispersion and finer emulsion droplets size in addition to the aqueous segment. Campus PG-8 and Kolliphor RH 40 withinside the proportion of 1:1 indicated higher microemulsion region and rapid emulsification compared with 2:1 and 1:2 Smix selected for formulation development.

Different ratios, as according to figure 4A-4C were considered for optimization of the proportion of oil, surfactant, and cosurfactant for microemulsion. As per figure 4A, a 1:1 ratio was used. The data showed that up to 1:9 to 4:6 parts of Oil to Smix ratio gave a clear solution when it was titrated with 100 parts of the water because Smix parts are higher than oil parts. However, after 4:6, parts of oil to Smix produced turbidity in the solution, indicating that SEDDS was unstable on dilution. Therefore, as above figure 4B, SEDDS were prepared by using a 2:1 Smix ratio. The data found that from 1:9 to 3.5:6.5, parts of oil to Smix give clear solution when it was titrated up to 100 parts of water because oil parts are less than Smix parts. Therefore, surfactant reduces the interfacial tension between oil and water lead to a clear solution. From figure 4C, a 1:2 ratio was used. The data found that up to 1:9 to 3:7, parts of the oil to Smix ratio gave a clear solution when it was titrated up to 100 parts of water. The nature of microemulsion formed in the aqueous medium depends on the concentration of Smix (a mixture of Kolliphor RH 40 and Transcutol P) in the formulation. Figure 3A-3C observed that as the concentration of Smix was increased with decreasing concentration of oil, it improved the clarity of the self-micro emulsifying system. In addition, the surfactant reduced the oil-water interface, which made rapid dispersion of SEDDS in an aqueous medium and reduced in particle size when diluted with water.

Effect of ezetimibe in the phase diagram

As per the phase diagram (Figure 4D-4F), different ratios considered optimizing the proportion of oil, surfactant, and co-surfactant for the Ezetimibe microemulsion system. The microemulsion was prepared using Capmul PG-8, Kolliphor RH 40, and Transcutol P as the main three constituents of the formulation. A formulation was designed with a S/Co-surfactant ratio of 1:1, 2:1, 1:2. When the amount of oil is less than 10%, the water content is 90%. At this point, microemulsion can be diluted to infinite. Which fulfilled the requirements of SEDDS, and also droplet size is less than 100 nm.

The hydrophobic drug was entrapped in the SEDDS, which affects the self-emulsifying performance. Also, precipitation of the drug may occur in the gut during the dilution by body fluids due to the loss of solvent capacity in a co-surfactant present in the microemulsifying drug delivery system. In the present study, a reduction in the efficient self-emulsifying domain was observed by incorporation with Ezetimibe.

Optimization of Ezetimibe-loaded SEDDS using desirability function

Three highlights are required for streamlining: cutoff esteem (upper or lower), dispensed objective value, and information showing whether every response should be better or limited. When a streamlining interaction is combined with the increase, an incentive for the lower bound is figure 5B. Impacts of independent factors on the reaction factors, Y1, Y2, Y3, Y4. Three-dimensional response surface plot for the impacts of the independent factors. Contour plots for the impacts of X1 and X2 on the reactions. (Y1, percentage drug content; Y2, the rate of drug discharge in 20 min; Y3 emulsification time in second, and Y4, the drop size in nm).

Conversely, when a response variable is limited, an incentive for the upper bound is required. Y1 was set to be amplified, and an objective worth and the cutoff worth of 95 and100%, individually, were chosen. The lower bound of 95% was chosen dependent on the most reduced level of medication content in the SEDDS formulation. Y2 was set to be boosted, and an objective worth and the cutoff worth of 100 and 80%, individually, were chosen. The lower bound of 80% was chosen dependent on the most reduced medication delivered from SEDDS formulation in 20 min in pH 1.2 medium. The objective worth of 100% was chosen depending on the most noteworthy medication discharge quickly (Table 5). A little drop size permits better medication absorption since it gives an expanded surface region to the retention and permits quicker medication discharge. Dissolution is a rate-restricting advance for the oral absorption of inadequately water-dissolvable medications, particularly drugs having a place with class II of the Biopharmaceutics Classification System, including Ezetimibe. Further, to expand proficiency and decline work escalation, a disintegration time of 20 minutes was embraced to decide the connection between independent factors and dissolution. Y3 was set to be a target match and an objective worth and lower bound of 20 seconds and 60 seconds individually, were chosen. The lower bound of 20 seconds was picked dependent on the miscibility in the water and the structure of a steady emulsion (Table 5). Y4 was set to be limited because the more modest the globule sizes of SEDDS formulation, the better the G.I. assimilation. A few examinations on SEDDS and microemulsions proposed that the ideal distance across a steady microemulsion ought to be < 200. In our exploratory run of blend plan, the base trial worth of drop size was 56.25 nm (Table 7). In this manner, both the objective worth and upper bound were 50 and 100 nm, individually, for the enhancement. All reaches for the reaction factors were among the upsides of our trial run of a combination plan. The independent factors were improved for the reaction esteems by utilizing the attractive quality capacity. Figure 5A addresses the contour and surface plot for the impacts of the three free factors on all response factors.

Parameters	Y1	Y2	Y3	Y4
R Square	0.952	0.995	0.998	0.990
R Square Adj	0.958	0.975	0.998	0.996
Root Mean Square Error	1.16	8.6	0.03	16.6
Mean of response	99.06	69.08	25.56	63.92

Table 9: Summary of fit.

The Optimized formulation proportions of Ezetimibe-loaded SEDDS of X1, X2, and X3 were 18%, 40.97%, and 40.95%, individually. An attractive quality capacity worth of 0.000196 upheld these qualities (Figure 5A). The anticipated and noticed upsides of Y1, Y2, Y3, and Y4 for the improved Ezetimibe-loaded SEDDS formulations appear in table 10. Upsides of expectation blunders were determined to assess the dependability and precision. The model approval was done through the ternary mixture profiler. The ternary blend profiler gives the ideal space in the ternary graph (Figure

5C). The distinctive proportion of oil, surfactant, and cosurfactant inside the perfect area doesn't influence the dependent factors (Responses) of the SEDDS definition. Albeit the expectation blunder of Y1 and Y2 was moderately high, its variable achieved a size that appeared to be good thinking about the exploratory scope of the SEDDS plan. The forecast mistakes of both Y3 and Y4 were a little greater than usual. Consequently, these outcomes showed that the D-Optimal mixture design technique utilized for optimizing Ezetimibe -stacked SEDDS in this investigation was dependable and exact. For the level of medication delivered in 20 min (Y2), the improved Ezetimibe-stacked SEDDS showed Y3 worth of 99.4%. Also, it showed attractive upsides of percentage drug content (99.98%) , % drug release in 20 minute (80.25%) emulsification time (Y3 = 21 second). And bead size (Y4 = 56.25 nm). Along these lines, F2 optimize formulation was exposed to promote *in vitro* dissolution study. The model was analyzed in JMP software. After Analysis, actual by predicted profile plot and contour profiler for globule size and % medicament release were obtained as shown in figure 5B. The summary of fit and parameter estimates was also obtained, shown in table 9 and table 11. In the meantime, the transmission electron microscopy investigation for the optimized formulation SEDDS showed that the emulsion drops were round in the nanometer range, as demonstrated in figure 8.

Responses	OF-SEDDS		
	Predicted value	Experimental value	% prediction error
% Drug content (Y1)	98.52	99.98	1.48
% Drug release in 20 Minutes (Y2)	64.75	80.25	20.93
Emulsification time (sec) (Y3)	27.2	21	-22.42
Droplet size (nm) (Y4)	66.88	56.45	-15.59

Table 10: Experimental and predicted values for the optimized SEDDS formulation.

Difference % = [(Experimental value – Predicted value) / (Predicted value)] x 100.

Parameter Estimate for Response Y1					
Components	**Coefficient Estimate**	**Std Error**	**DFDen**	**t Ratio**	**p-value**
A (Capmul PG-8-0.15)/0.25	490.70	808.3	5.707	0.051	0.63
B (Kolliphor RH 40-0.3)/0.25	102.43	1.927	1.389	53.34	0.0029*
C (Transcutol P-0.3)/0.25	102.34	2.56	5.08	39.97	<.0001*
A*B	-526.37	1354.38	5.714	-0.39	0.7116
A*C	-530.15	1337.79	5.68	-0.40	0.7063
B*C	-15.25	7.52	5.661	-2.03	0.0921
A*B*C	479.70	1135.69	5.69	0.42	0.6882
A*B*(A-B)	-202.36	575.95	5.723	-0.35	0.7379
A*C*(A-C)	-203.09	546.04	5.528	-0.37	0.7238
B*C*(B-C)	7.661	10.04	5.182	0.76	0.4790
Parameter Estimate for Response Y2					
A (Capmul PG-8-0.15)/0.25	1900.34	31148.83	5.802	0.06	0.9534
B (Kolliphor RH 40-0.3)/0.25	90.95	27.19	4.416	3.34	0.0247*
C (Transcutol P-0.3)/0.25	63.44	20.70	5.075	3.06	0.0274*
A*B	-7811.9	13911.74	4.787	-0.56	0.5997
A*C	-7171.1	13788.65	4.844	-0.52	0.6259
B*C	-42.34	77.16	5.987	-0.55	0.6030
A*B*C	5992.64	11681.21	4.78	0.51	0.6308
A*B*(A-B)	-3416.48	5906.61	4.745	-0.58	0.5893

A*C*(A-C)	-2645.1	5691.58	4.969	-0.46	0.6618
B*C*(B-C)	-107.83	106.96	5.995	-1.01	0.3523
Parameter Estimate for Response Y3					
A (Capmul PG-8-0.15)/0.25	1900.3478	31148.83	5.802	0.06	0.9534
B (Kolliphor RH 40-0.3)/0.25	27.272403	41.28062	3.222	0.66	0.5530
C (Transcutol P-0.3)/0.25	18.942631	105.1214	5.573	0.18	0.8634
A*B	-3224.652	51002.37	5.788	-0.06	0.9517
A*C	-2997.076	54762.99	5.844	-0.05	0.9582
B*C	7.8657411	327.9032	5.565	0.02	0.9817
A*B*C	2680.9995	44120.79	5.822	0.06	0.9536
A*B*(A-B)	-1422.454	19608.58	5.695	-0.07	0.9447
A*C*(A-C)	-1154.253	27096.04	5.93	-0.04	0.9674
B*C*(B-C)	-16.99015	85.29569	0.141	-0.20	0.9350
Parameter Estimate for Response Y4					
A (Capmul PG-8-0.15)/0.25	15403.815403.566	16073.96	1.48	0.96	0.4678
B (Kolliphor RH 40-0.3)/0.25	93.51104	74.41515	2.104	1.26	0.3304
C (Transcutol P-0.3)/0.25	-50.08988	61.35265	2.594	-0.82	0.4825
A*B	-24916.68	26734.35	1.462	-0.93	0.4791
A*C	-25395.87	26757.59	1.505	-0.95	0.4696
B*C	177.54653	233.7684	2.458	0.76	0.5137
A*B*C	20277.438	22333.29	1.462	0.91	0.4884
A*B*(A-B)	-9336.423	11237.48	1.417	-0.83	0.5226
A*C*(A-C)	-11415.34	11182.32	1.602	-1.02	0.4366
B*C*(B-C)	-239.8532	286.8052	1.945	-0.84	0.4931

Table 11: Parameter estimate.

Figure 5A: Prediction profiler for multiple responses optimization SEDDS Formulation.

Figure 5B: Contour and surface plots exhibiting the impact of formulation components on the responses.

Figure 5c: Ternary Mixer profiler displaying the impact of formulation components on the responses.

Figure 6A: Results of droplet size and PDI for the Optimization SEDDS Formulation.

Figure 6B: Results of droplet size and PDI for the Optimization SEDDS Formulation.

Figure 7: Dissolution profile of SMEDDS formulations F1-F16 and pure drug.

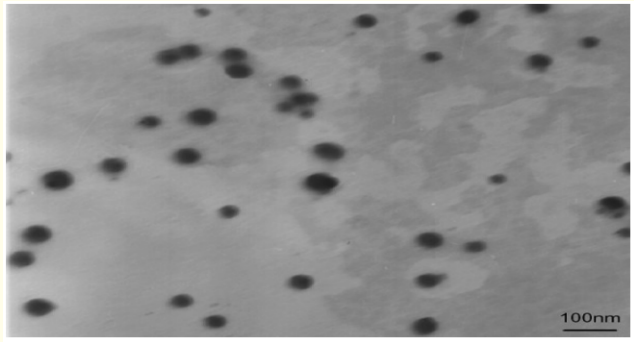

Figure 8: TEM result of the optimized SEDDS Formulation indicating the narrow particle size with uniform distribution.

JMP software was used to fit all responses to linear models. The sequential P-values for Y1, Y2, Y3, and Y4 were 0.00181 0.00189, 0.0185, and 0.0195, respectively. A sequential P-value of less than 0.05 indicates that the model terms are significant. ANOVA resulted in 12.65 F value with Prob > F value of 0.00181 for Y1 and 45.00 F value for Y2 with Prob > F value of 0.0189, 12.89 F value with Prob > F value of 0.00185 for Y3 and 35.00 F value for Y2 with Prob > F value of 0.0195, R^2 values for the responses Y1, Y2, Y3, and Y4 were approximately 0.952. 0.995, 0.998 and 0.990 respectively. The adjusted R^2 values for the responses Y1, Y2, Y3, and Y4 were approximately 0.958, 0.975, 0.998, and 0.996, respectively. For a good model, similar values of R^2 and adjusted R^2 are desirable.

Evaluation of SEDDS formulation

Visual assessment

The tendency to form emulsion was judged qualitatively as "good" if the droplets spread in water easily and developed a fine transparent emulsion. It was rated inadequate when there was milky or no emulsion formation, with immediate coalescence of oil droplets. Formulations from F1 to F16 are rapidly forming microemulsions, apparent in appearance and an appropriate SEDDS formulation asset. The grading system identifies it. Results were shown in table 7.

Transmission test

The clarity of SEDDS was determined through transparency, which was measured in the form of percentage transmittance. It was examined by directly taking the absorbance of the diluted SEDDS. Results present in table 7.

As per table 7, Optimize SMEDSS formulation shows the highest % transparency, which was 99.8%. Therefore, the value of transmittance is close to 100%. It indicates that each one of the formulations had been transparent. Therefore, the sizes of the globules in all the formulations are withinside the nanometer proportion. In turn, these specify that the drug within the formulations has a large surface area for drug release.

Drug content

The drug content of all SEDDS formulations was in the proportion of 98.4% to 99.98%. F5 formulation indicates the highest drug content is 99.98%. Therefore, it indicates good drug distribution in the formulation. Results were shown in table 7.

Determination of self-emulsification time:

The performance of self-emulsification could be estimated primarily by detecting the rate of emulsification. The SEDDS require dispersing absolutely and rapidly when added to aqueous dilution under slight agitation. The emulsification time of those formulations had been found among the 21 to 30 sec. Results had been proven in table 7.

Robustness of dilution

Robustness to dilution was performed. First, 1 ml of SEDDS was diluted with 1000 ml of water, 0.1N HCL, and acetate buffer pH 4.5. Then, it was stored for 12 hours. All those formulations indicated no precipitation or phase separation after 12 hours. Results were shown in table 8.

Droplet size and polydispersibility index (PDI)

The droplet size of the microemulsion is a vital parameter in self-emulsification performance. Droplet size needs to be <100 nm as it indicates the rate of drug release and absorption. Based on the review, there is no specified limit between SEDDS and SEDDS. The average droplet size found in water varies from 56.45 to 89.58 nm, indicating all the particles were within the nanometer range and homogeneous distribution of size. The tiniest particles were ascertained for formulation F2 (56.45nm), and the largest particles were observed for formulations F15. Results were shown in table 7.

Polydispersibility index (PDI)

Polydispersibility Index indicates the size variety of droplets within the system.

PDI = No. of particles having a size greater than 100 nm/ No. of particles having a size much less than 100 nm. As ideal SEDDS formulation must be widely distributed with particles much less than 100 nm, PDI needs to be less than 0.3. If particles have a size greater than 100 nm, they should be maximum up to 23%. The data (Table 7) shows that most formulations have a PDI less than 0.3 while F3, F4, F9, and F13 have PDI greater than 0.3. The clarity of microemulsion was characterized by transparency, measured in segments of % transmission. SEDDS forms o/w microemulsion since water is in the external phase.

Zeta potential

Almost all macroscopic materials come in contact with liquid media having an electronic charge on their surfaces. It is a vital indicator of this charge, which may be utilized to predict and control emulsion stability. Due to stable suspension, the charged particles repel every various, conquering the tendency to aggregate. The standard value of Zeta potential is less than -30 mV. It implies that formulations have been stable for a long time. Results are depicted in table 7.

In vitro dissolution study

In vitro dissolution studies were performed to identify the drug release from the six various formulations (F1-F16) and pure drugs. Dissolution studies were performed for the SEDDS in pH 1.2 buffer solutions. There are no significant differences in the dissolution study of six SEDDS formulations. Around 100 % of the drug is released within 45 to 60 min in SEDDS, which is greater than the drug release from a pure drug (47.12%). The dissolution studies were evaluated for 1 hr. SEDDS in the spontaneous development of a microemulsion with a small droplet size let in the faster release rate of Ezetimibe in dissolution media. The optimized formulation F2 gives a release in 45 minutes because of the smallest particle size (56.45 nm) and much less PDI value (0.230). The *In vitro* dissolution research shows that formulations of Ezetimibe with inside SEDDS formulation increase the dissolution properties. In *in vitro* release study, F2 formulations were optimized, giving 99.98% release in 45 minutes. Results display in figure 7.

Thermodynamic stability study

Thermodynamic Stability study determined using temperature and centrifugation. The temperature stability study was carried out by keeping the samples at two different temperatures (2-4 ℃ and Room temperature) for 48 hours and performed a visual inspection. Evidence of phase separation, flocculation, and precipitation became determined in the formulation. All formulations are stable at each temperature are shown in Table 8. SEDDS are thermodynamically steady, having a specific centralization of oil, surfactant, and water, with no phase partition, creaming, or breaking. Results display in table 8.

Conclusion

SEDDS were a promising approach for the formulation of poorly water-soluble drugs. It achieved the predetermined quality characteristics of Ezetimibe-SEDDS with the implementation of QbD concepts throughout the development process. We studied the detailed Analysis of the three independent variables called Capmul PG-8, Kolliphor RH 40, Transcutol P and their effects on the quality attributes such as droplet size, emulsification time, % drug content, and % drug release with the application of statistical mixture design. This study showed the potential of QbD in SEDDS development. This study successfully developed an optimized Ezetimibe-loaded SEDDS formulation using the d-optimal mixture design, a statistical optimization tool based on response surface methodology. The preformulation study of Ezetimibe -loaded SEDDS formulation included Capmul PG-8 (99.73 mg/ml) (oil; X1), Kolliphor RH 40 (57.21 mg/ml) (surfactant; X2), and Transcutol P (135.2 mg/ml) (cosurfactant; X3) and showed excellent *in vitro* dissolution study. Optimized SEDDS formulation include Capmul PG-8 (18%,) (oil; X1), Kolliphor RH 40 (40.97%,) (surfactant; X2), and Transcutol P (40.97%,) (cosurfactant; X3) having particle size (56.45 nm), Polydispersibility index (0.230) zeta potential (- 13.6 mV) and the narrow droplet size with uniform distribution. SEDDS formulation showed 99.99% *In vitro* release of the drug in 45 minutes compared to the pure drug that demonstrated a 42.12% drug release like wisely. A good understanding was observed between model prediction and experimental values of percentage drug content (Y1), % drug release in 20 minutes (Y2), and emulsification time in second (Y3), and droplet size in nm (Y4). Thus, the finding shows that optimizing Ezetimibe-loaded SEDDS formulation is potentially used to improve the oral absorption of poorly water-soluble drugs.

Conflict of Interest

The authors declare that they have no known competing personal and no financial interests that could have appeared to influence the work reported in this paper. The authors alone are responsible for the content and writing of this article.

Bibliography

1. Gahlawat V., *et al*. "Application of D-optimal Mixture Design for Development and Optimization of Olmesartan Medoxomil Loaded SEDDS". *Current Drug Therapy* 15 (2020): 548-560.

2. Carlos T., *et al*. "Improvement in the oral bioavailability and efficacy of new ezetimibe formulations- Comparative study of solid dispersion and different micellar systems". *Pharmaceutics 12* (2020): 617.

3. Yeom DW., *et al*. "Development and optimization of a self-micro emulsifying drug delivery system for atorvastatin calcium using d-optimal mixture design". *International Journal of Nanomedicine* 10 (2015): 3865-3878.

4. Karavasili C., *et al*. "Self-Nanoemulsifying Drug Delivery Systems (SNEDDS) Containing Rice Bran Oil for Enhanced Fenofibrate Oral Delivery: In-Vitro Digestion, *Ex Vivo* Permeability, and *In-Vivo* Bioavailability Studies". *AAPS Pharmaceutical Science and Technology* 21 (2020): 1765-1972.

5. Dash R., *et al*. "Design, optimizes and evaluates glipizide solid self-nano emulsifying drug delivery for enhanced solubility and dissolution". *Saudi Pharmaceutical Journal* 23.5 (2015): 528-540.

6. Patel M., *et al*. "Self-micro emulsifying drug delivery system of lurasidone hydrochloride for enhanced oral bioavailability by lymphatic targeting: In-Vitro, Caco-2 cell line *In-Vivo* evaluation". *International journal of Pharmaceutical Science* 138 (2019): 105027.

7. Patel MS., *et al*. "Microemulsion Based Gel: A Review". *International Journal of Universal Pharmacy and Biomedical Sciences* 3 (2014): 63-78.

8. Kavitha AN., *et al*. "A Structural Framework for Developing SEDDS through Quality by Design Approach". *Asian Journal of Pharmaceutics* 14 (2020): 308-318.

9. Kavitha AN., *et al*. "Quality by design-based development of etravirine self-micro emulsifying drug delivery system". *International Journal of Applied Pharm* 13 (2021): 103-111.

10. Holm R., *et al*. "Optimization and self-micro emulsifying drug delivery system (SEDDS) using a D-optimal design and the desirability function". *Drug Development and Industrial Pharmacy* 32 (2006): 1025-1032.

11. Son HY., *et al*. "Optimization of self-micro emulsifying drug delivery system for phospholipid complex of Telmisartan using D optimal mixture design". *PLoS ONE* 13 (2018): e0208339.

12. Kavitha AN., *et al*. "Design and Development of Darunavir loaded SEMDDS using Extreme Vertices Mixture Design in a Quality by Design Framework". *Industrial Journal of Pharmaceutical Education and Research* 54 (2020): 337-3348.

13. Gowthami K., *et al* "Quality by Design based Development of Self Nano Emulsifying Drug Delivery System of Ritonavir". *Journal Young Pharmacist* 12 (2020): 215-220.

14. Sarwar., *et al*. "QbD-based systematic development of novel optimized solid self-nano emulsifying drug delivery systems (SNEDDS) of lovastatin with enhanced biopharmaceutical performance". *Drug Delivery* 22 (2014): 765-784.

15. Gardouh AR., *et al*. "Design and evaluate combined Atorvastatin and Ezetimibe optimized self-nano emulsifying drug delivery system". *Journal of Drug Delivery Science and Technology* 60 (2020): 102093.

16. Huo T., *et al*. "Preparation and comparison of tacrolimus-loaded solid dispersion and Self-Microemulsifying Drug Delivery System by In-Vitro/In-Vivo Evaluation". *European Journal of Pharmaceutical Science* 114 (2017): 74-83.

17. Huang W., *et al*. "Enhanced anticancer effect of Brucea javanica oil by solidified self-micro emulsifying drug delivery system". *Journal of Drug Delivery Science and Technology* 48 (2018): 266-273.

18. Kantaporn K., *et al*. "Development and characterization of clove oil nanoemulsions and self- micro emulsifying drug delivery systems". *Journal of Drug Delivery Science and Technology* 46 (2018): 330-338.

19. Kamboj S., *et al*. "Quality-by-design based development of a self-micro emulsifying drug delivery system to reduce food effect of Nelfinavir mesylate". *International Journal Pharmaceutical Science* 30 (2016): 311-325.

20. Akiladevi D., *et al*. "Preparation and *In Vitro* Evaluation of Self-Emulsifying Drug Delivery System for Oral Delivery of Glibenclamide". *International Journal of Pharmacy and Pharmacology* 1 (2017): 105-112.

21. Kazi M., *et al*. "Enhancing Oral Bioavailability of Apigenin Using a Bioactive Self-Nano Emulsifying Drug Delivery System (Bio-SNEDDS): In-Vitro, In-Vivo, and Stability Evaluations". *Pharmaceutics* 12 (2020): 749.

22. Li S. "Effect of ionization of drug-on-drug solubilization in SEDDS prepared using Capmul MCM and caprylic acid". *Asian Journal of Pharmaceutical Science* 2 (2017): 73-82.

23. Liu H., *et al*. "Improving the Oral Absorption of Nintedanib By A Self-Microemulsion Drug Delivery System: Preparation and In-Vitro/In-Vivo Evaluation". *International Journal of Nanotechnology* 14 (2019): 8739-8751.

24. Mundada VP., *et al*. "Enhanced Oral Bioavailability and Anticoagulant Activity of Dabigatran Etexilate by Self Microemulsifying Drug Delivery System: Systematic Development, *In-vitro, Ex vivo*, and *In-vivo* Evaluation". *Journal of Nanomedicine and Nanotechnology* 9 (2018): 480.

25. Nan., *et al*. "Formulation and evaluation of luteolin supersaturate self nano emulsifying drug delivery system (S-SNEDDS) for enhanced oral bioavailability". *Journal of Drug Delivery Science and Technology* 58 (2020): 101783.

26. Pramanik S., *et al*. "Development of Solid Self-Microemulsifying System of Tizanidine Hydrochloride for Oral Bioavailability Enhancement: *In-Vitro* and *In-Vivo* Evaluation". *AAPS Pharmaceutical Science and Technology* 21 (2020): 182.

27. Reddy MS., *et al*. "Formulation and in-vitro characterization of self-micro emulsifying drug delivery systems of rivaroxaban". *International Journal Pharmaceutical Science and Research* 8 (2017): 3436-3445.

28. Ratnaparkhi MP., *et al*. "Design, Development, and Characterization of Self Micro Emulsifying Drug Delivery System for Aceclofenac". *International Journal of Pharmaceutical Science and Research* 9 (2018): 3815-3826.

29. Sahoo S., *et al* "Design and Development of Self Microemulsifying Drug Delivery Systems (SEDDS) of Telmisartan for Enhancement of *In-Vitro* Dissolution and Oral Bioavailability in Rabbit". *International Journal of Applied Pharma* 10 (2018): 117-126.

30. Wagh MP., *et al*. "Solid self-emulsifying drug delivery system: preparation technique and dosage forms". *International Journal of Biopharmaceutics* 5 (2014): 101-108.

31. Wang CY., *et al*. "Self-Nano emulsifying Drug Delivery Systems for Enhancing Solubility, Permeability, and Bioavailability of Sesamin". *Molecules* 25 (2020): 3119.

Volume 5 Issue 8 August 2021