"SELF EMULSIFYING DRUG DELIVERY SYSTEMS (SEDDS) OF EZETIMIBE FOR ENHANCEMENT OF ORAL BIOAVAILABILITY: FORMULATION, OPTIMIZATION AND CHARACTERISATION"

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in Partial Fulfillment for the Award of the Degree of

MASTER OF PHARMACY

IN

PHARMACEUTICS

BY

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June 2020

CERTIFICATE

This is to certify that the dissertation work entitled "Self-Emulsifying Drug Delivery Systems (SEDDS) of ezetimibe for enhancement of oral bioavailability: Formulation, Optimization and Characterisation" submitted by Ms. Mitali Paryani with Regn. No. (18MPH107) in partial fulfillment for the award of Master of Pharmacy in "Department of Pharmaceutics" is a bonafide research work carried out by the candidate at the Department of Pharmaceutics, Institute of Pharmacy, Nirma University under my guidance. This work is original and has not been submitted in part or full for any other degree or diploma to this or any other university or institution.

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CERTIFICATE OF ORIGINALITY OF WORK

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Ms. Mitali Paryani

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LIST OF ABBREVIATIONS

1. SEDDS: Self-Emulsifying Drug Delivery Systems
2. LCT: Long Chain Triglycerides
3. MCT: Medium Chain Triglycerides
4, SCT: Short Chain Triglycerides
5. UV: Ultraviolet
6. Vd: Volume of Distribution
7. BCS: Biopharmaceutical Classification System
8. BA: Bioavailability
9. PWSD: Poorly Water-Soluble Drugs
10. LBF: Lipid Based Formulations
11. HLB: Hydrophilic Lipophilic Balance
12. PG: Propylene Glycol
13. PEG: Polyethylene glycol
14, TG: Triglycerides
15. SMEDDS: Self Micro-emulsifying Drug Delivery Systems
16. SNEDDS: Self Nano emulsifying Drug Delivery Systems
17. GIT: Gastro-Intestinal Tract
18. LC: Long Chain
19. MC: Medium Chain
20. SC: Short Chain
21. HDL: High Density Lipoprotein
22. LDL: Low Density Lipoprotein

ABSTRACT

Ezetimibe is BCS class II drug with low water solubility and good permeability having Log P value 4.14. It exhibit very low bioavailability with high intersubject variability and lack of dose proportionality. Various formulation strategies have been tried for BCS class II drugs such as solid dispersion, complexation, lipid-based systems for improving their solubility and dissolution profile. In this research we tried lipid-based formulation called SEDDS for ezetimibe drug to enhance their bioavailability by improving its solubility. On the basis of UV, DSC & FTIR purity of drug was checked, after which saturation solubility studies of ezetimibe was done in numerous Oils, Surfactants and Co-surfactants for selection of all three components. The ratio of Smix was selected using ternary phase diagram using water titration method. Formulation was prepared using Capryol 90 as oil phase, Tween 80 as Surfactant and Transcutol P as Co-surfactant. Characterization was done on the basis of result, optimization was performed using simplex centroid design of Mixture design. The finding was to see the effect of each component with their ratio on self-emulsification process and to evaluate. The aim of the study was to formulate SEDDS with small droplet size to increase the bioavailability of ezetimibe in the body given by oral route of administration.

1. INTRODUCTION

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1.1. INTRODUCTION TO LIPID BASED FORMULATIONS

Identification of various new chemical entities (NCEs) having high potency came into existence with the help of high throughput screening by combinatorial chemistry, but with lower aqueous solubility and high molecular weight lead compounds which ultimately result to poor and highly variable bioavailabity (BA) (O'Driscoll & Griffin, 2008). In GI tract, when poorly soluble drugs exist in crystal-like state, have dissolution as rate limiting step for its absorption. These types of drugs majorly belong to class II/IV type in BCS. Class IV of BCS have less absorption with low solubility & permeability so they are not considered as good candidates for formulation except when the dose is low, while compounds which belong to class II of BCS have poor solubility and good permeability so its absorption is highly dependent on its formulation development (Pouton, 2006). So, lipophilic candidates are increasing with new lead compounds and in response many new formulation strategies are being developed by formulation scientist to increase solubility, absorption and ultimately bioavailability of these compounds (Trevaskis, Charman, & Porter, 2008).

Oral formulation of such compounds can be formulated but by ensuring consistency in BA. Strategies such as particle size reduction, drug solution, amorphous type system and lipid formulation are considered for such formulation (Pouton, 2006). It was observed that food affect absorption of drugs, lipids when digested with poorly water-soluble drugs (PWSD) result in improving bioavailability by increasing its absorption and gave evidence about lipid's beneficial role on absorption of drug in the body. Thus, new formulation strategy gain interest academically and commercially where use of natural and synthetic lipids is done for improving bioavailability of PWSD (Humberstone & Charman, 1997). There are main three ways by which lipophilic excipient and lipids affect absorption, BA and disposition after oral BA which are:

a) increasing solubilization of drug by composition alteration in intestinal milieu

b) lymphatic drug transport in intestine which reduce first pass metabolism

c) interaction of lipids with transport process based on enterocytes (Porter, Trevaskis, & Charman, 2007)

1.1.1 Lipid excipients and lipid formulation classification

There are many excipients available for lipid-based formulation, so the screening of such excipients and selecting the best among them is very important. Lipid excipients currently

marketed includes natural product oil, fatty acids, semisynthetic monoglycerides, diglycerides, triglycerides, semisynthetic PEG derivatives of fatty acids & glycerides, synthetic lipid excipients, surfactants and co-solvents. Main factors that are considered important for lipids excipients selection are its chemical stability, solvent capacity, regulatory issues, morphology, digestibility, miscibility, self-dispersibility purity, and cost. There are many systems in LBF, starting from simple oil solutions to use of complex mixtures including oil, surfactants, cosolvents and co-surfactants. In 2000 pouton introduced a system called "Lipid Formulation Classification System" or LFCS. The system was mainly classified to identify and interpret critical parameters and *in vivo* studies respectively so that best formulation can be identified for every specific drug. (Hauss, 2007; Pouton, 2006; Pouton & Porter, 2008)

TABLE 1.1: LIPID FORMULATION CLASSIFICATION SYSTEMS

(Pouton, 2006),(Pouton & Porter, 2008)

Category	Components	Attributes
Type I	Only Oils	Non- Dispersing and requires digestion
Type II	Oils + water insoluble surfactants	SEDDS with no water-soluble components
Type III A	Oils + surfactants + co-solvents (water insoluble + water soluble excipients)	SMEDDS/ SEDDS with water soluble components
Type III B	Oils + surfactants + co-solvents (water insoluble + water soluble excipients) with low oil	SMEDDS with low oil and water-soluble components
Type IV	Surfactants (water soluble) + co-solvents	Oil free, form micellar solution

a) Type 1 system:

In this system, drug is present in solution form in TGs &/or in o/w emulsion form having emulsifier in low concentration for stability or in mixed glycerides. Generally, emulsifier used are lecithin and polysorbate 60. Type 1 system tend to exhibit poor dispersion thus it need to undergo digestion by the components in GIT called co-lipase/ pancreatic lipase to generate amphiphilic products of lipid digestion consequently to stimulate transfer of

compound in colloidal phase. These are better known as simple oil solution type of formulation and is better option for potent/lipophilic drugs where drug is sufficiently soluble in oil to allow sufficient drug loading.

b) Type 2 system:

In this system, formulation form SEDDS. By using surfactant > 25 %, self-emulsification takes place. At larger content of surfactant above 50-60%, emulsification doesn't takes place it gets compromised by viscous gel formation i.e. liquid crystalline at o/w interface. This system have the advantage of overcoming slow dissolution which is seen in solid dosage form by generating interfacial area which will allow drug partioning efficiently b/w oil droplets and water phase from where absorption of drug will occur.

c) Type 3 system:

This system is known as SMEDDS and it consist of hydrophilic type surfactants with HLB value greater than twelve with PG, ethanol and PEG as co-solvents. In these types of formulations, systems further categorized into A and B systems. In Type III B system, quantity of lipid is less plus amount of hydrophilic surfactants, cosolvents is more which will have greater rate of dispersion if competed to III A system. One of the major concerns in III B is, by decreased lipid content risk of precipitation also increases.

d) Type 4 system:

In this system, larger amount of hydrophilic surfactants as well as co-solvents is present as per recent trend. This system does not have natural lipid present and thus known as hydrophilic type of formulation. These types of system have good drug loading compared to other system which contain oil. Inspite of this, it tend to produce very fine type of dispersion when diluted in aqueous media. However, drug remain in solution form or not when introduced in body and pass through GI tract is still a concern when compared to other system which comprises of oils.

TABLE 1.2: EXAMPLE OF LIPID-BASED FORMULATION AVAILABLE IN MARKET (Hauss, 2007)

Sr.no	Drug	Type of formulation	Lipid excipients and surfactants	Non-Lipidic excipients
1.	Amprenavir (Agenerase®	Oral solution (15mg/ml)	TPGS (~12%)	PEG 400 (~17%), PG (~5%)

	GSK in 2000 UK)			
2.	Ciprofloxacin (Cipro®/Bayer)	Microcapsules as constitution to be prepared for suspension (500r 100 mg/ml suspension)/ (5-10% solid)	Medium chain TGs, lecithin, sucrose, strawberry flavor, water	PVP, methylacrylic acid copolymer HPMC, polysorbate 20, Mg stearate
3.	Cyclosporin A Neoral® in 1995 in Novartis	Soft-gelatin capsules (available in 10,25,50 and 100mg)	dl- α tocopherol, Cremephor RH 40, corn oil mono, di, triglycerides	Ethanol 11.9%, PG, glycerol
4.	Progesterone as Prometrium®	Available as soft gelatin capsule in micronized form (100 and 200 mg)	Peanut oil	-
5.	Ritonavir as Norvir® by Abbott in 1999 in UK	Available in form of soft gelatin capsule with 100 mg drug	Oleic acid, Cremephor EL	Ethanol, BHT
7.	Tocopherol nicotinate as Juvela® by Eisai Co. in 1984	Viscous suspension / semisolid form in soft gelatin capsule	Medium chain TGs, glycol ester of FA	Aspartic acid

1.1.2. Lipid based formulations for oral route of administration

For poorly soluble BCS class II compounds, dissolution in GI tract act as rate limiting step when given by oral route while in lipid-based formulation drug gets pre-dissolved in

Lipidic components which omit this rate limiting step and thus makes it suitable type of formulation for oral administration of drugs. Selection of lipid in oral route is one of most important criteria for better formulation as it does not only affect the solubilization of drug in prepared formulation but also during lipid digestion in GI tract which ultimately affect absorption as well as BA. As shown in figure 1, lipid digestion starts with partially digested lipids which undergoes emulsification in presence of pancreatic juice and bile salt and form emulsion with bile salt micelles. After which in presence of phospholipase, mixed micelles are formed having lamellar vesicular structure. These formed micelles will deliver drug and digested lipid to the enterocytes where both will be absorbed subsequently.

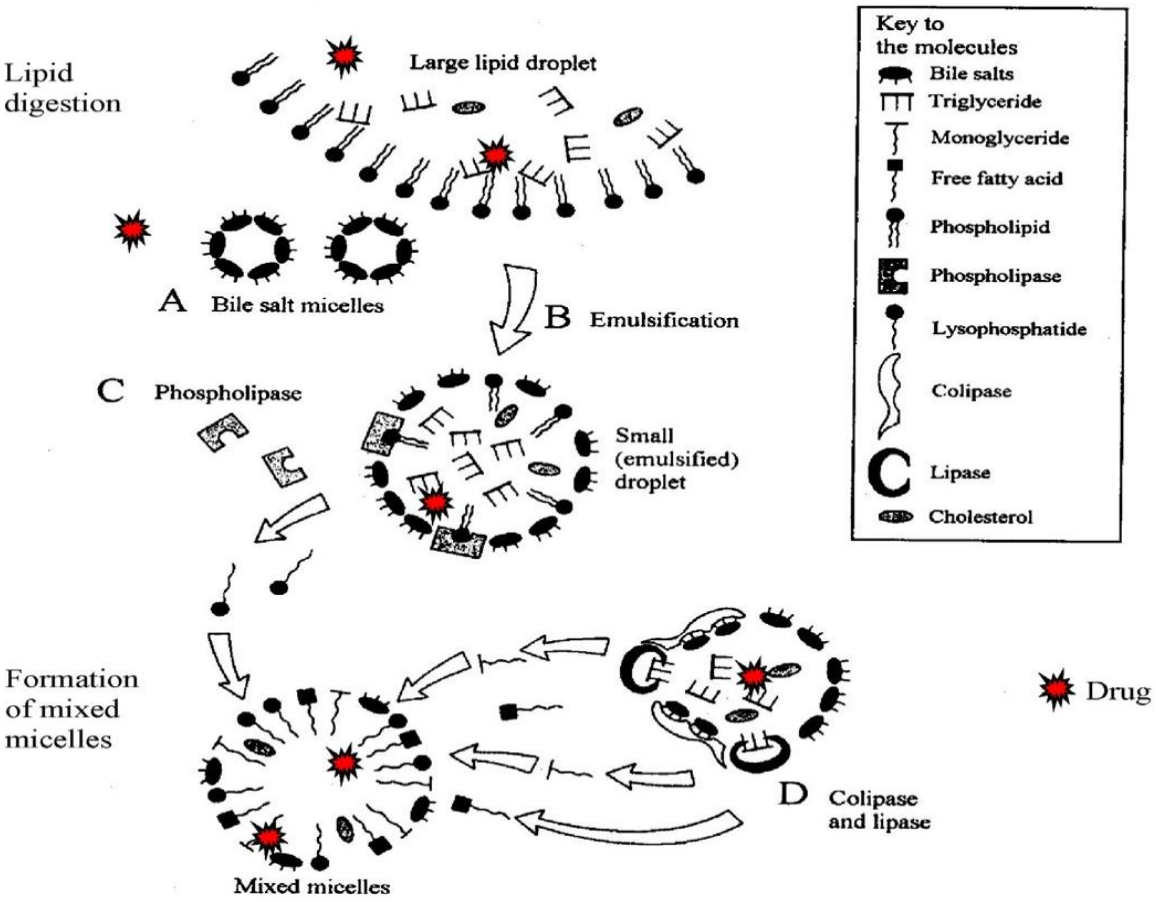


Figure 1.1. Lipid digestion process from (Mu et al., 2013)

1.2. INTRODUCTION TO SELF-EMULSIFYING DRUG DELIVERY SYSTEMS

Self-emulsifying drug delivery system is lipid-based formulation also known as isotropic mixture, composed of oil mixture(natural/synthetic), surfactants, cosolvents and/or co-surfactants which finally make O/W spontaneous emulsion in stomach. In GI tract, SEDDS formulation dispersed rapidly by the agitation provided from the motility of small intestine

and stomach. Being one of new delivery systems, formulators have identified some factors which affect drug bioavailability such as composition, droplet size, HLB value of the components, concentration of each component. One of the major considerations is lipid digestion dynamic which affect majorly on the drug absorption and thus it's bioavailability (Atef et al., 2008; Balakumar, Vijaya Raghavan, Tamil Selvan, & Habibur Rahman, 2013; Gibson, 2007). Typically, SEDDS formulation have size between 100-300 nm and SMEDDS have size of around 50 nm or less than that. As compared to conventional emulsion which are thermodynamic unstable, SEDDS are metastable form and easy to manufacture compared to simple emulsion with good physical stability. (Gursoy & Benita, 2004; Mahmoud, Bendas, & Mohamed, 2009).

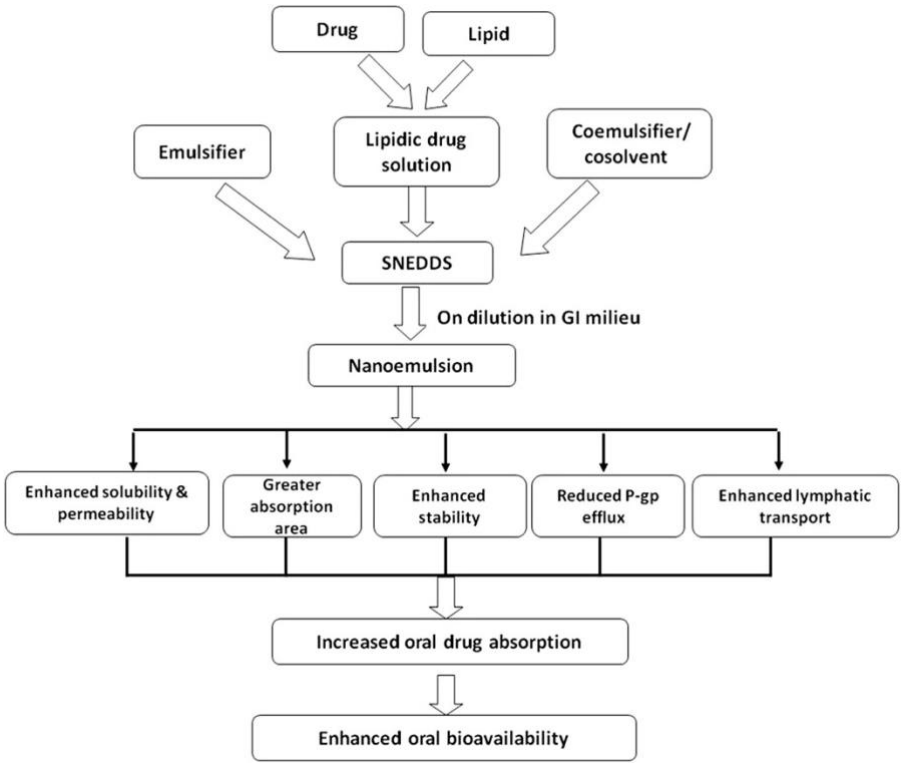


Figure 1.1: SEDDS formulation strategy and pathways (B. Singh et al., 2016)

1.2.1. Components of SEDDS:

SEDDS is an isotropic mixture which consist of oils, surfactants, cosurfactants and/or Co-solvents. Self-emulsification process totally depends on the oil: surfactant ratio, surfactant pair: nature of oil, concentration of surfactant and importantly temperature of the system at which self-emulsification takes place (Rahman, Hussain, Hussain, Mirza, & Iqbal, 2013). It is concluded by many scientists that only specific blend of oil, surfactant, co-surfactant and/or co-solvent can form self-emulsifying system efficiently. Apart from this interaction

between excipients as well as lipid digestion should also be considered as important parameters. Apart from the temperature HLB value of each component with HLB value of system also affect majorly on the stability of the self-emulsifying system.

1.2.1.1. Oil:

Oils can majorly affect the solubility of drug, size of droplet, stability of emulsion, in-vitro drug release by its physicochemical properties. So, selection of oil is very critical for the formulators in SEDDS formulations. Mostly, oil with maximum solubility is chosen for the formulation which can give nano-size droplets as well as stability. Oil is chief component of SEDDS having two advantages, one is it facilitates formation of SEDDS and other is drug loading can be increased as it absorbed by lymphatic route which have high solubilization for lipophilic drug. Oils are classified as below:

A) Natural oils/lipids:

Plant is primary source of natural oils from which abundant number of oils have been derived. Oils obtained from plant needs to go processing where isolation for various fractions are done and impurities is removed (Rahman et al., 2013). Apart from this, vegetable oils which are modified and hydrolyzed also used in formulation as these excipients have ability to form good emulsion system with other approved excipients as surfactants, giving good solubility of drug. Natural oil is preferred because they provide same end product as intestinal digestion end product which eventually give physiological advantages (Elgart, 2012).Examples of natural oils are olive oil, coconut oil, soybean oil and corn oil. Oils are generally less preferred in the formulation even after having great physiological advantage because of the poor solubility of lipophilic drugs (Kyatanwar, Jadhav, & Kadam, 2010).

Mixture of TG (Triglycerides) are present in the natural oils also known as triacylglycerol which means they are chemically fatty acid tri-esters of glycerol. These TGs of natural oil contain degree of unsaturation and different chain lengths. It is important to consider that melting point of TGs decreases by the increase in degree of unsaturation, while increases with the length of chain in fatty acids. Based on the HC chain length, TGs are divided as long chain (LC) TG with >12 carbons, medium chain (MC) TGs with 6-12 carbons and short chain (SC) TGs with < 5 carbons.

Triglycerides are hydrogenated so that the unsaturation decreases and so thus the oxidation. The whole process of isolation to fractions, removal of impurities and hydrogenation is done to decrease the susceptibility of TGs to degradation by oxidation and increasing better absorption with better physical properties. TGs are regarded as sage ingredient by GRAS because it is fully digested as well as absorbed from body without providing any safety issues (Greenberger, Rodgers, & Isselbacher, 1966; Rahman et al., 2013; Watkins, 1985).

B) Semisynthetic lipids:

Basically, semisynthetic lipids are prepared by the combination of plant derived MC Saturated fatty acids / glycerides with hydrophilic chemical entities available for oral formulations as pharmaceutical excipients. These excipients often available in liquid or as thermos-softening semisolid form. Applications of these excipients includes as wetting agent, solubilizing agent, surfactants, emulsifiers in SEDDS type of formulation. Due to their liquid and semi-solid form, they can be filled in both types of capsules including soft-gelatin capsules and hard gelatin capsules. In addition to that compared to natural TGs, semisynthetic lipids offer better uniform compositions. Though semi-synthetic lipids have better uniformity, variation in some parameters are seen. Variability in the position of glycerol backbone is also expected as which fatty acid is to be esterified. All the above discussion, give us idea that how formulator should be careful for choosing excipient as well as grade of excipient for the formulation by understanding composition variability and by using this variation potential of excipient (Gibson, 2007).

C) Synthetic Lipids:

There are many fully synthetic lipids that are used in formulations for very poorly soluble compounds. Mostly these lipids are monomeric or polymeric present as liquid or semisolid form and are of glycolic nature with relatively less toxicity. Most common example of this class is polyethylene glycols (PEGs), they are most versatile type of excipient which are present in liquid as well as in semisolid form used widely as solubilizer. PEGs are available in liquid form at molecular weight 200-600 and in semisolid form with more than 1000 molecular weight at ambient temperature. These are most used excipient but have several disadvantages when compared to natural oil such as chemical reactivity, relatively more GI irritation and peroxide impurities. They also contain other impurities by auto-oxidation which can further affect stability of drug incorporated in the system. Due to hygroscopicity,

PEGs have limited use in hard gelatin capsule as it affect integrity of the capsule while used greatly in soft gelatin capsule.

Other excipients mainly used are propylene glycol (PG) and poloxamers. Both of them are also available in various range of m.w. same as PEGs. PG is monomeric solvent which pharmaceutically acceptable and possess plasticizing and humectant properties. They are used for PWSD in soft gelatin formulation. While poloxamer composed of copolymers (polyoxypropylene and polyoxyethene) have surfactant and solvent properties and used widely for PWSD via oral delivery. All these excipient improve bioavailability of drugs which are poorly soluble and thus found many applications in various formulations (Rowe Raymond C, 2009).

1.2.1.2. Surfactants:

Surfactants also called as surface-active agents are molecules which are amphiphilic in nature consist of both polar as well as non-polar region. Having amphiphilic nature, surfactant can help lipophilic drug to It is generally divided on the basis of its HLB value. This includes detergent, foaming agent, wetting agent, dispersing agent, penetrating agent. In pharma field it is used as solubilizer, emulsifier and surfactant. There are various compound that are exhibiting property like surfactant but only very few are being used in SEDDS according to their potential. Surfactants help to extend drug time in GIT by precipitation prevention so give more absorption time. Some of the Surfactants also have been noted to increase food emptying time which ultimately gives more time for absorption. The chosen surfactants should have the capacity of decreasing the interfacial tension to a certain value where dispersion process can be facilitated during SEDDS formation. It should have negative free energy which can form film which is flexible in curvature shape and can deform easily around droplets. Most important surfactant property while forming an SEDDS in-vivo is prevention of precipitation by dilution in gastric conditions so that drug can easily absorbed by being in solubilized form until absorption.

To form stable SEDDS, concentration of surfactants is chosen to use between 30-60 %, mostly high HLB non-ionic surfactants are preferred for the SEDDS formulation while surfactant having low HLB are generally used as co-surfactants. GI irritation is one of the concern at use of large concentration surfactant so the safety aspect is major consideration. There are various mechanism known to scientist which can increase bioavailability of lipophilic drug by surfactants such as: improved dissolution of drug, increase in

permeability at tight junction and increasing permeability at intestinal epithelia. Examples of non-ionic surfactants that are used in market for oral formulations are Tween 20 , Tween 80, Labrasol, Labrafil M 1944 CS (Chavda & Shah, 2017; Kyatanwar et al., 2010).

1.2.1.3. Co-surfactants:

In SEDDS formulation, surfactants play significant role for reducing the interfacial tension but large amounts of surfactant may create toxicity and can be harmful to the patient. So, use of cosurfactants started as an alternative of surfactants. Co-surfactants decreases interfacial tension by increasing elasticity of film formed by surfactant between external phase and dispersed droplets. Medium chain amide, alcohol or acids containing C_8 to C_{10} are generally used as Co-surfactants (Chavda & Shah, 2017; Krstić, Medarević, Đuriš, & Ibrić, 2018).

1.2.1.4. Co-solvents:

Generally, high concentration of surfactants are less preferred for narrowing their use for formulation stabilization so co-solvent is alternatively used to that. Co-solvents are used to dissolve the hydrophilic surfactants and/or drug in lipid. By combining with co-surfactants, these can impart stability to interfacial film. Co-solvents includes organic solvents and some other compounds which are proved to be suitable for oral application. Some examples are propylene glycol, ethylene glycol, caprolactam, tributyl citrate and ethyl propionate. One of the disadvantage of alcohol based compounds is that it can migrate into shell of gelatin capsule which can result in precipitation of drug .while in alcohol free type of so-solvent there is limited drug dissolving property and thus choice of co-solvent should be done properly during screening (Chavda & Shah, 2017; Krstić et al., 2018).

1.2.2. Self-emulsification theories:

None of the Self-emulsification theory are yet proved but there are many hypotheses about the mechanism of self-emulsification. Reiss have suggested that when there change in entropy favors the dispersion is greater than the energy required for dispersion to increase its surface area, then the process of self-emulsification can take place (Reiss, 1975).

While Pouton observed self-emulsification under light microscopy and suggested that the mechanism involved in it is, fine cloud erosion of small droplets from larger droplet surface rather than gradual droplet size reduction. This can be due to large amount of surfactants (Pouton, 1997).

According to other theory given by Dabros, it is suggested that in SEDDS energy required for emulsification is very less and thus emulsion form spontaneously while in conventional emulsion, several emulsifying agents are required to diminish the interfacial tension plus free energy which is necessary for creating different surface between the 2 phases of oil and water. On the other hand, in absence of emulsifying agents, both phases gets separated to decrease the free energy between them and form coalescence (Dabros T., Yeung, A., Masliyah, J., 1999).

Lopez and its co-workers said that ease of emulsification formation is associated with ease of water penetration in water-oil interface by the liquid crystalline phase formation which finally results into swelling at interface. While in presence of surfactants, co-surfactants mechanism known as diffusion & stranding is proposed to occur where components partitioning occurs significantly between aqueous phase as well as oil phase, and oil gets solubilized leads to migration of oil into water or aqueous phase (López-Montilla, Herrera-Morales, Pandey, & Shah, 2002).

It was also suggested that by moderate agitation of the system, water can able to penetrate rapidly in aqueous cores which ultimately leads to interruption of interface and development of droplet. This results to development of interface to liquid crystal surrounding the droplets of oil and tends to decrease coalescence and form stable SEDDS (B. Singh, Bandopadhyay, Kapil, Singh, & Katare, 2009).

For further understanding, Gursoy used PSA (Particle Size Analyzer) & LFDS (Low Frequency Dielectric Spectroscopy) to observe the self-emulsification properties of systems by using Imwitor 742 series which includes mixture of Tween 80 with monoglycerides and diglycerides of capric & caprylic acids. From this result it was proposed that there is relationship between formation of emulsion and formation of liquid crystal. However, it also suggested the drug presence may affect the properties of emulsion due to interaction of drug to Liquid crystalline phase (D. P. Maurya, Yasmin Sultana, 2016; Gursoy & Benita, 2004)

Another phenomenon was described by Sjoblom, where initially surfactant, surfactant mixture and/or co-surfactant favors the oil as continuous phase for microemulsion. But when GI fluid/ water comes in contact with the surfactant, chemical reaction with water diffusion occurs. This process led surfactant to become surfactant more hydrophilic which then tries to increase water solubilization and decrease oil solubilization capacity. On result

of which oil tends to nucleate and microemulsion inverse the phase where water becomes continuous phase & oil droplets gets dispersed in aqueous phase (Sjoblom, 2005).

Deliberating all the theories, we can say that the Self-emulsification is a complex process, where drug, oils, surfactants, co-surfactants/co-solvent and water can influence to large extent to formulations and thus clear understanding is very necessary for preparing any emulsion and considering them as important factors.

1.2.3. Regulatory aspects:

For many years, lipids were used in many formulations mainly as fillers, diluents, binders, solvents, lubricants as an inert substance. But after advances in technology and pharmaceutical application, many novel excipients were available and many interactions were seen like drug-excipient, excipient-excipient, excipients with environment as well with container system which finally concluded that not all the excipients are inert and potential toxicities may be observed (Chen, 2008).

USFDA regulatory agency has published CFR listing for GRAS (Generally Recommended as safe) substance list which are considered as safe. The agency not only have list of substance but also limit decided for each inactive ingredient to be used known as IIG (Inactive ingredient guide) that are used in market products. This gives an idea to the formulator about maximum dose given by specific administration route and dosage form. One of the important considerations given by guidance is, if any inactive ingredient got approved for drug product by particular route then it will not be treated as new and less extensive assessment will be included in next product.

TABLE 1.3: LIPID EXCIPIENTS EXAMPLES AND THEIR REGULATORY ASPECTS (Rahman et al., 2013)

Sr no.	Lipid	Regulatory status	HLB value
1.	Capryol™ 90	USP-NF, FCC, JSFA, USFA	6
2.	Capryol™ PGMC 90	USP 31-NF 26 supp 1	5
3.	Labrafil® M 1944 CSS	US-NF, EP	4
4.	Labrafil® M 2125 CSS	US-NF, EP	4
5.	Labrafil® M 2130 CSS	EP	4
6.	Labrasol®	US-NF, EP	14
7.	Cremophore® RH 40	FDA inactive ingredients	14-16

8.	Cremophore® EL	USP	12-14
9.	Cremophore® A25	-	15-17
10.	Transcutol® HP	US-NF, EP, USIFA	-
11.	Transcutol® P	US-NF, EP, IIG, USIFA	-
12.	Capmul® MCM 8	EP	5-6
13.	Capmul® MCM 10	EP	5-6
14.	Capmul® MCM	EP	5-6
15.	Plurol Oleique® CC497	FCC, USFA, E471, JSFA	6
16.	Plurol® Diisostearate	EP	-
17.	Peceol™	US-NF, EP, FCC, GRAS, E471, JSFA	3.3
18.	Captex ® 355	USP	-
19.	Capttex® 200	-	-
20.	Miglycol® 818	-	-

Further, guidance is also given by FDA about the testing of excipient by various strategies. For many biological and drug products premarketing approval is done for excipients as components of the formulation. This was particularly for lipid excipients used in formulation because it's properties and complex interaction with other excipients, drug as well as physiological environment. Apart from this, oil tends to become cytotoxic when developed in nano size and thus scientist needs to be more careful for using it in the formulations. Other than oils, surfactant may also lead to GI irritation at higher concentration which can be considered before using it in the systems (Kohli, Chopra, Dhar, Arora, & Khar, 2010).

1.2.4. Biopharmaceutical Aspects

It is well known that foods/lipids have the ability to increase bioavailability of PWSD but mechanism by which bioavailability increases is still not well understood. Some of the possible mechanism are as follows:

1) By altering the gastric transit, slowing the drug absorption at site and increasing available dissolution time in GI tract.

2) Increasing the solubilization capacity in GI tract via formation of mixed micelles in presence of lipids which increases bile salt, cholesterol and endogenous lipid secretion and finally increases luminal solubility of drug.

3) Increasing the extent of lymphatic transport in intestine by using lipids with lipophilic drugs which decreases drug loss via first pass metabolism and leads to increase in bioavailability directly or indirectly. It may not be the case with hydrophilic drug which directly diffuse to portal supply.

4) By reducing the enterocyte-based metabolism using certain surfactants and lipids which lessen the activity of efflux transporter in intestine as directed via P-gp efflux pump. Thus, affecting the biochemical barrier of GI tract

5) Reducing physical barrier in the GI tract, by increasing drugs permeability in intestine using different combination of lipids, it's digestion products and surfactants. For most of lipophilic drugs, intestinal permeability is not majorly affecting bioavailability. (Constantinides, 1995; Jill, 2017).

1.2.5. Factors to be considered in formulation of SEDDS

a) Physicochemical properties of drug:

Drugs with low solubility are suitable candidates for lipid-based formulations, but it is important to consider that if compounds have low solubility in lipid application of SEDDS and other lipid-based formulation are limited. Lipophilic drugs are good candidates for SEDDS while drugs having Log P <2 are difficult to deliver by SEDDS (Pouton, 2000).

b) Dose of drug:

High dose drugs are not appropriate for SEDDS formulation except it have good solubility in Lipidic component. Drug solubility in oil phase is considered very important as it influence the time for which drug will be in solubilized form in the system. It is also said that SEDDS system take almost five days for reaching equilibrium so the drug remain in super saturated form for 24 hours after first emulsification event. Thus, it is said that such system will not precipitate in gut before the process of absorption and also enhance the absorption by remaining in super saturation state by increased thermodynamic activity of drug (Constantinides, 1995).

c) Miscibility of excipients:

Miscibility of all the excipients to be used in the system is required to form stable system. It is generally seen that LCT are not miscible with co-solvents/ hydrophilic surfactants so

need of third components such as co-surfactant is necessary to blend this system and promote the miscibility. Adding polar oil or co-surfactant generally make the system more beneficial in terms of enhancing dispersibility in the system. While water insoluble surfactants are miscible with LCT and MCT. On long term storage, several lipid excipients with diverse composition leads to immiscibility (Pouton & Porter, 2008).

d) Drug incorporation:

For many PWSD, hydrophobicity is major problem which prevent drug from dissolving in the common solvents approved. There are several new surfactants and synthetic oils which can dissolve these type of drug at a better extent. Apart from this, by adding solvents such as ethanol, PEG and PG also tends to contribute in betterment of drug solubility in lipid. Efficiency for the incorporation of drug is mainly dependent on physicochemical compatibility between drug/system. It is generally observed that drug may interfere with process of self-emulsification which leads to change in optimal ratio of oil: surfactant, results in change of size distribution.

e) Positively charges SEDDS:

It is well proven that most of absorptive cells with other cells are having negative charge with respect to the lumen mucosal solution. This gives us idea that emulsion with positive charge can be more beneficial as it will be naturally attracted to the negative charge of physiological compounds. Good interaction will be possible of system with biological components in GI environment.

f) Capsule compatibility:

Polar molecules with low m.w. can penetrate in the capsule shells made of gelatin, which restrict the use of PG and related solvents to be used in the system. Some surfactants are also able to destabilise shell which can affect the integrity of capsule. So, it is important that caution to be made for choosing the right excipients and also to hold integrity of capsule till shelf life (Pouton & Porter, 2008).

1.2.6. Enhancement of bioavailability by SEDDS

Absorption of drug from an oral route is a consecutive step of dissolution & permeability. It has been proven that bioavailability of a compound majorly depends on the solubility & permeability. Thus, poor absorption is result of inadequate dissolution rate or less permeation. On the basis of compound's solubility and permeability, it has been classified in four BCS class named as BCS class I which have good solubility and good permeability,

BCS class II having low solubility and good permeability, BCS class III having high solubility and low permeability and BCS class IV having low solubility and low permeability. It can be seen that in class II of BCS, solubility and dissolution are rate limiting step. Various formulation strategies are been tried from micronization, solid dispersions, co-solvency, complexation and lipid-based formulations. While for BCS class III, permeation enhancers have been successful while for BCS class IV is still problematic for formulation scientist. While SEDDS have been found to have beneficial for BCS class II and IV drugs via improving both solubility & permeability (B. Singh et al., 2009).

Drug release from SEDDS occurs during transport of droplets and its disintegration in GI tract by partitioning in intestinal fluids. Efficiency of drug release from system is based on two parameters including particle size of droplets and polarity of droplets. However, in O/W type of emulsion polarity does not play major role as drug reaches to the capillary in incorporated form in oil droplets. Some of factors responsible for increasing oral bioavailability are as follows:

a) Effect of Lipids:

Lipids have a great effect on drug's oral bioavailability by exerting its effect via complex mechanism which can alter drug properties including drug dissolution rate, solubility of drug in intestinal fluid, formation of lipoprotein which promotes the lipophilicity of compound to lymphatic transport as well as drug protection from enzymatic and chemical degradation. By promoting lymphatic transport of the lipophilic drug, lipids avoid first pass metabolism which can result to increase in absorption and thus bioavailability.

Drug absorption and distribution is majorly affected by the lipid chain, it's saturation and administered lipid volume. When drug is transported via lipid system, presence of lipoprotein is essential which can stimulate bile salt production with lipoprotein. It should be noted that only long chain FA & mono-glycerides can re-esterified to TG within cells of intestine while incorporated in chylomicrons & secreted via exocytosis into lymph vessels. Further formation of small droplets of size 0.5-1 µm via emulsification of large droplets in intestine by presence of cholesterol, lecithin, monoglycerides and bile-salts. These small droplets than metabolize by using pancreatic lipase as catalyst which further form mixed micelle. These microemulsion will then get absorbed by diffusion, pinocytosis or endocytosis process and reach systemic circulation by lymphatic/portal system (Gursoy & Benita, 2004; B. Singh et al., 2009).

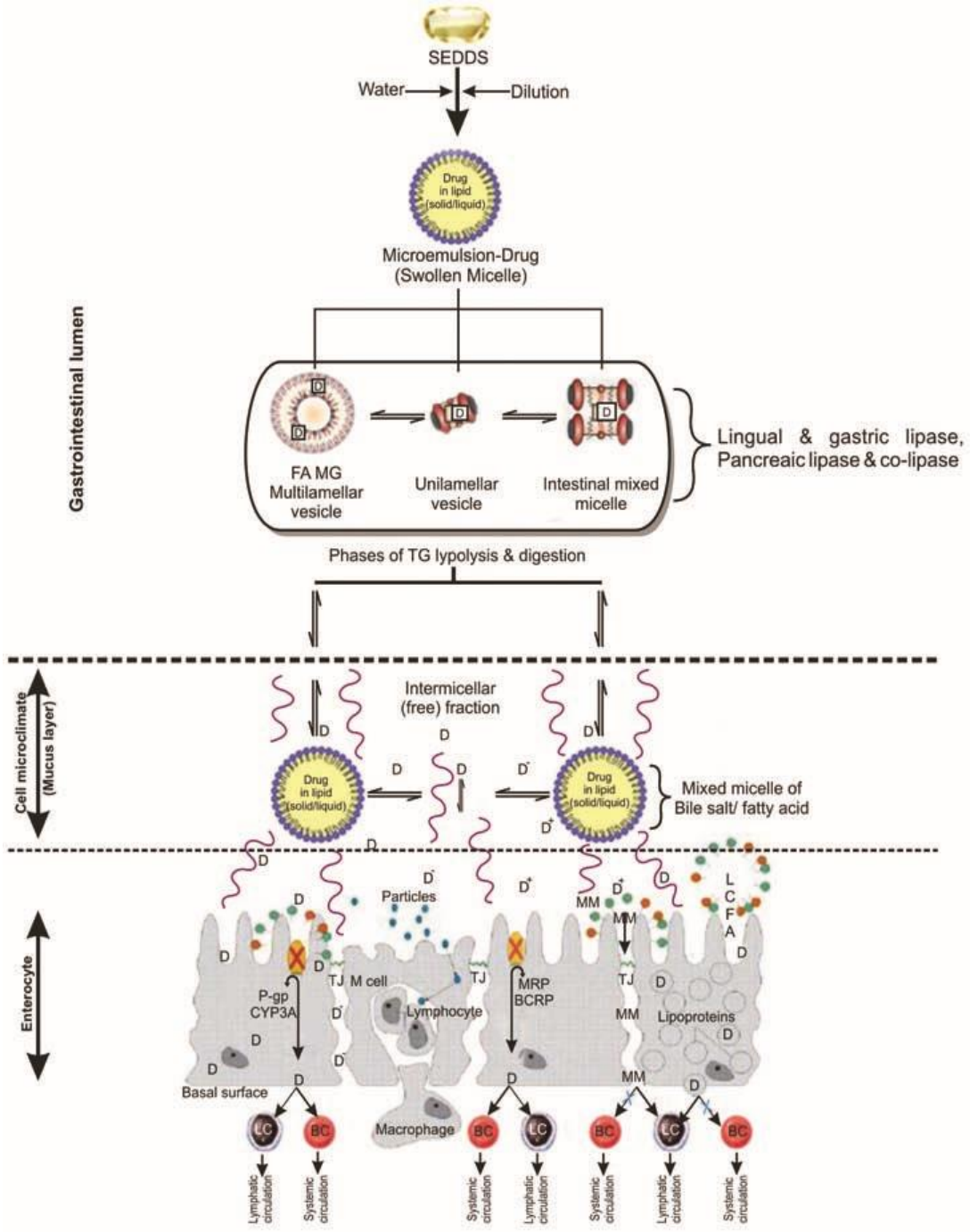


Figure 1.2: SEDDS Mechanistic pathways across GI lumen for drug transportation

(B. Singh et al., 2009)

b) Effect of Surfactants:

There are several mechanisms by which surfactants improve bioavailability of drugs including increasing permeability in tight junction, intestinal epithelia, decreasing p-gp efflux and improving dissolution of drug. By interfering the lipid bilayer present on

epithelial cell membrane, permeability was increased. Here, lipid bilayer was barrier for absorption of drug with unstirred aqueous layer and thus passive route was major route for drug absorption. By partitioning in the cell membrane structural organization was disorder using surfactant which led to permeation enhancement. Apart from this, use of surfactant decreases the globule size of the droplet which further increases dissolution. These small oil droplets will have large area for hydrolyzation of TGS by pancreatic lipase which can also result to increase release of drug by mixed micelle formation of drug and bile salts (Gursoy & Benita, 2004; Kommuru, Gurley, Khan, & Reddy, 2001).

c) Effect of P-gp inhibition:

P-gp is multidrug efflux type pump which is responsible for phase I metabolism via cytochrome P450s of intestine. It is now recognised as one of the major factor affecting drug's oral bioavailability. In SEDDS type of formulation, excipients inhibit p-gp efflux transporter which thus inhibits pre-systemic metabolism as well as intestinal efflux resulting into increase in uptake of drug from GI tract and thus oral absorption. Certain surfactant of category non-ionic are reported as p-gp inhibitor including cremephor, Tweens & Spans. BCS class II drugs do have good permeability but still P-gp inhibition significantly impact oral bioavailability of drug (Gursoy & Benita, 2004; Hauss, 2007).

1.2.7. Evaluation of SEDDS

Evaluation of SEDDS is challenge as it is complex and dynamic type of system due to presence of colloidal size particles. Some of the important parameters for evaluation are discussed below:

A) Construction of Pseudo ternary phase diagrams:

As per drug's solubility in numerous lipids (oils), surfactants, cosurfactants and co-solvent screening is done. Once selected, water is used for phase diagram as an aqueous phase. Smix (surfactants & co-surfactants) are taken in various ratio from 1:2 to 2:1 with increasing concentration of surfactant and then increasing concentration of co-surfactants with co-surfactants and surfactants respectively. In every phase diagram, oil: Smix ratios are taken starting from 9:1 to 1:9. These diagrams are created using aqueous titration method in which slow addition of water is done and observed visually if emulsion is transparent or not. These results are then recorded on diagram where one axis point have oil, second have Smix and third have water on it.

B) Particle Size:

Particle size is one of the important parameters whose accurate estimation is necessary as it can affect highly on the in-vivo functioning of the SEDDS. The particle size of the SEDDS is done by diverse methods involving dynamic light scattering (DLS), Transmission electron microscopy (TEM), Photon correlation spectroscopy (PCS), Scanning electron microscopy (SEM), laser diffraction (LD), Atomic force microscopy (AFM). DLS is applied method for routine evaluation. DLS is based on principle of diffusion where time variation of scattered light by Brownian motion of particles are counted. These motions of particles are described by stokes equation. Intensity of the particles are collected through detector and deconvoluted to determine particle size (Elgart, 2012; Ujhelyi et al., 2018).

In orally administered lipid formulations, particle size is one of key factors which directly impact on stability, kinetic studies and in vivo performance. It has been studied several times that nano-size particles are enable to efficiently hydrolyze TGs which will promote solubilization of drug which will ultimately increases the bioavailability. Thus it is important to measure particle size and its distribution which will be beneficial to oral bioavailability of drug and gives formulator better understanding for properties of the system (Elgart, 2012).

C) Zeta Potential:

It can be defined as potential between dispersing liquid medium and droplet surface, also known as potential measured in double layer. It is used to measure the surface charge of the droplets which is responsible for stability of the particles. It is also considered that positive value more than 130 mV and negative value more than 230 mV shows that droplet have good stability and not tends to coalescence. It is calculated by measuring the droplets electrophoretic mobility where it is indicated that FFA presence leads to negative charge on SEDDS surface droplet and thus zeta potential. It is also said that being a preconcentrate of emulsion zeta potential is not important assessment for SEDDS which upon digestion convert to emulsion for very short time and get absorbed. But relevant to bioavailability, positive charged SEDDS are more favorable as it interact to negative charge membrane leads to improved absorption of drug. To resist the flocculation of dispersion, dispersion should have zeta potential of ± 30 mV which indicates physically stable system (Krstić et al., 2018; Ujhelyi et al., 2018).

D) Assessment of Self emulsification and dispersibility:

Basically, self-emulsification is transforming one LCS to other. It is defined as time required to form dispersion completely. SEDDS should exhibit quick emulsification as soon as it comes in contact with water by gentle agitation. The assessment of self-emulsification is done by using USP II standard dissolution apparatus. The stated amount of preconcentrate is added i.e. 5ml of preconcentrate was added to 250 ml water in paddle apparatus rotating at speed of 50 rpm with temperature 37 ± 0.5 °C.

Visually self-emulsification is evaluated by the observation of changes in formulation upon dilution of preconcentrate with water and outcomes are assessed according to arranging system as follows:

Grade A: Rapidly forming emulsion within one minute and have clear/ bluish appearance, Grade B: Rapidly forming emulsion but comparatively less clear and have bluish white appearance, Grade C: Milky fine emulsion that forms within 2 minutes, Grade D: Dull emulsion (grayish white color) having oily appearance and slow to emulsify, Grade E: Poor or very less emulsification with oil droplets present on surface.

Stability of emulsion is also checked by visual observation only of phase separation or precipitation for different time periods between 12-48 hours (Kadu, Kushare, Thacker, & Gattani, 2011; Krstić et al., 2018; Ramasahayam, Eedara, Kandadi, Jukanti, & Bandari, 2015)

E) % Transmittance:

Transmittance is basically % of light impinging on formulation and passes through it which then detected by instrument. It starts from 0% when light is completely absorbed as in case of opaque solution to 100 % when light is fully transmitted with no absorption.

Transmittance is also known as measurement of optical clarity of SEDDS dilute formulation from 10 to 100 times with water. It is done by using UV spectrophotometer by putting distilled water in both cells and adjusting 100 % transmittance. After that, in standard cell SEDDS diluted formulation is added which is measured for transmittance at around 650 nm using distilled water as blank

F) Thermodynamic stability:

The SEDDS formulation are subjected to diverse thermodynamic stability studies to check the stability of emulsion and also to assess phase separation. In this study, phase separation, change in appearance, drug-excipient interaction, incompatibility, etc. are checked

i) Cloud point determination:

It is important for the determination of stability during storage. The temperature at which emulsion break is identified as cloud point. For determination of cloud point, the formulation is diluted with water 1:100 ratio (SEDDS: water). This sample was then kept in water bath with gradually increasing temperature of around 5°C and the temperature at which turbidity appears first is noted. Other than visual method, spectrophotometric analysis is done and the temperature at which there is rapid decline of transmission was observed is noticed.

ii) Heating cooling cycle:

In this study, samples are kept for heating-cooling cycle between temperature of 4 °C and 40°C for at least 48 hours in storage. The sample then tested for creaming and cracking of sample. If sample passed this test, it will be further chosen for freeze-thaw cycle.

iii) Free-thaw cycle:

Freeze thaw cycle is thermodynamic study for stability purpose only, in which sample is kept for -21°C and +25°C is kept for NLT 48 hours as free-thaw cycle for three times. After, cycles get completed sample is centrifuged for five minutes at 3000 rpm and phase separation was observed. The sample which are stable in phase separation are then selected for further studies of dispersibility (Chavda & Shah, 2017; B. Singh et al., 2009).

G) in-vitro drug release studies

For, drug release studies in SEDDS suitable biorelevant media for dissolution studies is required. Generally, SEDDS formulation is kept in semipermeable membrane specifically dialysis membrane and then kept in USP dissolution apparatus I (basket)/ II (paddle). Use of III & IV USP type are also used nowadays as they can have biorelevant media used in study efficiently and give more relevant data. Aliquots are taken periodically to check drug release from oil droplets in the medium. These samples are then checked by different analytical techniques(Suthar, 2016).

H) In-vitro lipolysis

Based on the number of parameters affecting and can influence gastrointestinal absorption with complexity of the lipid digestion & absorption in intestine, conventional methods were not appropriate for in-vitro testing. Thus, in-vitro lipolysis model was constructed which can incorporate as many parameters as possible to mimic body environment to study the process and give more relevant result.

Here, lipolysis is performed in reaction vessel attached to magnetic stirrer at 37°C temperature maintained via thermostatically controlled jacket. Lipolysis is done by using a media which contain pancreatic lipase, bile salt, buffer formulation. Process of lipolysis is started by addition of lipase in solution form, where pH and free concentration of calcium is maintained in reaction mixture by computer controlled by NaOH addition and Calcium chloride addition respectively.

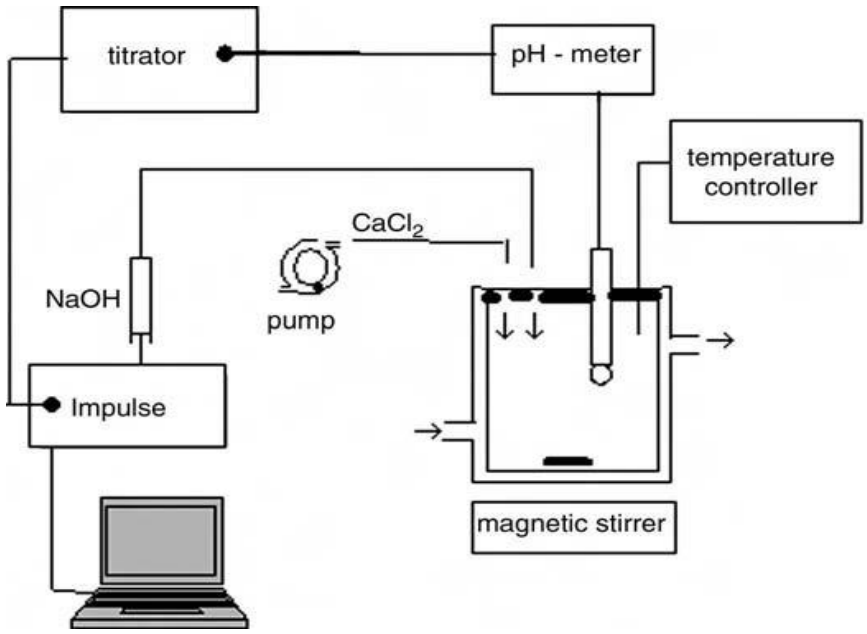


Figure 1.3: Lipolysis apparatus (Hauss, 2007)

First sample is withdrawn immediately after lipase addition and then at specific point of time after initiation of lipolysis process. Lipolysis inhibitors are added in the lipolysis process for quenching of process and samples are then withdrawn, followed by ultracentrifugation. This consist of three phases including:

1. First phase contain insoluble form of calcium soaps of fatty acids in a large pellet form.
2. Second phase contain aqueous layer which consist of lipid vesicles and mixed micelles of bile salts.
3. third phase which is upper most contain oily layer contain diglycerides and unhydrolyzed triglyceride (Hauss, 2007; Suthar, 2016)

I) In-vivo bioavailability

To predict the behavior of SEDDS formulation in the body, in-vitro studies are not generally sufficient. It is very difficult to mimic body conditions such as gastrointestinal

environment which have different environment for every individual. So, it is now essential to carry in-vivo BA studies to prove successful formulation by giving proper result. Appropriate animal models are used for testing to check bioavailability of drug and effect of SEDDS formulation on body.

1.2.8. Advantages and Disadvantages of SEDDS over conventional formulations (Sarpal, Pawar, & Arvind, n.d.)

1.2.8.1. Advantages:

1. Reduced dose and improved bioavailability.
2. Consistent absorption of drug in the body.
3. Bypassing the first pass metabolism by using different lipids which can deliver drug through lymphatic system.
4. Drug protection from GI environment by incorporating them in oil droplets.
5. Decreased inter-subject variability.
6. Formulation of SEDDS can be possible in liquid as well as solid form according to patient compliances.
7. Easy scale up and manufacturing which make SEDDS the most advantageous lipid-based system as compared to liposome, SLN, NLC.
8. SEDDS require simple and in-expensive manufacturing facility as compared to other lipid systems.
9. SEDDS have good absorption with increase in AUC, as it is present in dissolved state at absorption site.
10. Compared to conventional emulsion, SEDDS have good thermodynamic stability and can be autoclaved.

1.2.8.2. Disadvantages:

1. For proper assessment and predictability, lack of proper in-vitro models.
2. Large quantity of surfactants and co-surfactants may be used which can lead to toxicity.
3. Limited drug solubilizing capacity by the lipids alone.

1.2.9. Substitute to SEDDS and modification of SEDDS

Self-emulsifying formulations have been in research for longer time now. Researcher are now coming up with more and better formulation to overcome its limitations such as precipitation or to make formulations with other valuable properties.

1.2.9.1. Supersaturable SEDDS:

Severe side-effects in GI tract is one of the major limitations by using high surfactant concentration in SEDDS. But in case of low concentration of surfactant, system leads to precipitation on dilution. To overcome this, alternative of this system was introduced known as Supersaturable SEDDS (S-SEDDS). In S-SEDDS, low concentration of surfactant was used with addition of new excipient called polymer precipitation inhibitor (PPI). PPI maintains in-vivo metastable state i.e. Supersaturable state which surround droplet maintaining hydrophilic nature and prevent Ostwald ripening. These PPI prevent crystallization which basically have capability to make system Supersaturable and also maintained for extended period of time.

In S-SEDDS, dilution lead to microemulsion formulation which will undergo slow crystallization, indicating system remains in supersaturated system by PPI addition in the formulation. Some of the PPI examples are PVP, HPMC and NaCMC. Apart from addition of PPIs, sonication also achieved via sonication followed by short term heating at moderate temperature and cooling (Morozowich & Gao, 2009).

1.2.9.2. Solid SEDDS:

Several problems associated with liquid SEDDS such as high cost, low drug loading, low stability led to search for alternative which is now extensively used known as solid SEDDS. For SEDDS solidification method such as spheronization/extrusion is used which allow more loading of drug and content uniformity. It is solvent-free process and used extensively for solidification. Combining the advantage of both, conventional SEDDS and solid system are used in this formulation.

Here liquid preconcentrate is incorporated on powder by mechanism of adsorption using solid carriers. Colloidal silica, MCC and HPMC can be used as solid carriers. Techniques used for S-SEDDS formulation includes melt granulation, spray drying, extrusion. These can be formulated in form of tablets or pellets (Jannin, Musakhanian, & Marchaud, 2008).

1.2.9.3. Self-double emulsifying drug delivery systems (SDEDDS):

SDEDDS shows very good potential for BCS class III type of drugs for stimulating their oral absorption. It is water in oil in water (w/o/w) type of double emulsion also known as multiple emulsion with high surfactant concentration where, drug is present in droplets of water which is dispersed in oil, whereas oil is additionally dispersed in the water which upon gentle agitation in GI form emulsion spontaneously. The major advantage of

SDEDDS as compared to conventional emulsion is stability on long term storage (Qi, Wang, Zhu, Hu, & Zhang, 2011).

1.2.9.4. Positively charged SEDDS:

Absorptive cells have been proven to have negative charge by various physiological studies when compared to other cells, this is respect to lumen mucosal solution. Bioavailability enhancement of drug was observed in positive charge SEDDS when compared to other conventional type of SEDDS. It was also observed that cationic SEDDS have more binding compared to anionic SEDDS which means cationic SEDDS have better adhesion of droplets by electrostatic attraction to cell surface. This proves that emulsion with positive charge can be more beneficial as it will be naturally attracted to the negative charge of physiological compounds. Good interaction will be possible of system with biological components in GI environment which ultimately lead to better formulation. Some of the examples of positive charge inducer are stearylamine, chitosan, oleylamine which is widely used in lipid based formulation (Chavda & Shah, 2017).

TABLE 1.4: EXAMPLE OF SELF EMULSIFYING FORMULATION FOR LIPOPHILIC DRUGS

Type	Oil	Surfactants	Solvent	Model drug	Ref
1. SEDDS	mixture of mono and diglyceride (oleic acid)	Polyglycolyzed mono, di and triglycerides HLB=14	-	Ontazolast	(Hauss, 2007)
2.Sandimmun® SEDDS	Olive oil	Polyglycolyzed glycerides HLB=3 or 4	Ethanol	Cyclosporin A	(Grevel, Nüesch, Abisch, & Kutz, 1986)
3. SEDDS	Medium chain FA (saturated), peanut	PEG-25 glyceryl trioleate , Tween 80, Polyglycolyzed glycerides, medium chain	-	Ro-15-0778 (napthelene derivative)	(Shah, Carvajal, Patel, Infeld, & Malick, 1994)

		mono and diglycerides HLB=6-14			
4. Neoral® formulation (SMEDDS)	Corn oil hydrolysed	Polyglycolyzed glycerides, castor oil derivative (POE)	Glycerol	Cyclosporin A	(Constantinides, 1995)
5. Positively charged SEDDS	Ethyl oleate	Tween 80	Ethanol	Progesterone	(Tarr & Yalkowsky, 1989)

1.3. Optimization by Design of Experiments (Bolton & Bon, 2004; Politis, Colombo, Colombo, & Rekkas, 2017):

To optimize the process or formulation is to make it effective, perfect and as functional as possible. It is a way which is used to make best formulation under given set of conditions. Initially, optimization was to change one variable at a time and see its effect. But, modern pharmacy includes DOE (Design of Experiment) by which various variables/parameters are checked and improvement is done such that best formulation is formulated.

DOE is a organised, structured method for determination of the relationship between various factors affecting the process and its output. In different words, it means achieving proper knowledge by using different mathematical relations to process inputs as well as its outputs.

1.3.1. Need of optimization:

a) Assessing the outcome, by knowing which factor affecting the response.

b) Determining the relationship between factors, levels and their response quantitatively.

c) Decreasing the experimental trials.

d) Reducing the experimental time.

e) Minimising the use of resources.

f) Choosing the best formula.

1.3.2. Steps of DOE:

1. Setting the objectives.
2. Selecting the proper factors (variables) and responses.
3. Selection of experimental design.
4. Execution of design.
5. Checking data for consistency with assumptions.
6. Analysing the results properly.
7. Interpretation of result.
8. Preparing the optimised batch.

1.3.3. Types of DOE:

Following are main experimental designs depending on the study and parameters involved in it:

TABLE 1.5: EXPERIMENTAL DESIGNS (H. Singh, 2018)

Sr.no	Type of design	screening	optimization	Design variables	Use
1.	Full factorial	+	-	2-6	To study effect of few variables which are independent of each other.
2.	Fractional factorial	+	-	3-15	To study number of variable to select which variable is important & should be investigated further.
3.	Placket-Burman	+	-	4-26	Fractional alternative design to study main effects.
4.	Central composite	-	+	2-6	Find the optimized level by adding few to full factorial design.
5.	Box-Behnken	-	+	3-6	Alternative of CCD where optimal is not present in extremes.
6.	D-optimal	+	+	2-6/2-12	Variables in multi-linear constraints
7.	Mixture	+	+	3-6	When overall amount of composition is same only proportion varies. It includes simplex lattice, axial, simple centroid and D-optimal design.

1.4. DRUG PROFILE

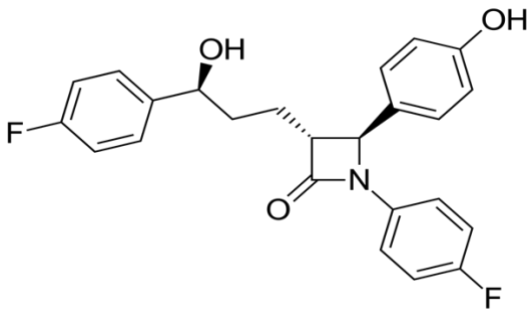
Name: Ezetimibe

1.4.1. Introduction:

Ezetimibe is an azetidinone derivative, the first drug in category of lipid lowering agent which can inhibit uptake of both dietary as well as biliary cholesterol in intestine without having any impact on the absorption of nutrients which are fat soluble. The terminated $t_{1/2}$ of ezetimibe and its metabolite is around 22 hours. It was approved in 2002 by FDA for decreasing cholesterol level in patients. This is given as an adjunctive therapy with healthy diet for decreasing/lowering the cholesterol level in mixed hyperlipidemia, primary hyperlipidemia, homozygous familial hypercholesteremia and phytosterolemia (Kosoglou et al., 2005).

1.4.2. Physicochemical Properties:

TABLE 1.6: EZETIMIBE PHYSICOCHEMICAL PROPERTIES (DRUG BANK)

1.	**Description**	It is white crystalline powder.
2.	**Molecular formula**	$C_{24}H_{21}F_2NO_3$
3.	**Chemical name**	1-(4-fluorophenyl)-3I-[3-(4-fluorophenyl)-3(S)-hydroxypropyl]-4(S)-(4-hydroxyphenyl)-2-azetidinone
4.	**Molecular Structure**	
5.	**Molecular weight**	409.4252 g/mol
6.	**State**	Solid
7.	**Solubility**	0.00846 mg/ml in water (Practically insoluble in water)
8.	**Pka (strongest acid)**	9.48
9.	**Pka (strongest base)**	-3
10.	**Log p**	4.14

11.	**Melting Point**	163 °C
12.	**BCS class**	II
13.	**Dose**	10 mg

1.4.3. Mechanism of Action:

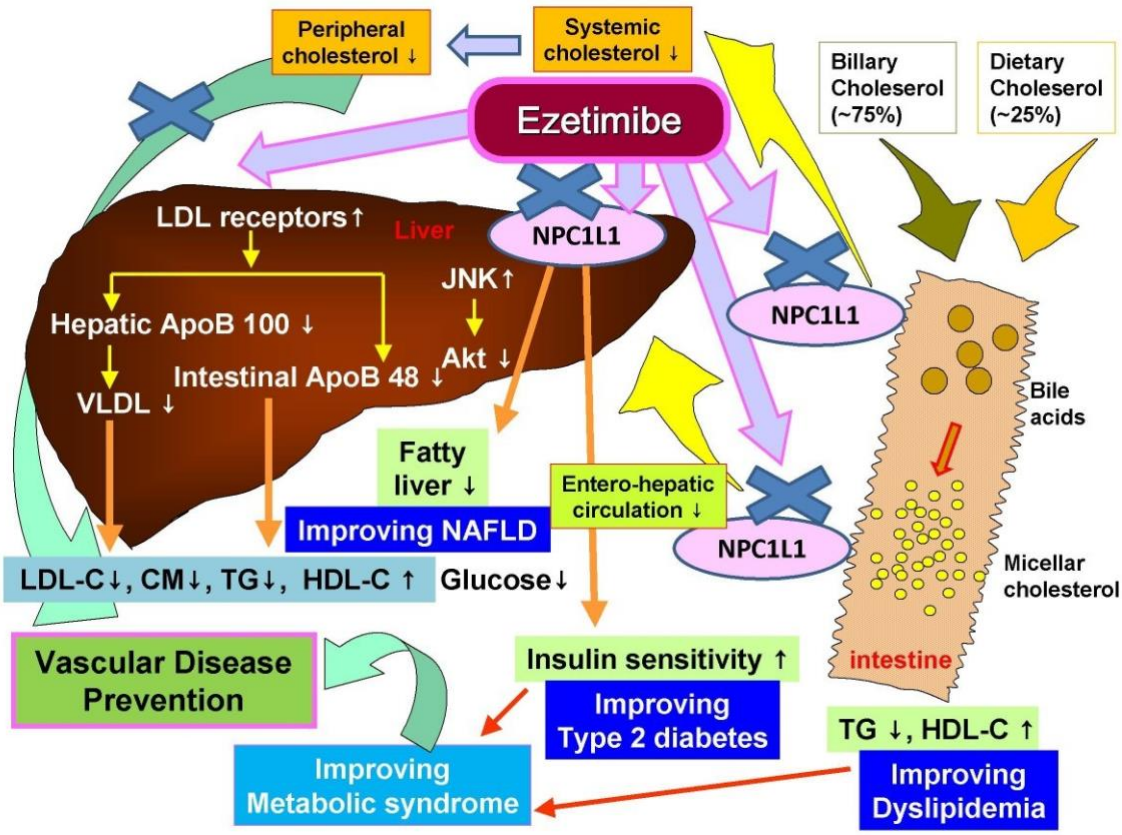


Figure 1.4: Schematic diagram of Ezetimibe Mechanism (Yamaoka-Tojo et al., 2009)

Ezetimibe inhibits phytosterol and cholestrol absorption in small intestine (SI) and lowers the cholesterol level. Ezetimibe inhibits Niemann-Pick C1-Like1 (NPC1L1) which is a transport protein for cholesterol and present on apical membrane at enterocytes and at canalicular interface i.e. hepatobiliary, it facilitates free cholesterol internalization into enterocyte in conjunction with clathrin & AP2 (adaptor protein 2). As soon as cholesterol incorporates in lumen of get or bile gets in enterocytes cell membrane, it starts binding with domain which is sterol sensing of NPC1L1 and makes a complex as NPC1L1/cholesterol complex. This complex then goes in internalization or endocytosis by AP2 clathrin joining which further forms a vesicle complex, it will then translocated in endocytic compartment for storage. Ezetimibe is independent of pancreas exocrine function for ezetimibe's

biological activity; rather it appears & localizes to act at small intestine brush border. It blocks NCP1L1 protein specifically in brush border of jejunum which then reduces lumen micelles uptake of intestine into enterocyte. This way, ezetimibe decreases intestinal cholesterol delivery to liver and this reduces storage of hepatic cholesterol and thus increase the cholesterol clearance from blood. The full working ezetimibe mechanism is still not understood at every extent. Some studies proposed that it prevent complex NCP1L1/sterol to interact with the clathrin AP2 and induces NPC1L1 conformational change (Kosoglou et al., 2005; Phan, Dayspring, & Toth, 2012).

1.4.4. Pharmacokinetics:

i) Absorption:

Single dose of ezetimibe is 10 mg, when fasted adult has taken single dose it reached Cmax of 3.4 to 5.5 ng/mL in span of 4-12 hours as Tmax. The metabolite of ezetimibe i.e. ezetimibe-glucuronide which is pharmacologically active reach Cmax 45 to 71 ng/mL with span of 1-2 hours as Tmax. It was observed that food has very minimum effect on the absorption of drug but ezetimibe when administered with very high fat meal it gets increased by 38%. The true BA cannot be determined as ezetimibe is insoluble in aqueous media (Schering Corporation, a subsidiary of Merck & Co., 2012).

ii) Volume of distribution (V_d):

V_d in case of ezetimibe is relatively around 107.5 Litre.

iii) Protein binding:

Ezetimibe and it's metabolite ezetimibe-glucuronide have bound of 90% to plasma protein. Protein binding in-vitro is ranged from 99.5%-99.8% for drug ezetimibe while 87.8%-92% for ezetimibe-glucuronide (Kosoglou et al., 2005; Schering Corporation, a subsidiary of Merck & Co., 2012).

iv) Metabolism:

Ezetimibe undergoes extensively metabolism via second phase of metabolism where conjugation reaction with glucuronide occurs in liver as well as in small intestine to form ezetimibe glucuronide which is considered as main phenolic metabolite. Ezetimibe glucuronide is around 80-90% of total drug that circulates in plasma & responsible for activity in inhibiting cholestrol uptake in intestine. Ezetimibe and it's glucuronide metabolite makes approximately 93% in human body of total drug present in plasma. Plasma profile suggest that ezetimibe undergoes enterohepatic recycling as it exhibits

multiple peaks and thus 20% drug gets reabsorbed from total drug distributed due to recirculation (Kosoglou et al., 2005; Schering Corporation, a subsidiary of Merck & Co., 2012).

v) Elimination:

Approximately 78% radiolabelled ezetimibe gets recovered in feces and 11 % gets recovered in urine when orally administered. In feces, 69% of drug was unchanged of total administered drug in feces while it's glucuronide metabolite was around 9% in urine as major component. High amount of unchanged drug suggest low absorption of drug in body or/and ezetimibe-glucuronide hydrolysis in bile (Kosoglou et al., 2005; Schering Corporation, a subsidiary of Merck & Co., 2012).

vi) Half-life:

Ezetimibe and it's glucuronide metabolite have around 22 hours of half-life.

vii) Clearance:

In context of clearance, no data is available on basis of pharmacokinetic.

viii) Toxicity:

Ezetimibe is considered as safe drug, as oral lethal dose and I/p lethal dose in rat is above 2000 mg/kg. In dog & mouse, it is more than 3000 mg/kg & 5000 mg/kg respectively. (Monograph, 2010).

1.4.5. Pharmacodynamics:

Ezetimibe reduces the level of LDL cholesterol, apoprotein B, total cholesterol, HDL cholesterol and non-HDL cholesterol in patients with hyperlipidemia. When given in combination with statin/fenofibrate it has shown better therapeutic effect compared to alone treatment. Patient having homo/heterozygous hypercholesteremia were given ezetimibe therapeutic dose in clinical trials and found to be effective in reducing 15-20% of LDL and increasing HDL levels by 2.5-5%.

Increased exposure can affect hepatic impairment from moderate to severe but not have been assessed clinically yet. Patient in these criteria should avoid ezetimibe use. Post marketing surveillance also suggest that patient taking ezetimibe have potential for rhabdomyolysis and myopathy. This risk further increases by receiving statin therapy recently and exacerbated if receiving concurrently(Nutescu & Shapiro, 2003; Schering Corporation, a subsidiary of Merck & Co., 2012).

1.4.6. Indications:

Ezetimibe reduces the level of LDL cholesterol, apoprotein B, total cholesterol, HDL cholesterol and non-HDL cholesterol in patients with hyperlipidemia by alone treatment or by combination with statins or fenofibrate. By using it with atorvastatin/simvastatin it can reduce total cholesterol and LDL cholesterol in patients having familial hypercholesterolemia (Schering Corporation, a subsidiary of Merck & Co., 2012).

1.4.7. Side-effects:

Muscle pain, weakness or tenderness usually when taken with statin drugs. In clinical studies, some side effects reported were diarrhea, fatigue and joint pain.

1.4.8. Dose:

General dose of ezetimibe is around 10 mg, with or without eating food.

1.4.10. Interaction (Schering Corporation, a subsidiary of Merck & Co., 2012):

a) Cyclosporine: when taken in combination, exposure of both cyclosporine and ezetimibe increases and thus monitored. Patients with renal insufficiency may have elevated levels of ezetimibe at higher degree and thus monitored more carefully.

b) Fibrates: In combination with ezetimibe, they tend to increase cholesterol excretion in bile which can lead to cholelithiasis.

c) Cholestyramine: AUC (Area under curve) of ezetimibe decreases to 55% when given with cholestyramine.

d) Coumarin anticoagulants: International Normalized Ratio (INR) is to be monitored when ezetimibe added to warfarin.

1.5. EXCIPIENT PROFILE

1.5.1. Tween 80 (Jill, 2017)

Description: It have somewhat bitter taste &characteristic odor

Nonproprietary name: Polysorbate 80

Mol.wt: 1310 g/mol

Synonym: Polysorbate 80, Capmul PEO-O, Cremephor PS80, polyoxyethylene 20 oleate.

Category: emulsifying agent, dispersing agent, nonionic surfactant, wetting agent, suspending agent.

Physicochemical properties:

a) Physical form: oily liquid

b) Color: yellow

c) HLB: 15

d) Solubility: soluble in water and ethanol, insoluble in vegetable and mineral oil

e) Melting point: -20.556

f) Boiling point: 100

g) Density: 1.06-1.09 g/ml

Stability: should be stored in well closed container, sensitive to oxidation, saponification with strong acid and strong bases and hygroscopic in nature.

Pharmaceutical application: as surfactant in many topical and oral emulsions, excipient to stabilize parenteral formulations.

Incompatibility: paraben antimicrobial activity decreases in contact with tween 80, discoloration and precipitation occurs on contact with phenols, tars, tannins.

1.5.2. Capryol 90 (Kuzminov, Koonen, Ponsard, & Nieuwenhove, 2003)

Description:

Chemical name: Propylene glycol monocaprylate

Mol.wt: 202.29

Synonym:

Category: co-surfactant, solubilizer, bioavailability enhancer, penetration enhancer

Physicochemical properties:

a) Physical form: oily liquid

b) Color: colorless

c) HLB: 5

d) Solubility: soluble in ethanol, methylene chloride, chloroform and insoluble in water

e) Specific gravity: 0.935-0.955

Pharmaceutical application: as surfactant in SEDDS and SMEDDS in oral & topical formulations.

1.5.3. Labrasol (Kuzminov et al., 2003)

Chemical name: Caprylocaproyl macrogol-8 glycerides

Category: surfactant and co-surfactant

Physicochemical properties:

a) Physical form: oily liquid

b) Color: roughly white

c) HLB: 12

d) Solubility: miscible with water

e) Boiling point: >150

f) Specific gravity: 1.060-1.070

Stability: not stable in presence of strong oxidants, strong acid and base

Pharmaceutical application: in oral and topical formulation, can be used as surfactant in self emulsifying formulations

Incompatibility: Incompatible with strong acid and base. Incomplete combustion can release monoxide, carbon or dioxide carbon.

1.5.4. Transcutol P (Kuzminov et al., 2003)

Description: Highly pure solvent, solubilizer for poorly soluble APIs.

IUPAC name: 2-(2-ethoxyethoxy) ethanol

Mol.wt: 134.2g/mol

Synonym: diethylene glycol monoethyl ether, ethyl diethylene ether, carbitol, ethyl carbitol.

Category: co-surfactant and penetration enhancer.

Physicochemical properties:

a) Physical form: Liquid, hydroscopic

b) Color: Colorless

c) HLB: 4.2

d) Solubility: Miscible in water

e) Density: 0.988 g/ml

f) Melting point: -76°C

g) Boiling point: 197-205 °C

Stability: Stable and can be stored between 40-120°F

Pharmaceutical application: as penetration enhancer in emulsions, topical ointments and in aqueous gels, as co-surfactants in microemulsion.

Incompatibility: with strong oxidants & Hydrofluoric acid

1.5.5. Capmul MCM C-8 (Kuzminov et al., 2003)

Chemical name: Monoglyceride of caprylic acid

Molecular weight: 218.29 g/mol

Category: surfactant, solubilizer, bioavailability enhancer, carrier

Physical form: liquid/semisolid

Color: colorless

Solubility: slightly soluble in water

HLB: 5.5-6.0

Storage and stability: In light resistant tight containers, keep away from flame and heat.

Pharmaceutical applications: in oral, topical, transdermal, ophthalmic preparation, used in SEDDS.

1.5.6. Labrafil M 1944 CS (Kuzminov et al., 2003)

Chemical name: oleoyl Macrogol-6 glycerides

Molecular weight: 765.15 g/mol

Category: solubilizer, water dispersible surfactant, bioavailability enhancer

Physical form: Liquid

Color: colorless

HLB: 9

Pharmaceutical applications: solubilizer for poorly soluble APIs, emulsifier and bioavailability enhancer

1.5.7. Plurol Oleique CC497 (Kuzminov et al., 2003)

Chemical name: polyglyceryl-3 Dioleate

Molecular weight: 726.93 g/mol

Category: solubilizer

Physical form: very viscous liquid

Color: yellow

Solubility: immiscible in water

HLB: 3.0

Pharmaceutical applications: co-surfactants for SEDDS and SMEDDS, solubilized for poorly soluble APIs

1.5.8. Tween 20

Chemical name: polyethylene glycol sorbitan monolaurate

Molecular weight: 1227.54 g/mol

Category: solubilizer, surfactant, emulsifier

Physical form: viscous liquid

Solubility: miscible in water

Density: 1.095g/cm3

HLB: 16.7

Pharmaceutical applications: excipient to stabilize emulsions, topic and ophthalmic. Route

1.5.9. PEG 400 (Jill, 2017)

Chemical name: Macrogol

Molecular weight: 380-420 g/mol

Category: emulsifier, co-solvent

Boiling Point:260-280

Melting Point: 4-8

Physical form: viscous liquid

Color: colorless

Solubility: miscible in water, acetone, alcohol, benzene.

HLB: 16

Density: 1.128 g/cm3

Storage and stability: chemically stable but Hygroscopic in nature.

Pharmaceutical applications: as water miscible co-solvents in self emulsifying formulations.

1.5.10. Span 20

Chemical name: sorbitan monolaurate

Molecular weight: 346.464 g/mol

Category: emulsifier, co-emulsifier and surfactant

Physical form: liquid

Color: pale yellow

Solubility: insoluble in water

HLB: 8.6

Pharmaceutical applications: in oral and topical formulations.

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2. AIM AND OBJECTIVE OF THE WORK

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2.1. AIM OF THE PRESENT WORK

SEDDS have tendency to spread in GI tract readily and motility in digestion provided by the stomach as well as intestine provide required agitation for the process of self-emulsification. Conventional emulsions are metastable and sensitive, compared to which SEDDS have good physical stability as well as easy to manufacture. Thus, for poorly soluble lipophilic drugs which have dissolution as rate limiting step for absorption, systems like SEDDS may serve as better alternative and provide an improvement in absorption rate & extent. These systems may result in more reproducible blood-time profile with less variation in bioavailability.

Ezetimibe is a first cholestrol inhibitor drug belonging to lipid lowering category. It is an azetidinone derivative which can inhibit uptake of both dietary as well as biliary cholesterol in intestine without having any impact on the absorption of nutrients which are fat soluble. Based on the solubility profile of ezetimibe it belong to BCS class II category which means it have low solubility and good permeability. The market formulation available for ezetimibe is tablet which have poor dissolution profile with very slow and limited release in the intestine. Because of the above properties, ezetimibe have very poor oral bioavailability with inter subject variability and lack of dose proportionality.

Ezetimibe is lipophilic drug with Log P value of 4.14 which means, by using lipid-based drug delivery systems, better formulation can be formulated with low inter subject variation and better bioavailability. Ezetimibe being a lipophilic drug have good solubility in diglycerides and triglycerides, and being a drug of low melting point around 163 °C, lipid-based system will be the best option for this drug. By the use of diglycerides and triglycerides absorption rate and extent may be increased as SEDDS get absorbed by the lymphatic route and avoid first pass metabolism.

2.2 OBJECTIVE OF THE PRESENT WORK

The main objective of this work is to formulate Ezetimibe SEDDS for better solubility and bioavailability enhancement of drug.

1. Rational selection of lipids, based on the solubility studies.
2. To select most suitable excipients with best ratio by using ternary phase diagram.
3. To develop formulation and evaluate their properties for optimization.
4. Optimization by using Design of Experiment concept by selecting proper experimental design.
5. To develop optimized and stable formulation of SEDDS in order to increase solubility and bioavailability.
6. To evaluate the optimized formulation.

2.3 PLAN OF WORK

1. Literature Survey
2. Selection of drug
3. Identification study for drug
   a) Appearance
   b) Melting Point
   c) FTIR
4. Analytic method for drug
5. Development of formulation
   a) Selection of oil
   b) Selection of Surfactants and Co-surfactants
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3. LITERATURE REVIEW

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1. REVIEW OF LITERATURE ON SELF EMULSIFYING DRUG DELIVERY SYSTEMS (SEDDS)

1. Mazzeti et al. prepared SEDDS of Benznidazole (BZ) as an alternative form of tablet for the treatment of Chagas disease in paediatric patient with same safety and efficacy. Chagas disease affects overall to 6-7 million patients and important health concern. While, BZ is one of the first line treatment in case of Chagas and only dosage form available is tablet which is needed to be crushed or divided which can result in non-suitable release of drug in GIT and have risk of toxicity. Compared to which, dose adjustment in liquid form will be easy and better alternative for BZ administration. The aim of the work was to prepare BZ SEDDS, as SEDDS can increase bioavailability, and decrease variation in absorption by using various lipids, surfactants and co-surfactants. Apart from this, they can also be filled in capsule to adults by which drug administration is easy for all population. This study was further focused on activity as well as toxicity evaluation of BZ SEDDS. The formula of optimized formulation is Capryol 90®, Miglycol 810 N®, Labrasol® and Lipoid® S75 and NMP. This optimized batch with dose of 25mg/mL of BZ induced no cytotoxicity in $Caco_2$ cells, HepG2 cells and H9c2 cells at 25 micromolar level. The developed formulation was stable and had high drug load with affordable preparation method with same efficacy in murine model with better safety than tablets. So, the study by Mazzeti concluded that SEDDS can provide bioequivalent formulation as tablet with better dose adjustment options and decreasing risk of toxicity with less absorption variation (Mazzeti et al., 2020)**.**

2. Balakrishnan et al. and his co-workers studied and prepared solid SEDDS for the most common drug dexibuprofen for enhancement of its bioavailability. The preparation of solid SEDDS was done by using spray drying technique using Aerosil 200 as solid carrier. The components of SEDDS selected were Labrasol as surfactant, Labrafil M 1944 CS as oil and Capryol 90 as co-surfactant and liquid SEDDS were prepared using 15% Labrafil M 1944 CS, 80% Labrasol and 5% Capryol 90 with drug loading of 20%w/v. Characterisation of Solid SEDDS was done using Differential Scanning Colorimeter (DSC), Scanning Electron Microscope (SEM) and X-Ray Diffraction (XRD). By using XRD and DSC, it was observed that Solid SEDDS remain in the dispersed state. In-vitro dissolution also shown good result suggesting faster rate of release than powder. In vivo studies in rats have shown that, AUC and Cmax of dexibuprofen Solid SEDDS have shown significant

increase. The factors may be responsible for better performance of formulation are the following: a) More surface area of fine droplets compared to conventional emulsion droplets. b) Better diffusion because of small droplets. c) use of surfactants result in increase in mucosal permeability Thus, in vivo studies showed improved bioavailability of dexibuprofen indicating SEDDS as better formulation. Thus, the study concluded that Solid SEDDS are better alternative dosage form for Poorly water soluble drugs (Balakrishnan et al., 2009).

3. Nipun et al. and Islam et al. have prepared and characterized Gliclazide SEDDS which belongs to 2nd generation of hypoglycaemic sulfonylurea. The evaluation was also done using various techniques including ex-vivo , in-vivo techniques and in-vitro techniques. Gliclazide is relatively insoluble in water with Pka of 5.8. The absorption rate of Gliclazide was slow with inter subject variation in bioavailability due to slow dissolution and permeability in GI membrane. Various oils, surfactants were screened for the selection on the basis of their solubilising capacity. Capryol 90 was selected as oil, while tween 80 and Transcutol HP was selected as surfactant and co-surfactant respectively. The 1:1 ratio of surfactant: co-surfactant was selected as final batch with droplet size of 50.959 micrometre. The final batch was further evaluated for drug release and shown 99% release of drug in 20 minutes and shown diffusion of 97.6% in 5 hour in chicken intestinal sac while in-vivo studies was done in albino mice has also shown decrease in plasma glucose level significantly when given by oral route. All above result in nutshell determined that SEDDS provide better result and is good alternative for oral administration of Gliclazide (Nipun & Ashraful Islam, 2014).

4. Zaichik et al. have developed SEDDS for increasing an absorption of vancomycin, an antibiotic drug which is used in infectious disease by oral administration for better mucosal permeation in intestine. Vancomycin is used in treating many bacterial infections including infection caused by methicillin resistant *S. aureus*. Vancomycin have very low bioavailability of around 5% and belong to hydrophilic molecule so hydrophobic ion - pairing method was used for lipidization in SEDDS. SEDDS containing vancomycin/CTAB complex was prepared by using Capryol 90/Captex as oil, Cremephor EL or/and Cremephor RH 40 as surfactant and Transcutol or/and DMSO as cosolvents. The lipophilicity and drug loading of vancomycin was increased by using hydrophobic ion-pairing method. The system was further evaluated for drug release, permeability in mucosa

and antimicrobial in-vitro activity. Permeability was checked via diffusion study using porcine intestinal mucus which showed promising and significant result with 4-8 fold increase in permeation as compared to free vancomycin solution. Moreover, high in-vitro anti-microbial activity was observed by vancomycin SEDDS against *S.aureus* compared to free vancomycin. Thus, their study concluded that SEDDS is promising tool for antibiotic oral delivery (Zaichik, Steinbring, Caliskan, & Bernkop-Schnürch, 2019).

5. Hong et al. have prepared self-emulsifying formulation for itraconazole to improve its dissolution profile and increase its absorption. By screening of various oils, surfactants tocopherol acetate, Pluronic L64 and Transcutol was selected for the formulation on the basis of their solubilizing capacity. By adding hydrochloric acid, itraconazole solubility was improved significantly. Size of droplets in emulsion was same in both the medium including simulated gastric and intestinal fluid having pH 1.2 and 6.8 respectively throughout the period of incubation. There was rapid and similar dissolution profile of itraconazole in every medium from SEDDS while the dissolution profile of Sporanox vary in different media depending on the pH and surfactant concentration during incubation period. The reason behind the similar profile in case of SEDDS was due to rapidly formed particles with 100-1000 nm size which solved dissolution profile and improved the absorption of drug. When both formulation were given in normal fed and fasted group, AUC and Cmax of SEDDS formulation was comparable with Sporonax. While, given in Lipidic group, AUC and Cmax of SEDDS was able to increase its bioavailability by 3.7 fold and 2.8 fold respectively. The result suggest that increase in bioavailability by SEDDS was because of tocopherol acetate, Pluronic L64 and Transcutol and not particularly by food intake and this system can provide dosage form which is useful for poorly soluble drug without having any food effect. (J. Y. Hong, Kim, Song, Park, & Kim, 2006).

6. Zaichik and his co-workers have developed SEDDS for the antibiotic named ciprofloxacin. The aim was to develop a system which can have better permeation and good anti-microbial activity for treatment of infectious disease. Ciprofloxacin is effective antibiotic for both gram positive and negative bacteria, this drug is helpful in respiratory infections including cystic fibrosis. But, permeation of drug via mucus is major obstacle. SEDDS can overcome this problem by increasing permeability due to droplet charge, size and surface area and thus SEDDS were developed for better efficacy of ciprofloxacin. SEDDS were prepared using HIP method for lipidization using oleic acid as lipid. The

study suggested that system was able to improve permeation of ciprofloxacin with insignificant effect on anti-microbial activity against E.coli, S.aureus and P.aeruginosa and can be better alternative for pulmonary infections specially in patients of cystic fibrosis and pneumonia (Zaichik et al., 2018).

7. Low solubility is big challenge faced by industries for many new candidates for preparation of effective formulation. Most common problem with low solubility drug is their low bioavailability with high inter/intra subject variation when administered orally. Phenytoin is prescribed in epilepsy and cardiac arrhythmia but only problem is its erratic absorption due to poor solubility. Many strategies including use of lipids, permeation enhancer are used to solve this problem and to prepare the right formulation. Atef used lipids and developed SEDDS for phenytoin drug to improve its oral bioavailability compared to marketed suspension. The optimised batch of SEDDS was prepared using Labrasol®, Labrafac CC®, Transcutol® and BHT. It was further evaluated and compared with marketed formulation known as Dilantin® suspension. The significant increase in absorption rate was observed in SEDDS, AUC $_{(-10\ min \rightarrow 10\ h)}$ was found to be increased by 2.3 times than Dilantin® while C_{30} was also reported 4.9 times higher in comparison. Apart from this, optimized batch was also found stable with significant improvement in bioavailability which further should be evaluated in human clinical trials to check variability issue (Atef & Belmonte, 2008).

8. Zupančič and his co-worker developed and evaluated enoxaparin SEDDS for oral adminstration. Enoxaparin is low Mol.wt Heparin and is most potent anticoagulant adminstered intravenously or sub-cutaneously as they are unstable in acidic environment of stomach.So, SEDDS were prepared as alternative for better patient compliance and reducing expenses. They were prepared using LC lipids, MC lipids and No-lipids (NL) which were further evaluated by measuring the droplet size. The avg size of droplet was between 30-40 nm was chosen for further studies. Diffusion study was done which suggested that MC-SEDDS and NL SEDDS exhibits higher mucus difussion by two fold and were further chosen for studies. 2% w/v payload of enoxaparin was done in MC-SEDDS and NL-SEDDS and were further studied. 97% of MC-SEDDS were degraded by pancreatic lipase while only 5% NL-SEDDS was degraded in 90 minutes. The sustained release was observed from both formulations in in-vitro studies. In-vivo studies were done and he bioavailability exhibited by MC-SEDDS was 2.02 % and NL-SEDDS was 2.25%.

Two fold increase in bioavailability was observed by delivering enoxaparin by SEDDS. Thus, SEDDS should be considered as potential drug delivery system for these type of drugs.(Zupančič, Grieβinger, et al., 2016)

9. Oderberg et al. have tried to study about SEDDS by preparing cyclosporine SEDDS using natural components by rational approach to understand their characteristic for oral drug delivery of drug. Galactolipids were used as surfactant component for the formulation. The optimised formulation was prepared using oat oil (fractionated) consist of 50% polar lipid and 50% neutral lipid, MC monoglycerides in 1:1 ratio and was bioequivalent to Neoral® formulation. Various factors were found affecting the cyclosporine absorption including ratio b/w lipid excipients, different lipid excipients combination and drug incorporation. Using experimental design and Multivariate analysis, number of experiments were reduced. The number of experiments were reduced to 17 formulation which was used in 3 clinical trial. Pharmacokinetic parameters and blood concentration was measured and showed equivalency in absorption of cyclosporine from SEDDS when compared with marketed Neoral® formulation (Odeberg, Kaufmann, Kroon, & Höglund, 2003).

10. Wei Y. explored Supersaturable-SEDDS for improving the oral bioavailability of drug silybin. S-SEDDS was prepared using Labrasol, Labrafac CC and Cremephor RH40 with HPMC as precipitation inhibitor. Ternary phase was used to identify the self-emulsification area by using above components. Droplet size of emulsion was characterised and it demonstrated that droplet size by S-SEDDS was smaller as compared to normal SEDDS when diluted with 0.1 N HCL. It was due to presence of HPMC which work as precipitation inhibitor. Precipitation of silybin was slow in S-SEDDS due to presence of HPMC while fast in normal SEDDS during dilution. The result demonstrated that HPMC presence can effectively sustain the emulsion in supersaturated state by hindering the kinetics of precipitation. For verification of precipitation crystallinity, both formulation were studied using X-ray scattering. The in-vivo study was also carried out, indicating there was nearly 3 fold increase in S-SEDDS as compared to SEDDS at 533 mg/kg drug dose. Pharmacokinetic studies suggested that S-SEDDS exhibited higher oral BA than SEDDS. Thus, it was demonstrated that S-SEDDS is an effective approach and can improve oral BA for poorly water soluble drug(Y. Wei et al., 2012).

11. Zupančič et al. have developed SEDDS for daptomycin which is a peptide drug. The preparation was done by HIP method using dodecylamine HCl as cationic surfactant in the 5:1ratio of surfactant to peptide. Various formulations were prepared and evaluated for the characterisation of emulsion including zeta potential, size of droplets and polydispersity index. The maximum paylod of 8% was achieved with 5.5 % pure daptomycin drug when the complex of daptomycin dodecylamine was dissolved in formulation. This formulation was consist of 30% capmul MCM, 30% Dermofeel MCT and around 35% cremephor RH 40. Log P was also raised from -0.5 to +4.8 by the use of complex formed by dodecylamine HCl with daptomycin. Lipase degrades the formulation in time span of 90 minutes. The daptomycin drug release study was also done in 50mM buffer of phosphate having pH 6.8. The release study suggested that daptomycin released is done in sustained manner for six hours. SEDDS exhibited protective effect against degradation of drug by alpha-chymotrypsin and mucus permeation. Drug payload is major limitation faced till date in case of peptide drug, and SEDDS were able to overcome that by 5 fold increase in drug load compared to previous studies. Thus , study suggest SEDDS as potential delivery system for delivery of daptomycin. (Zupančič, Partenhauser, Lam, Rohrer, & Bernkop-Schnürch, 2016)

12. Carvedilol is used in lowering the blood pressure of patient in hypertension by blocking beta adrenoreceptor activity and alpha receptor activity with oral bioavailability of 20%. It is reported that drug has been beneficial to the patient of angina or cardiac failure. But, carvedilol have low solubility in GI fluids with extensive metabolism in liver which in result decreases the oral bioavailability in humans. So, Wei and his co-workers developed an system i.e. SEDDS and SMEDDS to increase it oral bioavailability by increasing its solubility and dissolution rate. The preparation was done by using phase diagrams to identify the domain of microemulsion area and self-emulsification. Characterisation was done including PSD, zeta potential, in-vitro dissolution. The optimised composition included Tween 80, Labrafil M 1944 CS and Transcutol P. To get positively charged system, benzoic acid was used. It was beneficial in forming positive charged SEDDS as well as in improving self-emulsifying performance in 0.1 N HCl. There was improvement in release rate by using higher amount of tween 80. The dissolution rate was increased by 2 fold and oral bioavailability was increased by 413% when compared with tablets (L. Wei, Sun, Nie, & Pan, 2005).

2. REVIEW OF LITERATURE ON EZETIMIBE

1. Ezetimibe is the first member in this class which inhibits the absorption of cholestrol in intestine. It was approved in October of 2002 by FDA under brand name of Zetia for the patients having hypercholesteremia. Many studies including epidemiological and clinical trials were done which established the role of ezetimibe in reducing level of total cholestrol (TC) and LDL-cholestrol which are highly beneficial in coronary heart disease which is the leading cause of morbidity and death in the world. The series of study was done in the compounds based on azetidinone with extensive structural activity studies which led to discovery of ezetimibe. Ezetimibe showed good efficacy as cholestrol inhibitor in hamster model and other animal models and then chosen for further clinical development. Clinical studies were done and patient having hypercholesteremia were give ezetimibe orally at dose of 10 mg daily once in a day. It reduced levels of total cholestrol (TC) and LDL-cholestrol and other TGs responsible for promoting atherosclerosis. It was also observed that it can increase HDL-cholestrol which was again beneficial to reducing the development of atherosclerosis. Maximum response was reached in two weeks and further chronic therapy was maintained. Ezetimibe was given as monotherapy for 2 twelve week studies in 1719 patient and reduced the LDL-cholestrol by 18% compared to placebo which increased by 1%. When ezetimibe was given in 769 patient for eight weeks study with statin as combination therapy, which were not reached to their NCEP II LDL-Cholestrol goal it reduced LDL-cholestrol by 25% compared to placebo which reduced by 4%. Finally 4 twelve week trial was done in 2382 untreated patient and ezetimibe was given in combination with one of 4 statins using different doses of statins reduced LDL-cholestrol level more than statin alone indicating benefit of ezetimibe (Earl, Kirkpatrick, & Peter, 2003).

2. Toth et al. have studied about ezetimibe and its mechanism with clinical updates. Ezetimibe has been proven to reduce levels of total cholestrol (TC) and LDL-cholestrol by acting on the NPC1L1 protein , when used in patient as monotherapy or in combination with the statins. Altmann have reported in 2004 for the NPC1L1 (Niemann-Pick C1-Like 1 protein) discovery as transport protein for human sterol. He said that it is expressed at the hepatobiliary interface and enterocyte/apical gut lumen. This protein is having sterol domain in its structure. Evidences have been studied suggesting the NPC1L1 protein work in conjunction with clathrin and AP2 complex to facilitate free cholestrol internalisation.

Cholestrol present in bile or gut lumen gets incorporated in membrane so it can easily bind to NPC1L1 sterol domain. The complex is formed as NPC1L1/cholestrol which undergoes internalization or endocytosis and by creating vesicle complex with by AP2 clathrin. This complex can translocate easily by the help of myosin with microfilaments in cytosol to storage endosome. When intracellular cholestrol reduces, NPC1L1 gets released to regulate cholestrol level. Ezetimibe selectively block this transport protein and found to decrease circulatory and biliary cholestrol. It is suggested that ezetimibe prevent the complex of NPC1L1/cholestrol to interact with AP2 or by changing the conformation of protein inhibiting binding of cholestrol to domain. Ezetimibe have been reported to inhibits cholestrol absorption in intestine and as effective therapy with marked inter/intrasubject variability in patients with hypercholesteremia, insulin resistance and sitosterolemia. Growing data suggests ezetimibe as effective therapy against the controversies about its clinical effectiveness raised from reports of ENCHANCE and ARBITER 6 which had negative outcome in studies related to ezetimibe. It is also observed that ezetimibe has positive effect on reducing the progression of atherosclerosis, ultimately reducing cardiovascular events in subject which are at high risk of CHD including patients with CKD. Thus, the author have given clinical update of ezetimibe mechanism with its clinical safety as well as efficacy (Phan, Dayspring, & Toth, 2012).

3. In this study, shevalkar and his colleague have chosen NLC (Nanostructured Lipid carrier) as formulation system for enhancing the bioavailability of ezetimibe given by oral route. NLC is Lipidic drug delivery system and comes under 2nd generation of lipid nanoparticles prepared by using solid as well as liquid lipid. This system is more benifial for drugs which have maximum solubility in oil when compared to solid lipid. The reason behind this is on addition of oil, structure of NLC becomes amorphous which provide more place for drug accommodation and can load more drug. Apart from this, NLCs have many benefits including controlled release of drug, biocompatibility, protecting drug from degradation and scalability. NLCs does have stability issue which was further solved by preparing solidified NLCs using adsorption method. NLCs was prepared by microemulsion technique and ezetimibe nano-emulsion was prepared separately for comparision. The final optimised batch of NLCs were then converted to the free-flowing stable solidified NLC by using Neusilin® as inert carrier for adsorption. The prepared S-NLCs were then evaluated for dissolution profile, pharmacokinetic, pharmacodynamic studies and characterisation

was done using DSC, X-RD, and SEM. In SEM studies, no precipitation of drug was observed on surface of carrier in S-NLC and crystallinity was less in S-NLC compared to nanoemulsion when checked by DSC and X-RD. There was increase in release of drug from S-NLC compared to marketed tablet and pure drug. In-vivo data suggested reduced level of total cholestrol in rats from S-NLC compared to drug suspension and positive control group. There was no change in HDL cholestrol level. Thus, pharmacokinetic study suggested increase in oral bioavailability of ezetimibe by NLC formulation compared to marketed tablet and pure drug (Shevalkar & Vavia, 2019).

4. Kim et al. and his co-workers worked on improving the dissolution profile of poorly soluble drug ezetimibe by producing a solid dispersion tablets for ezetimibe using spray drying which was optimised to get physicochemical properties improved compared to conventional tablet form. Spray drying was used as it is very beneficial for heat sensitive because of short time solvent evaporation. Apart from this, spray drying can decrease size of particles and can also change a drug from crystalline to amorphous state. These changes can contribute to improving solubility, dissolution rate and may be bioavailability of drug. The spray drying process was optimised by controlling temperature of inlet, rate of feed , solid content which helped in optimising moisture content, yield, solubility using box behnken DOE design. Tablets which were made by using optimised solid dispersion of ezetimibe exhibited better dissolution profile compared to free drug or tablet made by just physical mixture. Therefore, optimisation on process of spraying drying was successful for producing ezetimibe solid dispersion with potentially improved dissolution profile as well as bioavailability (Kim et al., 2016).

3. TABLE 3.1: EXAMPLE OF PATENTS ON SEDDS

SR. NO	INVENTORS	TOPIC	DESCRIPTION	PATENT NO. & REF:
1.	Holmberg Christina and Siekmann Britta	Self-Emulsifying drug delivery system wherein fatty agent is optional	The patent claims for oral pharmaceutical composition as preconcentrate. The formulation is useful for pain treatment as well as in inflammation. Formulation consisting of surfactants (one or more),NO releasing NSAIDS (one or more) and oil/semi-solid fat (optionally)	EP1267832 B1 & (Holmerg & Siekmann, 2004)
2.	Bansal Arvind Kumar , Munjal Bhusan, Patel Sarsvat Babulal	Self-nanoemulsifying curcuminoids composition with enhanced bioavailability.	This invention is about novel curcuminoid composition consist of curcumin or curcuminoids, lipid carrier, surfactant, cosolvents, pH buffer, optionally polymeric aggregation inhibitor	WO2010/010431 Al (51) & (Bansal, Munjal, & Patel, 2010)
3.	Hong chung II, Shin Hee, Jong KI Min Hyo, Lee Seok Kyu, Kweon Don Sun	Self-emulsifying matrix type transdermal preparation	This invention is related to novel composition of self-emulsifying matrix type preparation for transdermal and transmucosal preparation in which SEDDS is grafted to matrix. This is prepared to maintain drug release at constant rate with improved	WO 01/72282 A1 & (chung I. Hong, Shin, Hee, KI, Min, Lee, Seok, & Kweon, Don, 2001)

			absorption of drug & minimum irritation	
4.	Premchand Nakhat, Mandaogade Prashant, Jain Girish Kumar, Talwar Munish	Self-emulsifying pharmaceutical composition of Rhein / Diacerein	This invention is related to the SEDDS based Rhein or diacerein composition and preparation, bioequivalent to marketed Art 50® formulation with reduced side effects & no variability during fast or fed state.	US,999,381. B2 & (Premchand, Mandaogade, Jain, & Talwar, 2015)
5.	Kenichi Sakai, Chugai Seiyaku Kabushiki Kaisya	Method for the design of Self-emulsifying drug formulations	This invention provide high throughput formulation screening system method for preparing self-emulsifying formulation which allow screening at high speed and gives optimum formulation	EP 1 879 013 A1 & (KenichI & Kaisya, 2008)
6.	Morozowich Walter, Shenoy Narmada	Self-emulsifying drug delivery system for extremely water-insoluble lipophilic drugs	The invention field of this patent is formulation for compounds which are water insoluble particularly SEDDS.	WO 02/07712 A2 & (Morozowich & Shenoy, 2002)

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4. EXPERIMENTAL SECTION

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4.1. MATERIALS AND REAGENTS

TABLE 4.1 : LIST OF MATERIALS USED

SR.NO	MATERIALS	VENDOR'S NAME
1.	Ezetimibe	Emcure Pharma
2.	Capryol 90 (Propylene glycol monocaprylate)	Gifted by Gattefosse, India.
3.	Labrasol (caprylocaproyl macrogol-8 glycerides)	Gifted by Gattefosse, India.
4.	Labrafil M 1944CS (oleoyl macrogol-8 glycerides)	Gifted by Gattefosse, India.
5.	Capmul MCM C-8	Abitec Corporation
6.	Tween 20 (polyoxyethylene sorbitan monolaurate)	S.D. Fine-Chem Ltd. India
7.	Tween 80 (polyoxyethylene sorbitan 20 monooleate)	S.D. Fine-Chem Ltd. India
8.	PEG 400	Central drug house Pvt. LTd, Delhi
9.	Span 20 (sorbitan monolaurate).	S.D. Fine-Chem Ltd. India
10.	Plurol Oleique CC497	Gifted by Gattefosse, India.
11.	Transcutol P	Gifted by Gattefosse, India.
12.	Methanol	S.D. Fine-Chem Ltd. India
13.	Concentrated HCl	

4.2. EQUIPMENTS USED

TABLE 4.2: LIST OF EQUIPMENTS USED

SR.NO	INSTRUMENTS	NAME
1.	Digital Balance	Citiweigh- Tejas exports, India
2.	Electronic digital weighing balance	Shimadzu corporation Ltd. Japan
3.	Magnetic stirrer	Remi motors Ltd. India
4.	Head Stirrer (propeller stirrer)	Remi motors Ltd. India
5.	Vortex mixer	Remi motors Ltd. India
6.	pH meter	Analab scientific instruments, India.
7.	(Differential Scanning Colorimetry) DSC	Hitachi DSC 7020
8.	Fourier Transform Infrared Spectrometer (FTIR)	Jasco FT/IR-6100
9.	UV Spectrophotometer	Jasco V-570
10.	Malverm Zetasizer	Nano ZS90, Malvern Instruments Ltd, UK

4.3 IDENTIFICATION OF EZETIMIBE

4.3.1. Melting point:

Figure 4.1 Differential Scanning Colorimetry

The melting point of ezetimibe was done by (differential scanning colorimetry) DSC. In this, sample was weighed accurately in crucible. The crucible was closed by pressing and kept on die. After that, crucible lid was applied and lever was rotated till plunger was down, and thus lid was cold welded on crucible. Then, sample was scanned and melting point was detected by peak identification.

4.3.2. FTIR

Figure 4.2 Fourier Transform Infrared Spectrometer

The structure analysis was done by Fourier transform infrared spectrometer. First of all, KBr and ezetimibe was kept under IR lamp to make sure that both are anhydrous to avoid moisture peak in FTIR. Sample cell, mortar pestle was cleaned by methanol and dried. KBr was used as reference or blank and scanned in FTIR. After that, drug sample and 100 times KBr was mixed in mortar pestle and then filled in sample cell. This sample cell was kept and scanned for peaks and interpreted. This interpretation was done using reference peaks of ezetimibe and available reference peaks of each functional group.

4.4. ANALYTICAL METHOD FOR EZETIMIBE

4.3.1. Absorption maxima determination:

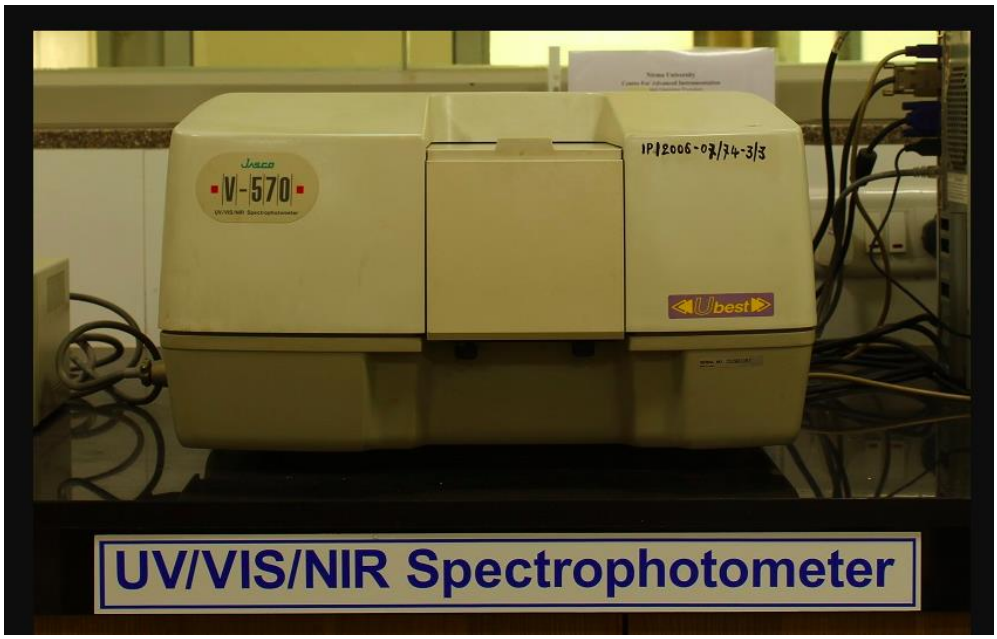

Figure 4.3 UV Spectrophotometer

Absorption maxima of ezetimibe was determined by using UV spectrometry method. Firstly, 100 mg of ezetimibe was accurately weighed, then it was dissolved in volumetric flask by diluting upto 100ml with methanol resulting into 1000 µg/ml solution. Further, it was diluted by taking 1 ml into 100 ml to make stock solution of 100 µg/ml. The prepared ezetimibe solution of 100 µg/ml was scanned from 200 to 400 nm using spectra manger as software in UV JASCO spectrophotometer. The absorption maxima was then checked for the same.

4.3.2. Ezetimibe stock solution:

Stock solution of ezetimibe was prepared in methanol as solvent.100 mg of ezetimibe was accurately weighed and dissolved in 100 ml of methanol to get 1000 µg/ml solution. 1 ml of 1000 µg/ml solution was diluted 10 times to get a stock solution of 100 µg/ml.

4.3.3. Calibration curve:

A series of dilution ranging from 5 µg/ml to 25 µg/ml were prepared from stock solution using methanol as solvent. Absorbance of diluted solution were measured in UV spectrophotometer against the blank sample. Four repetitions were made from making fresh stock solution to diluted aliquots and average was taken for construction of calibration curve. Calibration curve was constructed by taking absorbance at y-axis and concentration at x-axis.

4.5. DEVELOPMENT OF SEDDS

4.5.1. Selection of components for SEDDS:

Selection of all components used in SEDDS such as oil, surfactants and cosurfactants were based on the suitability of each component for oral formulation and its toxicity consideration in the formulation. With this, liquid state, compatibility, HLB value were considered for stable formulation.

4.5.2. Saturation solubility study:

Saturation solubility of ezetimibe was determined in oils, surfactants and co-surfactants were visually determined and was quantified. Excess amount of ezetimibe was added to Eppendorf tube containing 2 ml of all liquid excipients. After sealing, sample was mixed in vortex mixer for 5 minutes. It was kept for 72 hours to equilibrate at room temperature after which each Eppendorf tube was centrifuged for 15 minutes with rpm of 3000. The 0.1 ml of supernatant was taken by micropipette and analyzed by UV spectrophotometer for drug determination in methanol in range obtained by linearity by dilution with methanol.

4.5.3. Miscibility between selected Surfactants and Co-Surfactants by Visual Observation:

Co-surfactants were screened on the basis of their miscibility with surfactants selected. By visual observation, interphase between them was checked and the one with weaker interphase was selected because weaker interphase indicates higher miscibility between two liquids which was necessary to make bigger emulsion domain

4.5.4. Selection of Co-surfactant:

It was observed from literature review that use of co-surfactant with surfactant generally gives large domain area of emulsion in ternary diagrams. Therefore, use of co-surfactant is done in SEDDS.

It was also observed particle size tends to decrease by the use of co-surfactant because Co-surfactants decreases interfacial tension by increasing elasticity of film formed by surfactant between external phase and dispersed droplets.

From solubility studies, Capryol 90 was selected as oil component and Tween 80 was selected as surfactant. Combination of Capryol 90 and Tween 80 with two co-surfactants as suggested by preliminary studies were screened for the selection of component in SEDDS.

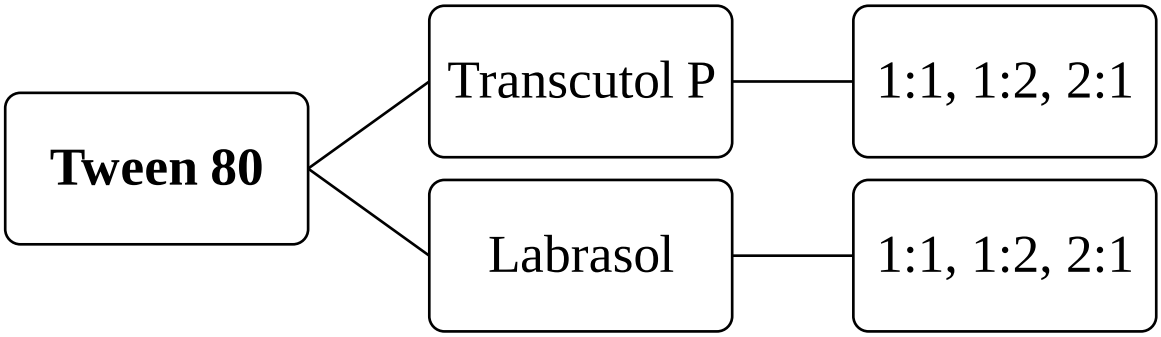

Figure 4.4.: Selection of Co-surfactants

Tween 80 was blended with both the surfactants named Labrasol and Transcutol P in a ratio of 1:2, 1:1 and 2:1. These blends of all three ratio was then mixed with Capryol 90. The ratio of S_{mix}: oil was then varied from 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2 and 9:1. The water was added in above mixtures by gradual increase of 5% at a time and was checked for the turbidity or clear emulsion. The resulting mixture was kept for 24 hours and stored at room temperature. The clarity of mixture was checked and if clear, further addition of water was checked till turbidity appears. The Co-surfactant which has clear solution for larger amount of water percentage was selected further for studies.

4.5.4. Construction of pseudo ternary phase diagrams:

On the basis of saturation solubility studies and selection of surfactant, Capryol 90 was taken as oily phase for development of SEDDS. Tween 80 was taken as surfactant and Transcutol P as Co-surfactant. The water titration method was employed to identify the proper oil-surfactant- cosurfactant ratio in the phase diagram. Here, surfactant to

cosurfactant was taken in three combination as 1:1, 1:2, 2:1. $S_{mix.}$ To oil was taken from 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7. 2:8, 1:9. Gradual addition of 5% water was done to this blend and till its turbidity was seen. Till the point which have clear mixture, all three components such as oil, S_{mix} and water were plotted on ternary phase diagram using online software. By help of ternary phase diagram, maximum emulsion area or isotropic region were identified.

4.5.5. Drug loading calculation:

Mostly, SEDDS formulation are given in capsule from 0.2 ml to 0.3 ml so drug loading calculation was necessary. In this, 10 mg dose was selected for each 0.2 ml i.e. 10 mg / 0.2 ml of formulation. S0, for each 0.5 ml preconcentrate 25 mg of ezetimibe was used.

4.5.6. Formulation of SEDDS:

From ternary phase diagram, it was determined that 1:1 and 1:2 ratio of surfactant: cosurfactant was not having significant difference in result and thus 1:1 ratio was selected. While for S_{mix} to oil emulsification area for all nine ratios i.e. 9:1 to 1:9, were determined by pseudo ternary phase diagram. For, preliminary formulation Capryol 90, tween 80 and Transcutol p was taken and drug was added according to calculated dose. Mixture was homogenized for 24 hours and was evaluated.

4.5.7. Optimization using Mixture Design

Mixture Design for three Components:

Mixture design comes under design of experiments used for the optimization of formulation with less number of experiments within less time and with best formulation. Mixture design is basically a statistical approach which is used to know the relationship between response and factors affecting that to get best result possible. In ternary diagram, the isotropic region was identified from the total plotted area and mixture design is applied on that region.

Simplex centroid design was used in which, seven runs are done using three corners (1,2,3), three halfway points from each corner (4,5,6) and one at center point (7) of the triangle. This way seven runs are done for selected ratio of surfactant to co-surfactant.

Each corner point is representing for one maximum component and other two minimum components. Halfway between two corner points represents average of minimum and maximum for two components while minimum of one component. While center point represents the one third part of all three components.

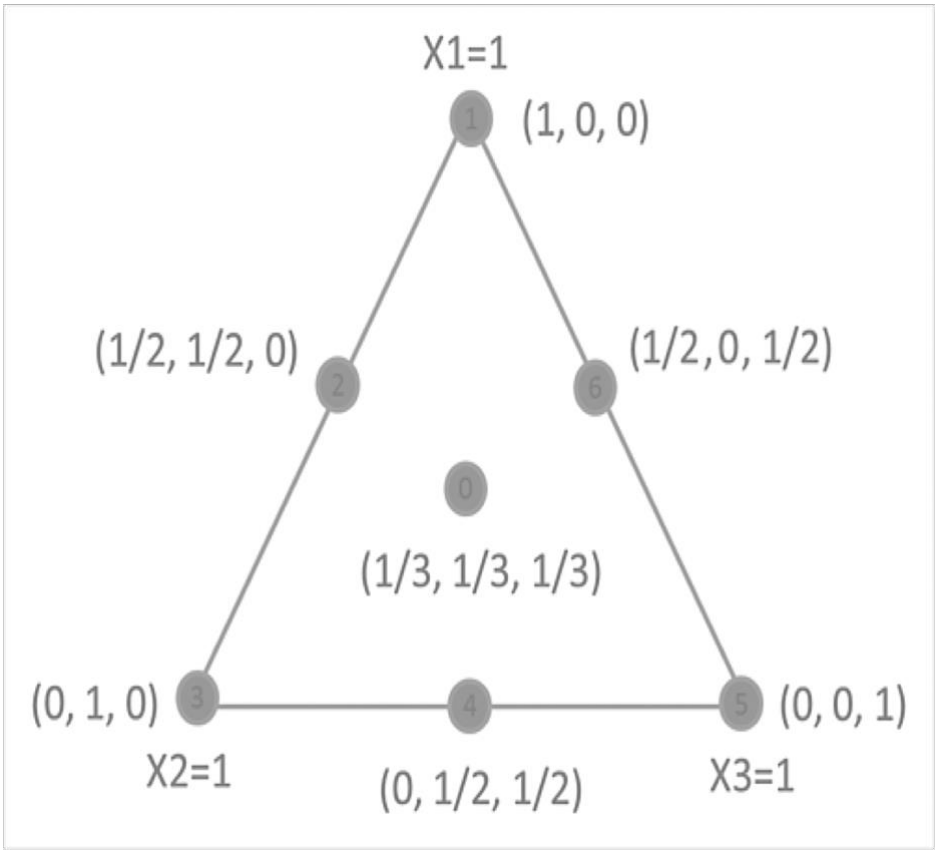

Figure 4.5: Mixture Design

The response such as particle size, self-emulsification time and PDI was taken as values and data treatment was done for the same in the Microsoft excel. The co-efficient of the variables were obtained from the data treatment done in Microsoft excel using regression model. These values were kept at the place of co-efficient in the equation so that it can be used to predict response. The coefficients obtained from Ms excel was compared with design expert software to validate it. Then with the help of Design expert analysis was done and contour plot was generated for interpretation

TABLE 4.3: SEVEN RUNS OF MIXTURE DESIGN

Batches	X1	X2	X3
A1	1	0	0
A2	0	1	0
A3	0	0	1
A4	0.5	0.5	0
A5	0.5	0	0.5

A6	0	0.5	0.5
A7	0.33	0.33	0.33

4.5.8. Equation for simplex mixture Design:

$$Y=b_1X1+b_2X2+b_3X3+b_4X1X2+b_5X1X3+b_6X2X3+b_{123}X1X2X3$$

Here, Y act as response, b as coefficients obtained from model and X1 as oil, X2 as surfactant and X3 as co-surfactant transformed values.

4.6 CHARACTERIZATION OF SEDDS PRECONCENTRATE:

For characterization, 0.1 ml of preconcentrate was diluted with 0.1 N HCl to produce nanoemulsion. Self-nanoemulsification time, dispersibility, cloud point determination, globule size, zeta potential, drug content and in-vitro release were performed.

4.6.1. Self nanoemulsification time and % transmittance:

The 0.1 preconcentrate was diluted by 0.1 N HCl at room temperature and emulsification time was observed by visual observation. The percentage transmittance was measured at 233 nm in UV spectrophotometer.

4.6.2. Cloud Point determination:

The resultant emulsion was subjected to see effect of temperature on emulsion stability by cloud point determination. Here, diluted formulation was initially maintained at 25 °C in water bath which was gradually increased by 5°C every minute and the first point at which turbidity appeared virtually was noted as cloud point.

4.6.3. Dispersibility studies:

Dispersibility study was done by using 1 ml of preconcentrate in 500 ml distilled water and in 500 ml 0.1 N HCl at 37 ± 0.5 °C using USP dissolution apparatus 2 with speed of paddle rotation at 50 rpm which provide gentle type agitation. Further, grading was done of each formulation using the following table.

TABLE 4.4: OBSERVATION TABLE OF DISPERSIBILITY STUDY

SR.NO	OBSERVATION	GRADE
1.	Rapidly forming emulsion within one minute and have clear/ bluish appearance	A

2.	Rapidly forming emulsion but comparatively less clear and have bluish white appearance	B
3.	Milky fine emulsion that forms within 2 minutes	C
4.	Dull and gray white emulsion, have slightly oily appearance and take longer than 2 minutes to form emulsion.	D
5.	Large globules on the surface with poor emulsification	E

4.6.4. Globule size determination:

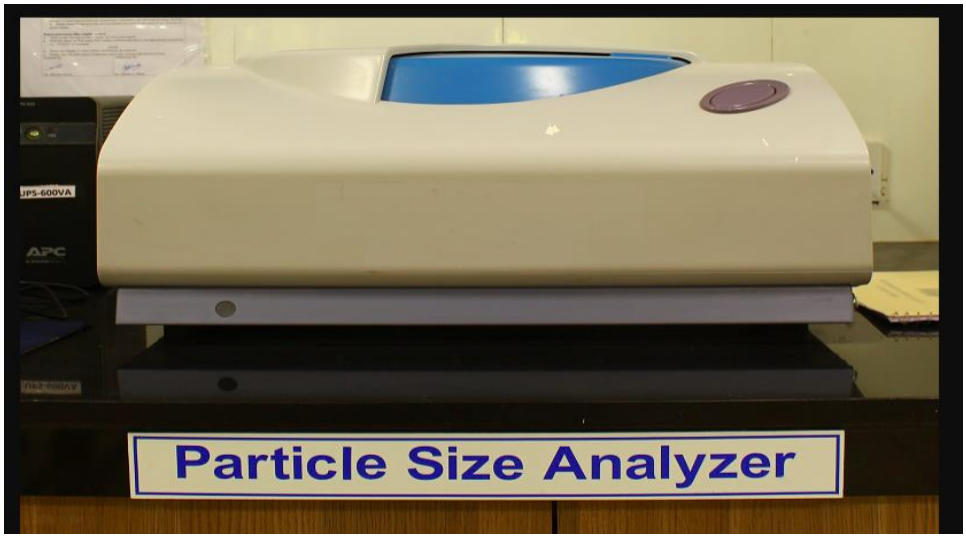

Figure 4.6. Particle Size Analyzer

The globule size of formulation was determined using a principle of Dynamic Light Scattering (DLS) technique. DLS analyses the globule size by fluctuations in intensity due to Brownian movement of formulation particles employing He-Ne red laser at an 90 ° angle at fixed temperature of 25 °C using disposable polystyrene cuvettes. For each sample, globule size with mean, Z-average, PDI was recorded.

4.6.5. Zeta Potential:

The same sample prepared for globule size determination was taken and used for zeta potential. Here, sample was filled by the use of syringe and surface charged was determined by the use of Zetasizer.

4.6.6. Drug Content:

The drug content was determined by taking 100 µl sample and diluting it by 100 times using methanol in volumetric flask. The solution was filtered and analyzed for drug quantity of ezetimibe using UV spectrophotometer at 233 nm.

4.6.7. *In-vitro* drug release:

The preconcentrate was filled into capsule with micropipette to conduct release in USP apparatus II taking 0.1 N HCl as dissolution media for the formulation. Speed of paddle apparatus was kept constant at 50 rpm. The samples were withdrawn at 5, 10, 15, 30, 45 and 60 minutes for further analysis using UV spectrophotometer.

5. RESULT AND DISCUSSION

TABLE OF CONTENTS

5.1 IDENTIFICATION OF EZETIMIBE

5.1.1. Organoleptic evaluation:

a) Color: White/colorless

b) Odor: Odorless

c) State: Solid Powder

5.1.2. Melting Point (M.P.):

When liquid state and solid state of the substance exist in equilibrium at particular temperature it is known as melting point. At this temperature solid state of substance converts to liquid state at atmospheric pressure. The melting point of ezetimibe was done by two methods that are digital thermometer and DSC as a part of Preformulation studies. In digital thermometer the melting point was measured using Thiel's tube and was observed while in DSC sample was prepared and scanned using software.

The reported melting point of ezetimibe is 164-166°C and actual melting point found to be from DSC was 163.8°C and digital thermometer was 164°C.

The resulted melting point is in the range of actual melting point which indicates that our drug sample has same property and pure as the standard ezetimibe drug.

5.1.3. Solubility

Ezetimibe belongs to BCS class II drug and practically insoluble in water with solubility less than 0.01 mg/mL while freely to very soluble in methanol.

5.1.4. FTIR:

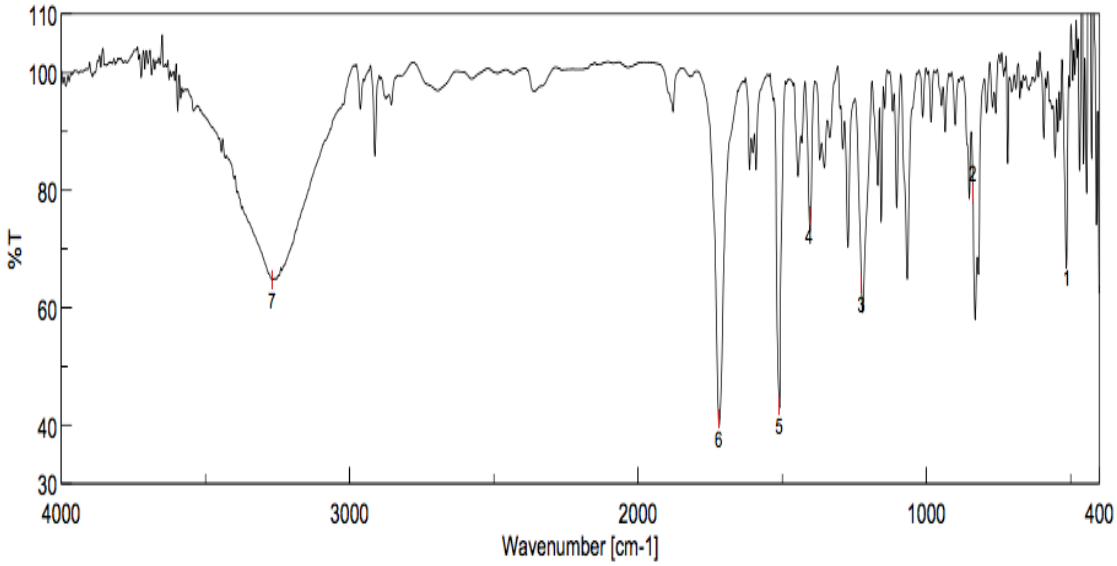

Figure 5.1. Ezetimibe sample FTIR data

IR spectra of dried ezetimibe drug powder with KBr pellets between 4000-400 cm^{-1} at moderate speed of scanning. Sample dried to reduce or neglect the moisture peak obtained during scanning.

TABLE 5.1 FTIR DRUG DATA

Functional groups	Standard frequency of functional group (cm^{-1})	Standard frequency of ezetimibe (cm^{-1})	Observed frequency in drug sample (cm^{-1})
Free (O-H) (stretching)	3397.7	3265.84	3268.75
Aromatic C-F (stretching)	1224.9	1221.69	1225.54
C=O (beta lactam) (stretching)	1721.7	1719.3	1721.16
Aromatic C=C	1559.8	1509.39	1509.99

The ezetimibe sample spectra was compared with Ezetimibe standard spectra data and individual functional group data. When these spectra were compared they found to have similar frequency peaks which indicated that ezetimibe was pure drug sample

5.2 ANALYTICAL METHOD FOR EZETIMIBE

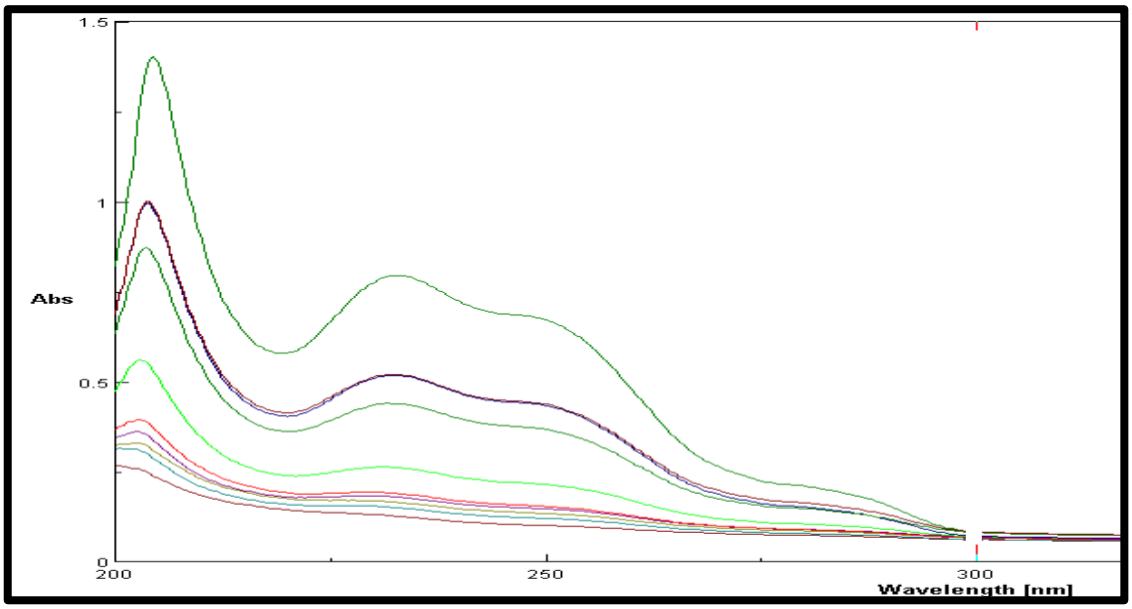

Figure 5.2. UV absorption maxima

5.2.1. Absorption maxima:

Media: Methanol

Concentration: 100 ppm

Absorption maxima refers to the maximum absorption of drug at particular wavelength which is known as λ_{max}. It is a quantitative parameter used in analysis for identification of molecules.

The absorption maxima of ezetimibe drug was estimated using 100 ppm solution of ezetimibe in methanol using JASCO UV Spectrophotometer and it was detected at 233 nm which indicates drug sample is pure.

TABLE 5.2. CALIBRATION CURVE OF EZETIMIBE IN METHANOL

Sr. no	Concentration (µg/ml)	Absorbance Trial 1	Absorbance Trial 2	Absorbance Trial 3	Absorbance (mean)
1.	0	0	0	0	0
2.	5	0.203	0.193	0.167	0.187
3.	10	0.398	0.303	0.171	0.230
4.	15	0.506	0.484	0.350	0.356
5.	20	0.64	0.612	0.483	0.437
6.	25	0.679	0.832	0.646	0.545

5.2.2 Calibration Curve:

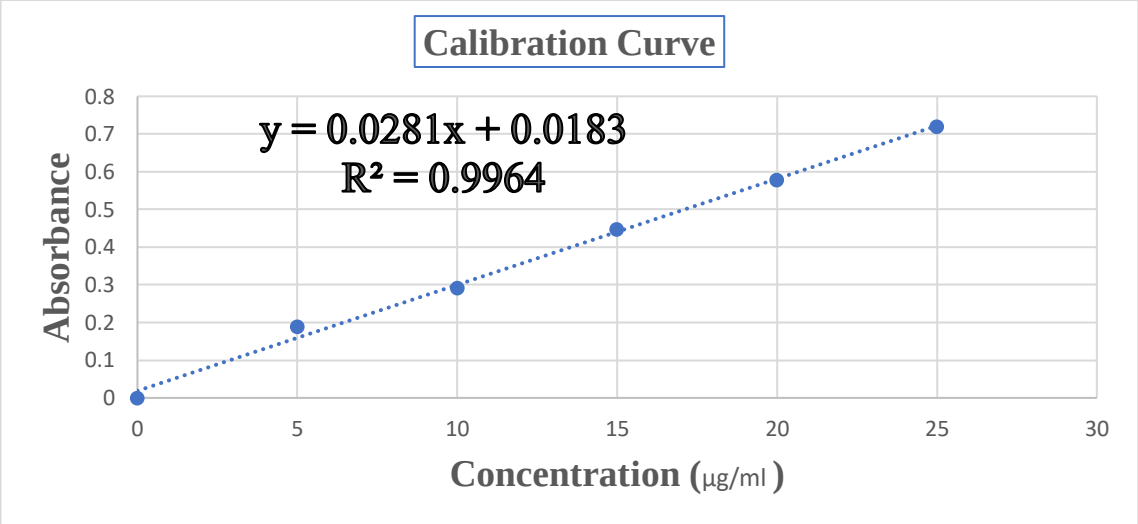

Figure 5.3 Calibration curve of Ezetimibe in methanol at 223nm.

TABLE 5.3 REGRESSION ANALYSIS

Sr. no	Parameters	Results
1	Regression equation	y= 0.0281x + 0.0183
2	Correlation coefficient	R_2=0.9964
3	Calibration range	5 µg/ml to 25 µg/ml

The regression equation obtained from standard curve is y=0.0281x+0.0187 and R_2=0.99 which is near to 1. The linear graph of conc v/s absorbance indicates that it follows the Beer Lambert equation from 5-25 µg/ml.

5.3. SATURATION SOLUBILITY FOR SELECTION OF COMPONENTS:

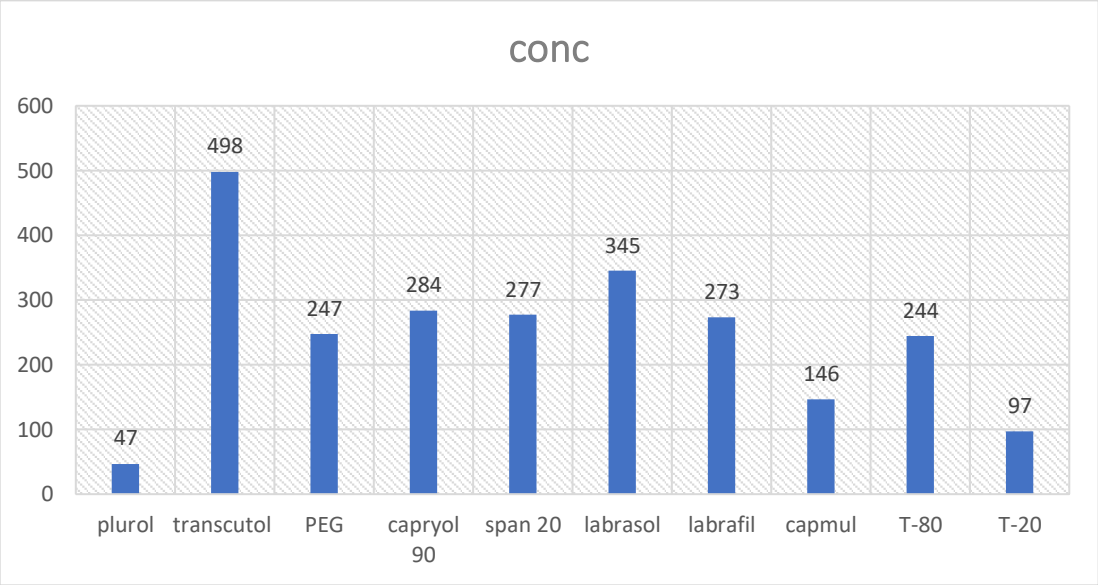

Figure 5.4.: Saturation Solubility of Ezetimibe

Saturation solubility of ezetimibe was determined visually in oils, surfactants and co-surfactants and quantified using UV analytical method. On the basis of solubility, Capryol 90 was selected as oil phase, Tween 80 as surfactant, Transcutol P and Labrasol as Co-surfactant. These components were then used for further studies.

5.4 MISCIBILITY BETWEEN SELECTED SURFACTANTS AND CO-SURFACTANTS

From solubility studies, Capryol 90 was selected as oil component and Tween 80 was selected as surfactant. Combination of Capryol 90 and Tween 80 with two co-surfactants named Labrasol and Transcutol as suggested by preliminary studies were screened for the selection of component in SEDDS. Ratio of oil to Smix was taken from 9:1 to 1:9 till

turbidity appears. As soon as turbidity appears on addition of water, further ratio study was discontinued. Following table is miscibility using water titration indicating C as clear and T as turbid.

TABLE 5.4 TWEEN 80: TRANSCUTOL (9:1)

% surfactant	%oil	%water	% Total	(1:1)	(1:2)	(2:1)
81.818	9.090	9.090	100	C	C	C
75.000	8.333	16.666	100	C	C	C
69.231	7.692	23.076	100	C	C	C
64.286	7.142	28.571	100	C	C	C
60.000	6.666	33.333	100	C	C	C
56.250	6.250	37.500	100	C	C	C
52.941	5.882	41.176	100	C	C	C
50.000	5.555	44.444	100	C	C	C
47.368	5.263	47.368	100	C	C	C
45.000	5.000	50.000	100	C	C	C
42.857	4.761	52.380	100	C	C	C
40.909	4.545	54.545	100	C	C	C
39.130	4.347	56.521	100	C	C	C
37.500	4.166	58.333	100	C	C	C
36.000	4.000	60.000	100	C	C	C
34.615	3.846	61.538	100	C	C	C
33.333	3.703	62.962	100	C	C	C
32.143	3.571	64.285	100	C	C	C
31.034	3.448	65.517	100	C	C	C
30.000	3.333	66.666	100	C	C	C

TABLE 5.5 TWEEN80: TRANSCUTOL (8:2)

% surfactant	%oil	%water	% Total	(1:1)	(1:2)	2:1
72.7272727	18.181	9.090	100	C	C	C
66.667	16.6	16.666	100	C	C	C
61.538	15.384	23.076	100	C	C	C
57.143	14.285	28.571	100	C	C	C
53.333	13.333	33.333	100	C	C	C
50.000	12.500	37.500	100	C	C	C
47.059	11.764	41.176	100	C	C	C
44.444	11.111	44.444	100	C	C	C
42.105	10.526	47.368	100	C	C	C
40.000	10.000	50.000	100	C	C	C
38.095	9.523	52.380	100	C	C	T.
36.364	9.090	54.545	100	C	C	T.
34.783	8.695	56.521	100	C	C	T.
33.333	8.333	58.333	100	C	C	T
32.000	8.000	60.000	100	C	T.	T
30.769	7.692	61.538	100	C	T	T
29.630	7.407	62.962	100	C	T	T
28.571	7.142	64.285	100	T	T	T
27.586	6.896	65.517	100	T	T	T
26.667	6.66666667	66.6666667	100	T	T	T

TABLE 5.6 TWEEN 80: TRANSCUTOL (7:3)

% surfactant	%oil	%water	% Total	(1:1)	(1:2)	(2:1)
63.6363636	27.272	9.090	100	C	C	C
58.333	25.000	16.666	100	C	C	C
53.846	23.0769	23.076	100	T	C	C
50.000	21.428	28.571	100	T TO C	C	C
46.667	20.000	33.333	100	T TO C	C	C
43.750	18.750	37.500	100	T	C	C
41.176	17.647	41.176	100	T	C	C
38.889	16.666	44.444	100	T	T.	C
36.842	15.789	47.368	100	T	T	T.
35.000	15.000	50.000	100	T	T	T
33.333	14.285	52.380	100	T	T	T
31.818	13.636	54.545	100	T	T	T
30.435	13.043	56.521	100	T	T	T
29.167	12.500	58.333	100	T	T	T
28.000	12.000	60.000	100	T	T	T
26.923	11.538	61.538	100	T	T	T
25.926	11.111	62.962	100	T	T	T
25.000	10.714	64.285	100	T	T	T
24.138	10.344	65.517	100	T	T	T
23.333	10.000	66.666	100	T	T	T

TABLE 5.7 TWEEN 80: TRANSCUTOL (6:4)

% surfactant	%oil	%water	% Total	(1:1)	(1:2)	(2:1)
54.545	36.363	9.090	100	C	C	T
50.000	33.333	16.666	100	T TO C	C	T
46.154	30.769	23.076	100	T TO C	C	T
42.857	28.571	28.571	100	T TO C	T.	T
40.000	26.666	33.333	100	T TO C	T.	T
37.500	25.000	37.500	100	T TO C	T. TO C	T
35.294	23.529	41.176	100	T	T. TO C	T
33.333	22.222	44.444	100	T	T	T
31.579	21.052	47.368	100	T	T	T
30.000	20.000	50.000	100	T	T	T
28.571	19.047	52.380	100	T	T	T
27.273	18.181	54.545	100	T	T	T
26.087	17.391	56.521	100	T	T	T
25.000	16.666	58.333	100	T	T	T
24.000	16.000	60.000	100	T	T	T
23.077	15.384	61.538	100	T	T	T
22.222	14.814	62.962	100	T	T	T
21.429	14.285	64.285	100	T	T	T
20.690	13.793	65.517	100	T	T	T
20.000	13.333	66.666	100	T	T	T

TABLE 5.8. LABRASOL: TRANSCUTOL (9:1)

% surfactant	%oil	%water	% Total	(1:1)	(1:2)	(2:1)
81.8181818	9.09090909	9.090	100	C.	C	C
75.000	8.333	16.666	100	C.	C	C
69.231	7.692	23.076	100	C.	C	C
64.286	7.142	28.571	100	C	C	C
60.000	6.666	33.333	100	C	C	C
56.250	6.250	37.500	100	C.	C	C
52.941	5.882	41.176	100	C	C	C
50.000	5.555	44.444	100	C.	C	C
47.368	5.263	47.368	100	C	C	C
45.000	5.000	50.000	100	C	C	C
42.857	4.761	52.380	100	C	C	C
40.909	4.545	54.545	100	C.	C	C
39.130	4.347	56.521	100	C	T	C
37.500	4.166	58.333	100	C	T	C
36.000	4.000	60.000	100	C	LH	C
34.615	3.846	61.538	100	C	T TO H	C
33.333	3.703	62.962	100	LH	T.	C
32.143	3.571	64.285	100	T	T	C
31.034	3.448	65.517	100	T	T	C
30.000	3.333	66.666	100	T	T	C

TABLE 5.9 LABRASOL: TRANSCUTOL (8:2)

% surfactant	%oil	%water	% Total	(1:1)	(1:2)	(2:1)
72.727	18.181	9.090	100	C	C	C
66.667	16.666	16.666	100	C	C	C
61.538	15.384	23.076	100	C	C	C
57.143	14.285	28.571	100	C	C	C
53.333	13.333	33.333	100	C	C	C
50.000	12.500	37.500	100	C	C	C
47.059	11.764	41.176	100	C	C	C
44.444	11.111	44.444	100	C	C	C
42.105	10.526	47.368	100	T	C	C
40.000	10.000	50.000	100	T	C	C
38.095	9.523	52.380	100	T	T	C
36.364	9.090	54.545	100	T	T	C
34.783	8.695	56.521	100	T	T	T
33.333	8.333	58.333	100	T	T	T
32.000	8.000	60.000	100	T	T	T
30.769	7.692	61.538	100	T	T	T
29.630	7.407	62.962	100	T	T	T
28.571	7.142	64.285	100	T	T	T
27.586	6.896	65.517	100	T	T	T
26.667	6.666	66.666	100	T	T	T

TABLE 5.10 LABRASOL: TRANSCUTOL (7:3)

% surfactant	%oil	%water	% Total	(1:1)	(1:2)	(2:1)
63.636	27.272	9.090	100	C.	C	C
58.333	25.000	16.666	100	C	C	C
53.846	23.076	23.076	100	C	C	T
50.000	21.428	28.571	100	T	C	T
46.667	20.000	33.333	100	T	C	T
43.750	18.750	37.500	100	T	C	T
41.176	17.647	41.176	100	T	T	T
38.889	16.666	44.444	100	T	T	T
36.842	15.789	47.368	100	T	T	T
35.000	15.000	50.000	100	T	T	T
33.333	14.285	52.380	100	T	T	T
31.818	13.636	54.545	100	T	T	T
30.435	13.043	56.521	100	T	T	T
29.167	12.500	58.333	100	T	T	T
28.000	12.000	60.000	100	T	T	T
26.923	11.538	61.538	100	T	T	T
25.926	11.111	62.962	100	T	T	T
25.000	10.714	64.285	100	T	T	T
24.138	10.344	65.517	100	T	T	T
23.333	10.000	66.666	100	T	T	T

Using the above result obtained from water titration method, ternary phase diagram was plotted to get the isotropic region and to finalize the Co-surfactant to be used.

5.5 Selection of formulation from phase diagram

Ternary phase diagram was plotted using online free software available. Ternary diagram consist of three components, whose total will be same although the proportion varies. The first component is water, second is oil and third is Smix. The points were plotted from 0 too 100 % of all components. Ternary Phase was plotted for both Transcutol P and Labrasol. Surfactant -cosurfactant ratio was selected from 1:1, 1:2 and 2:1.

From ternary phase diagram, selection of co-surfactant is done on the basis of maximum isotropic region obtained in it.

a) Tween 80: Transcutol (1:1)

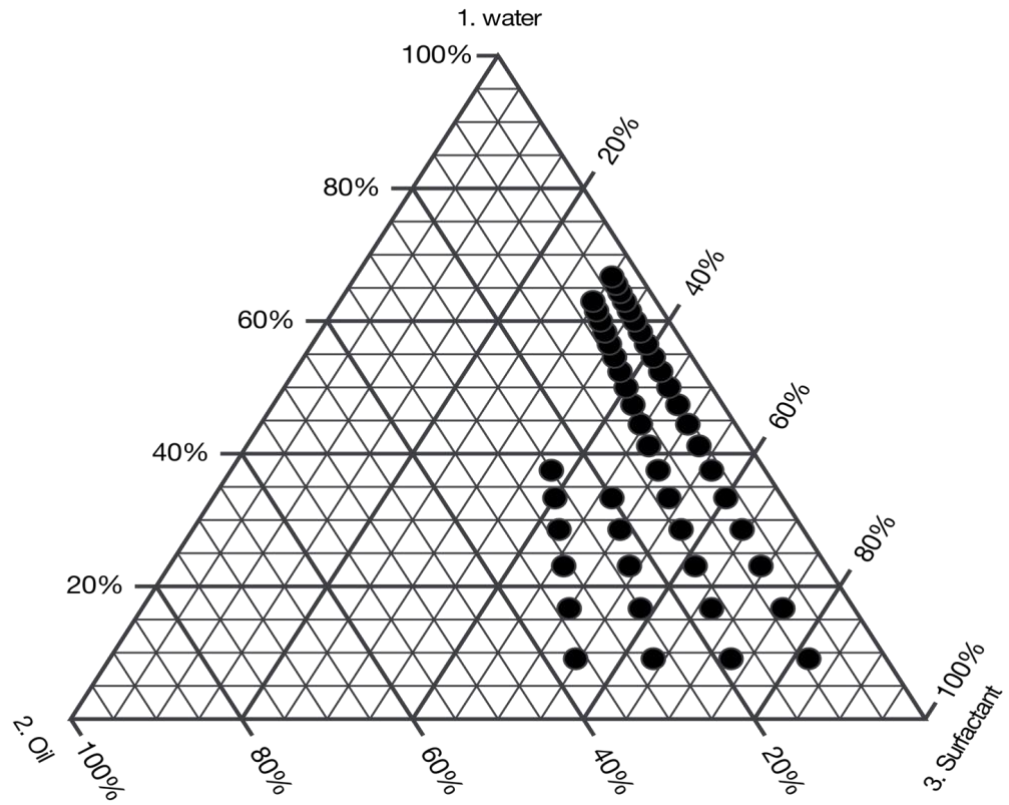

Figure 5.5 Tween 80: Transcutol (1:1)

b) Tween 80: Transcutol (1:2)

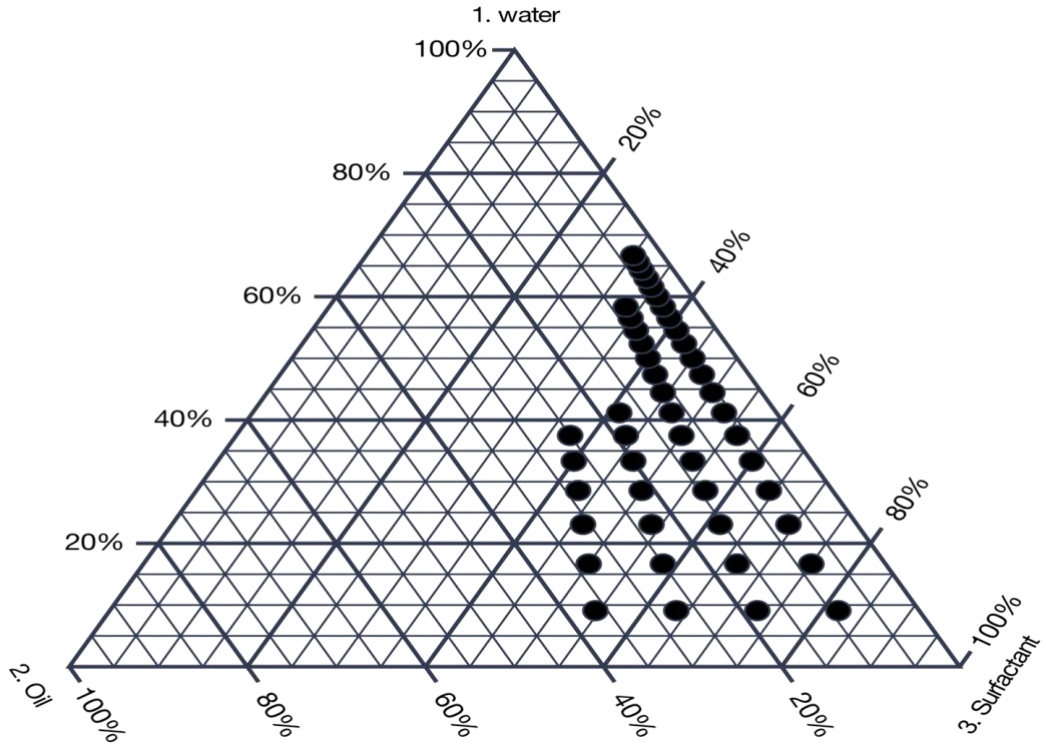

Figure 5.6 Tween 80: Transcutol (1:2)

c) Tween 80: Transcutol (2:1)

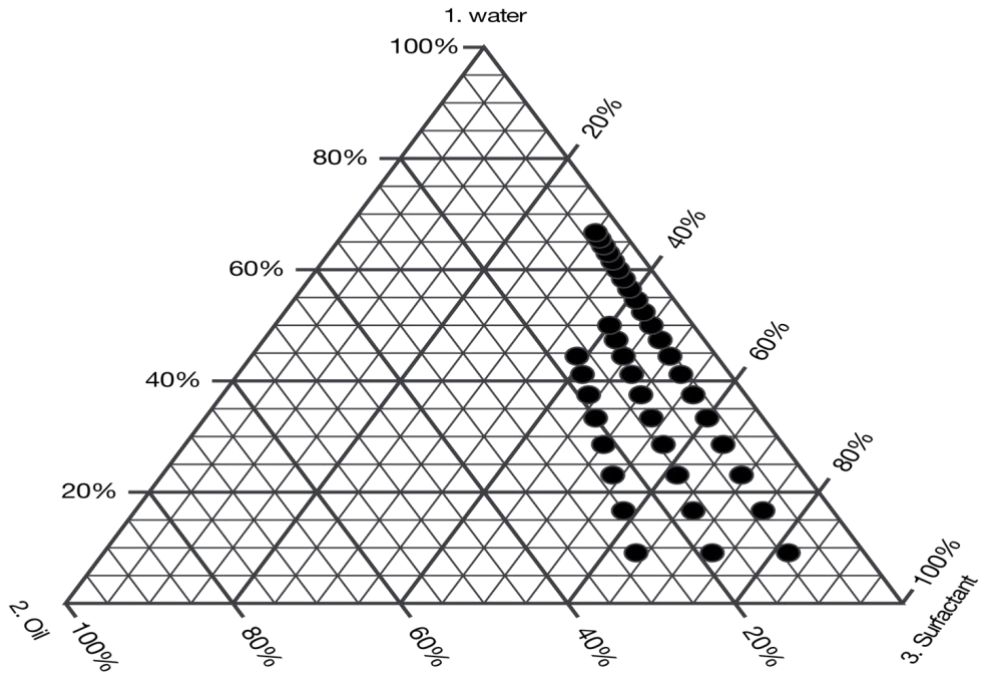

Figure 5.7 Tween 80: Transcutol (2:1)

Selection of formulation from phase diagram (Tween 80: Labrasol)

a) Tween 80: Labrasol (1:1)

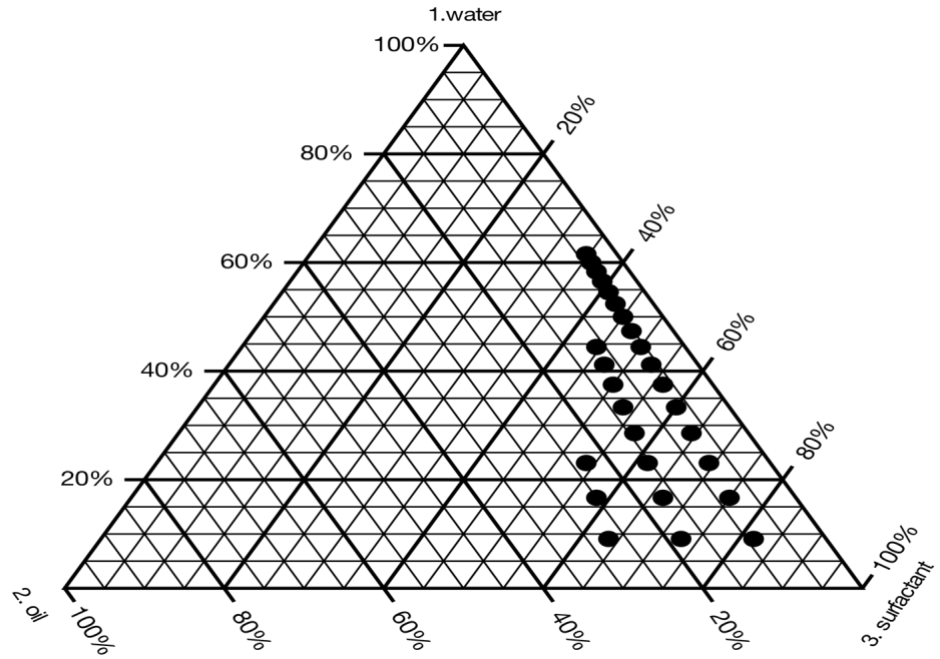

Figure 5.8. Tween 80: Labrasol (1:1)

b) Tween 80: Labrasol (1:2)

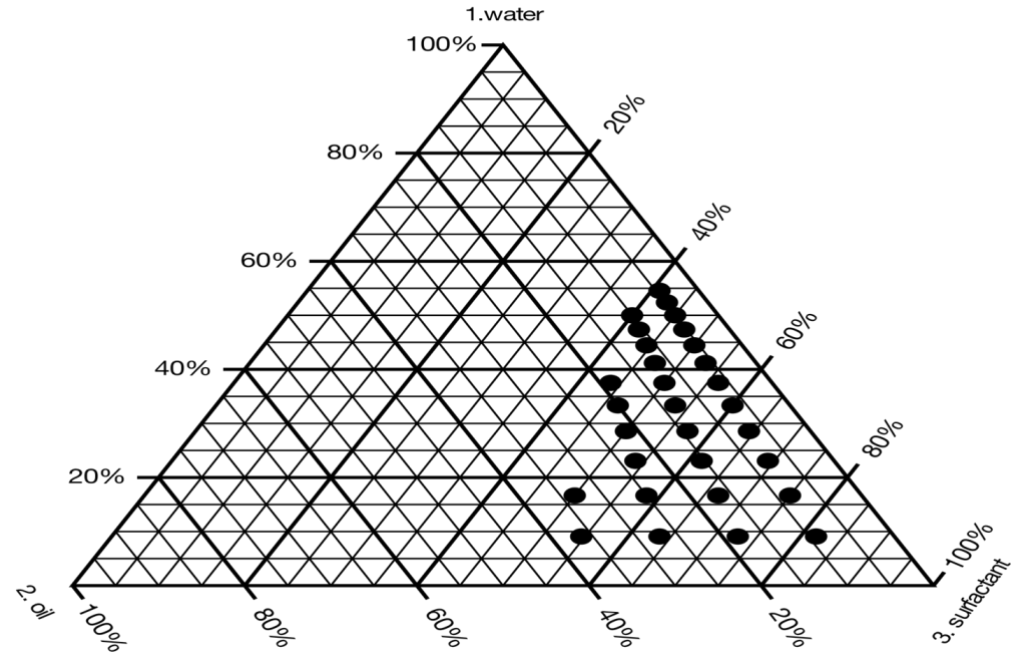

Figure 5.9 Tween 80: Labrasol (1:2)

c) Tween 80: Labrasol (2:1)

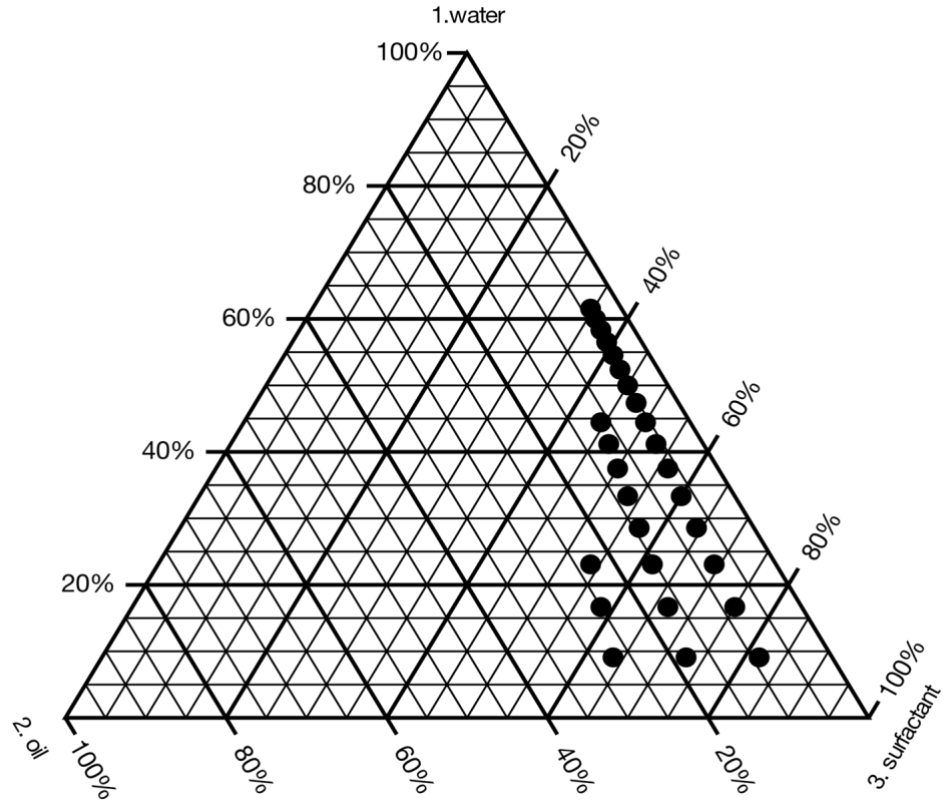

Figure 5.10 Tween 80: Labrasol (2:1)

From the Pseudo-ternary phase diagram, it was seen that by using Labrasol , very little emulsification area is obtained and that too in more than 40% of oil region which indicated Labrasol is not a choice of surfactant for formulation. It was also determined that Transcutol works better as Co-surfactant in compared to Labrasol. Transcutol have larger isotropic region which indicates emulsification by Transcutol is better than Labrasol.

From the Pseudo-ternary phase diagram, isotropic regions were identified with blend of excipients selected by solubility and maximum emulsification area was identified , where 1:1 (Tween 80: Transcutol) ratio was selected, as no major change was identified on increasing the amount of co-surfactant/ surfactant. The area of emulsification was further optimized by mixture design.

5.6. Formulation of SEDDS

From the ternary phase diagram, isotropic region was identified. The mixture design was then applied in isotropic region to get the range of oil, surfactant and co-surfactant. The higher and lower range of oil, surfactant and co-surfactant was then decided and mixture deign was applied.

TABLE 5.11 DEPENDENT VARIABLES:

Components	Low	High
Capryol	20	40
Tween 80	20	40
Transcutol	40	60

Seven batches were decided from mixture design by using higher and lower range of components and formulation was prepared accordingly. Dose was kept same as the tablet i.e. 10 mg.

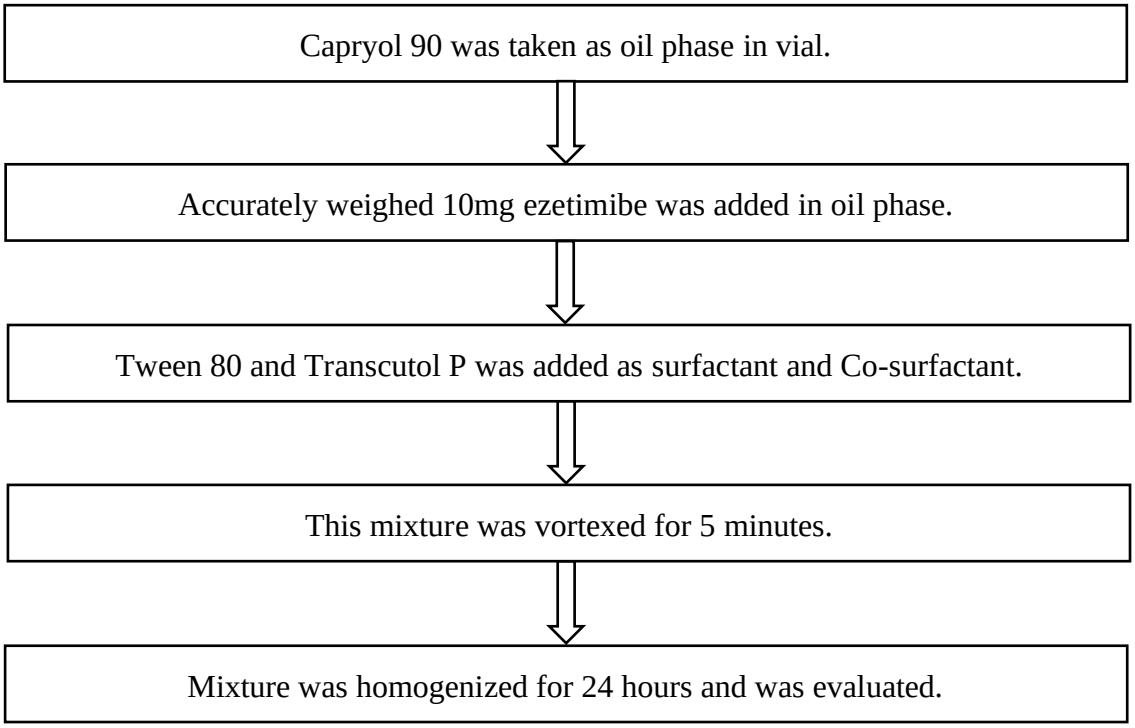

Figure 5.11 Formulation of SEDDS

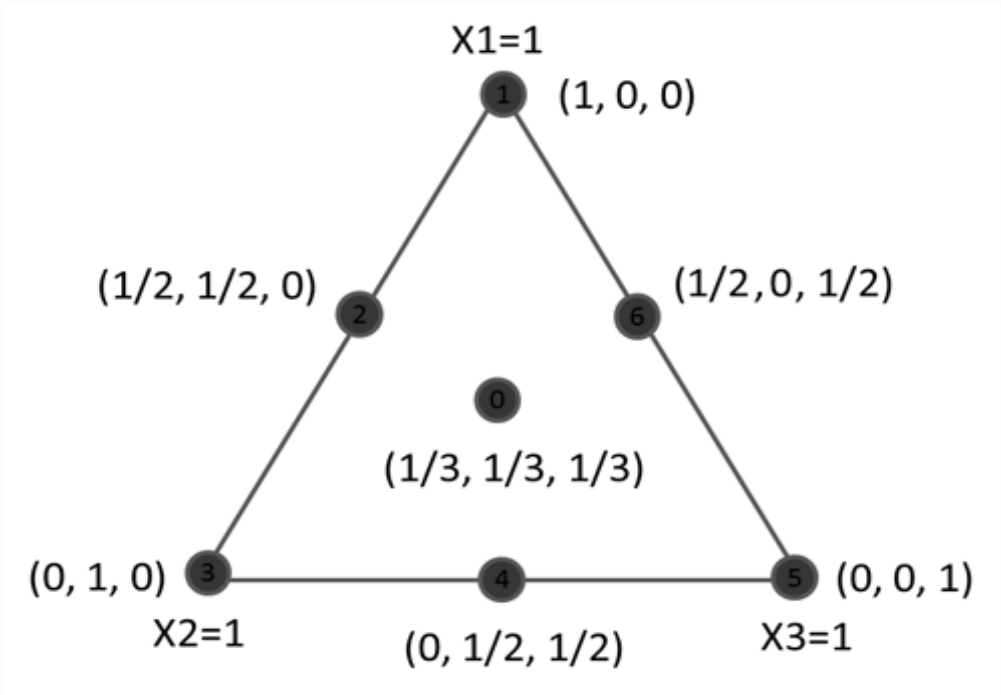

Figure 5.12 Mixture design runs

TABLE 5.12 CODED VALUES

Batches	X1	X2	X3
A1	**1**	**0**	**0**
A2	**0**	**1**	**0**
A3	**0**	**0**	**1**
A4	**0.5**	**0.5**	**0**
A5	**0.5**	**0**	**0.5**
A6	**0**	**0.5**	**0.5**
A7	**0.33**	**0.33**	**0.33**

TABLE 5.13 DECODED VALUES

Batches	X1	Capryol 90	X2	Tween 80	X3	Transcutol
A1	**1**	300	**0**	300	**0**	400
A2	**0**	100	**1**	500	**0**	400

A3	**0**	100	**0**	300	**1**	600
A4	**0.5**	200	**0.5**	400	**0**	400
A5	**0.5**	200	**0**	300	**0.5**	500
A6	**0**	100	**0.5**	400	**0.5**	500
A7	**0.33**	167	**0.33**	367	**0.33**	567

5.7. Characterisation of SEDDS:

For characterization, 0.1 ml of preconcentrate was diluted with 0.1 N HCl to produce nanoemulsion. Self-nanoemulsification time, dispersibility, globule size were performed.

a) Self nano-emulsification time:

The 0.1 ml preconcentrate of ezetimibe SEDDS and 0.1 N HCl 100 ml was blended with propeller stirrer at 50 rpm speed in room temperature in beaker and emulsification time was observed by visual observation.

TABLE 5.14 SELF-EMULSIFICATION

BATCHES	X1 (µL)	X2 (µL)	X3 (µL)	Self-emulsification time (seconds)
A1	300	300	400	9
A2	100	500	400	12
A3	100	300	600	7
A4	200	400	400	8
A5	200	300	500	10
A6	100	400	500	8
A7	167	367	567	9

Self-emulsification time in all seven batches was within several seconds indicating, the formulation as spontaneous emulsion can be formed via gentle agitation.

b) globule size:

The 0.1 ml preconcentrate of ezetimibe SEDDS was diluted with 100 ml of 0.1 N HCl which was stirred for 5 minutes at room temperature and droplet size was measured using Malvern Zeta sizer.

TABLE 5.15 GLOBULE SIZE, Z-AVG & PI

BATCHES	X1 (µL)	X2 (µL)	X3 (µL)	MEAN (nm)	Z AVG (nm)	PI
A1	300	300	400	519.9	1215.8	0.466
A2	100	500	400	200	1596.5	0.7
A3	100	300	600	124	627.1	0.855
A4	200	400	400	285.2	201.4	0.792
A5	200	300	500	322	240	0.739
A6	100	400	500	181.9	420.6	0.58
A7	167	367	567	288.8	356.4	0.577

The mean globule size, Z-average and PI was determined using Malvern Zeta sizer. The globule size of all seven batches was within 550 nm. It was also observed that on increasing the Smix or decreasing the amount of oil, globule size tend to decrease significantly. PI obtained from above batches were not very good which can further be solved by optimization.

c) Dispersibility studies:

Dispersibility study was done by using 1 ml of preconcentrate in 500 ml distilled water and in 500 ml 0.1 N HCl at 37 ± 0.5 °C using beaker with speed of 50 rpm which provide gentle type agitation. Further, grading was done of each formulation using the following table.

TABLE 5.16 GRADE OF DISPERSIBILITY STUDIES

SR.NO	OBSERVATION	GRADE
1.	Rapidly forming emulsion within one minute and have clear appearance	A
2.	Rapidly forming emulsion but comparatively less clear and have bluish white appearance	B
3.	Milky fine emulsion that forms within 2 minutes	C

TABLE 5.17 DISPERSIBLITY STUDIES RESULT

BATCHES	X1	X2	X3	Grade
A1	300	300	400	A
A2	100	500	400	A
A3	100	300	600	A
A4	200	400	400	A
A5	200	300	500	A
A6	100	400	500	A
A7	167	367	567	A

As objective of this formulation is to make oral, dispersibility studies are very important. From, dispersibility studies, it was observed that clear emulsion was formed spontaneously and rapidly This indicated that it will remain in emulsion form only when given in stomach and gets dispersed in GIT environment.

5.8 Optimization of SEDDS:

For preliminary studies, data treatment was done using Microsoft Excel using regression model. R square and co-efficients were calculated for all seven batches including all for all three variables.

TABLE 5.18 BATCH A1 TO A7 WITH 100 TIMES DILUTION AT A TIME AND EVALUATED

BATCHES	X1 (µL)	X2 (µL)	X3 (µL)	MEAN (nm)	PI	ST (sec)
A1	300	300	400	519.9	0.466	9
A2	100	500	400	200	0.7	12
A3	100	300	600	124	0.855	7
A4	200	400	400	285.2	0.792	8
A5	200	300	500	322	0.739	10
A6	100	400	500	181.9	0.58	8
A7	167	367	567	288.8	0.577	9

TABLE 5.19 REFRESSION ANALYSIS BY MS EXCEL

Sr.no	Variable	R_2 Obtained
1.	**Mean droplet size**	0.99979446
2.	**Polydispersity Index**	0.99896727
3.	**Self-emulsification time**	0.99989673

5.9 Optimisation by Design Expert:

Design Expert® was used for further data analysis and optimisation of SEDDS. The ratio of each component and concentration is critical to form stable and desired SEDDS formulation. Hence, concentration of Capryol 90, Tween 80 and Transcutol P was taken as independent variable in mixture design on the basis of literature survey. Simplex centroid design was used using Capryol 90 as X1, Tween 80 as X2 and Transcutol P as X3 as dependent variable. While, Globule size, Polydispersity Index and Self-emulsification time was taken as response Y1,Y2 and Y3 respectively.

The effect of all the variables was checked individually and data was analysed using the model suggested from software. ANOVA was used for further statistics and to calculate co-efficients from which effect of variable on each response is calculated. This help in optimization of ratio of each variable to get optimized formulation.

5.9.1. Actual Design run in design expert using simplex centroid design

	Std	Run	Component 1 A:capryol 90	Component 2 B:tween 80	Component 3 C:transcutol P	Response 1 R1 nm	Response 2 R2	Response 3 R3
	7	1	26.6667	26.6667	46.6667	289	0.6	9
	1	2	40	20	40	520	0.4	9
	6	3	20	30	50	182	0.6	8
	4	4	30	30	40	285	0.8	8
	5	5	30	20	50	322	0.73	10
	2	6	20	40	40	200	0.7	12
	3	7	20	20	60	124	0.8	7

5.9.2. Analysis for globule size response:

Parameter	Value
R2	0.952
P value	0.0023
Model Suggested	Linear

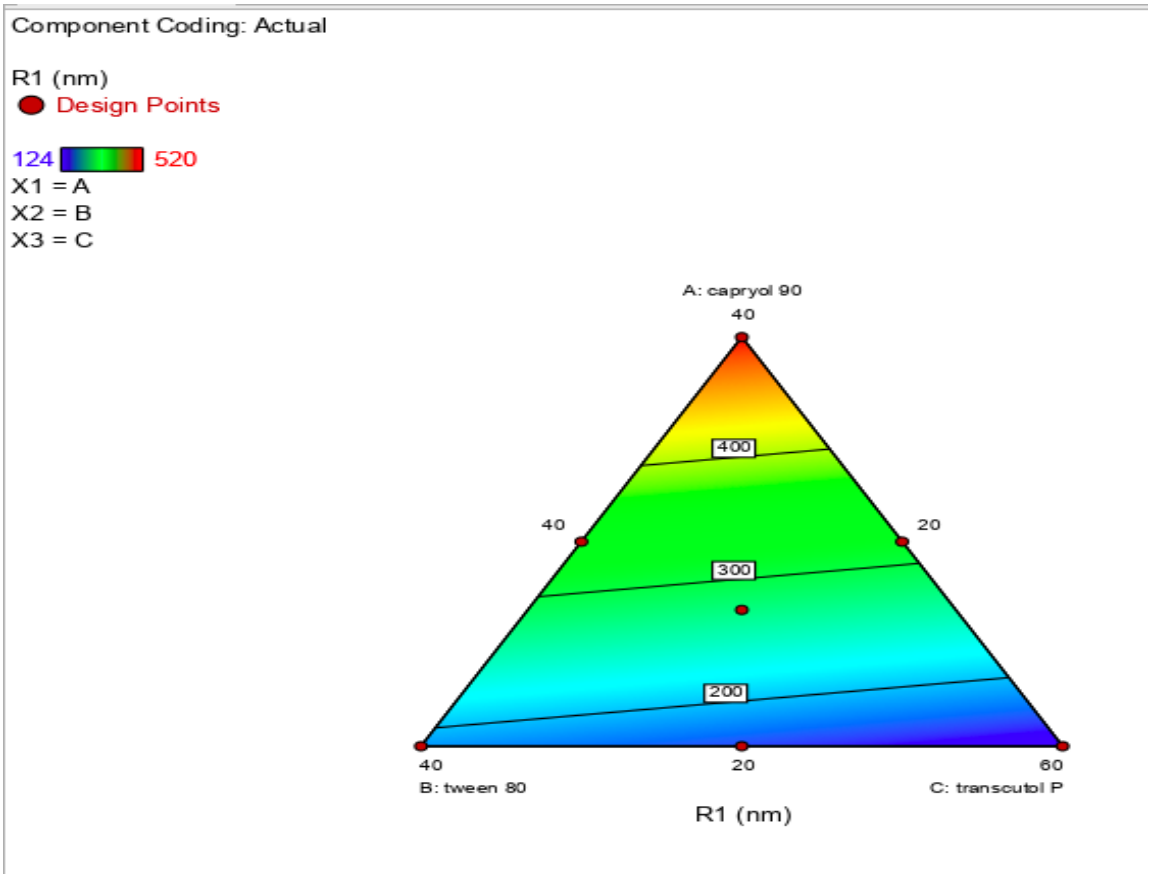

Figure 5.13 Contour plot for Droplet Size

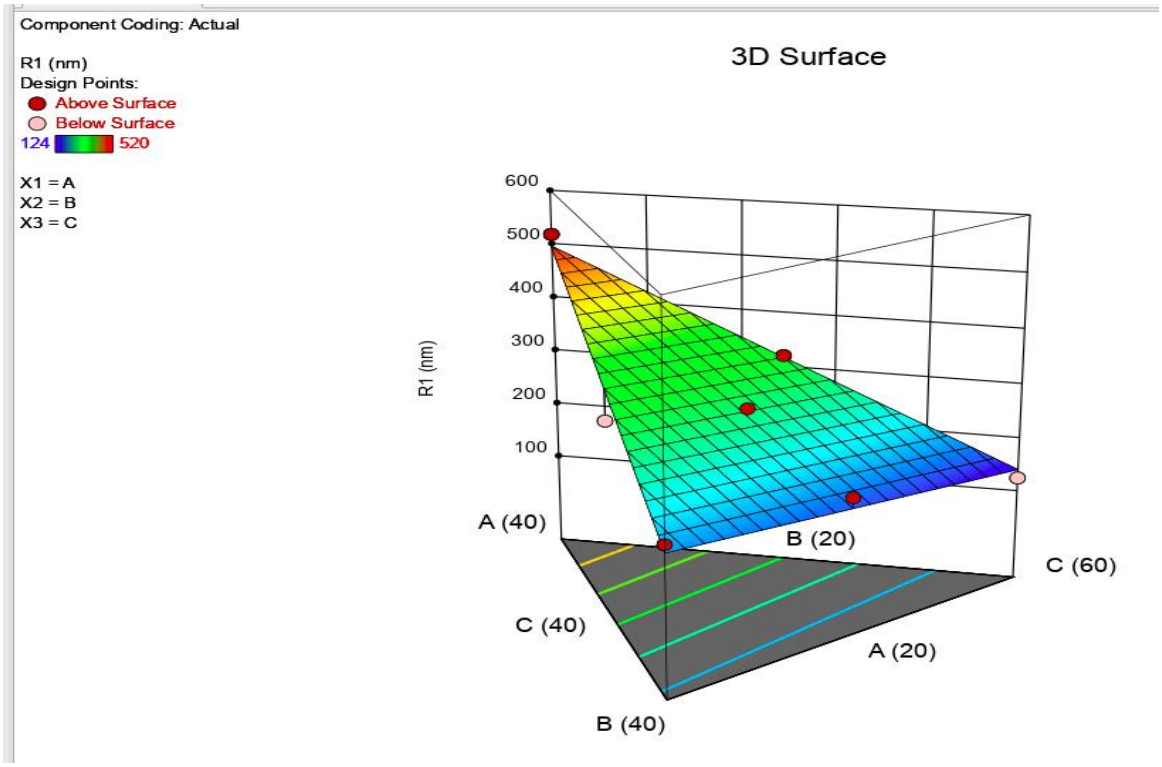

Figure 5.14 3D surface plot for Droplet size

Polynomial equation:

Y (particle size) = 497.90*A+185.90*B+139.90*C

Result and Discussion:

As per this graph and equation it can be observed that, independent variable are directly proportional to droplet size. From equation it can be noted that A is a significant term i.e. Capryol 90 (oil Phase) has more impact on particle size, as amount of Capryol 90 increases particle size also increases.

5.9.3. Analysis for PI response:

Parameter	Value
R2	0.906
P value	0.495
Model Suggested	Quadratic

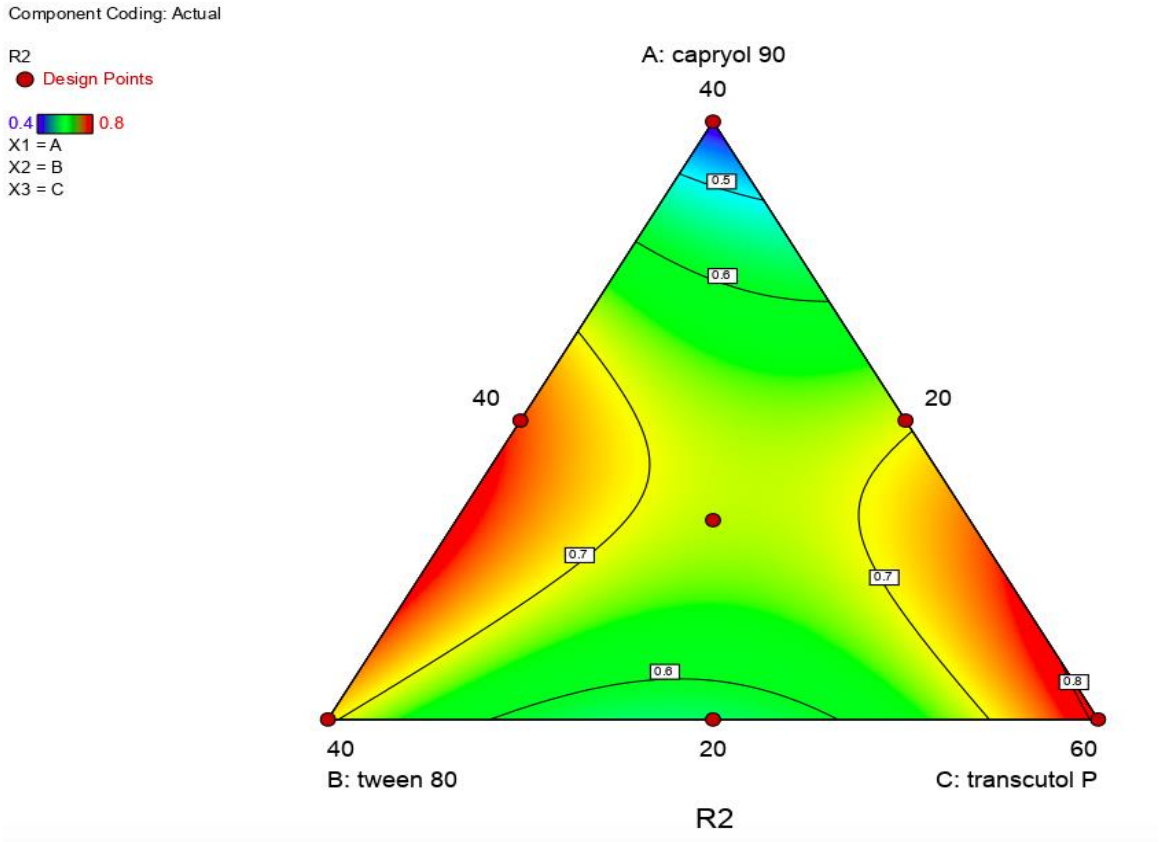

Figure 5.15 Contour plot for PI

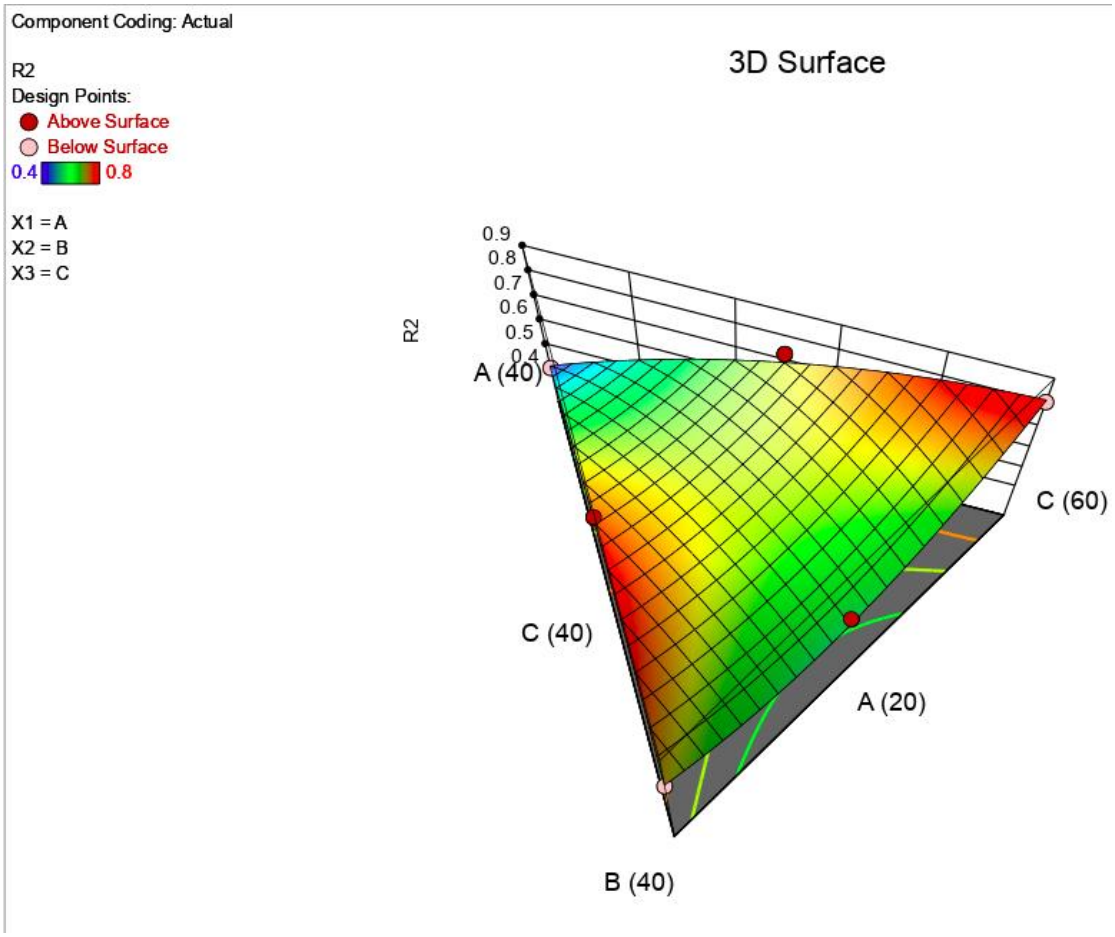

Figure 5.16 3D surface plot for PI

Polynomial equation:

Y(PI) =0.409*A+0.709*B+0.809*C+0.815*AB+0.3352*AC-0.7848*BC

Result and Discussion:

As per graph and equation it can be observed that term B & C are significant in this case. As the amount of B & C increases, the PI increases. All the terms gives positive effect except term BC which gives negative effect which means combined effect of term BC leads to decrease in PI.

5.9.4. Analysis for ST response:

Parameter	Value
R2	0.988
P value	0.183

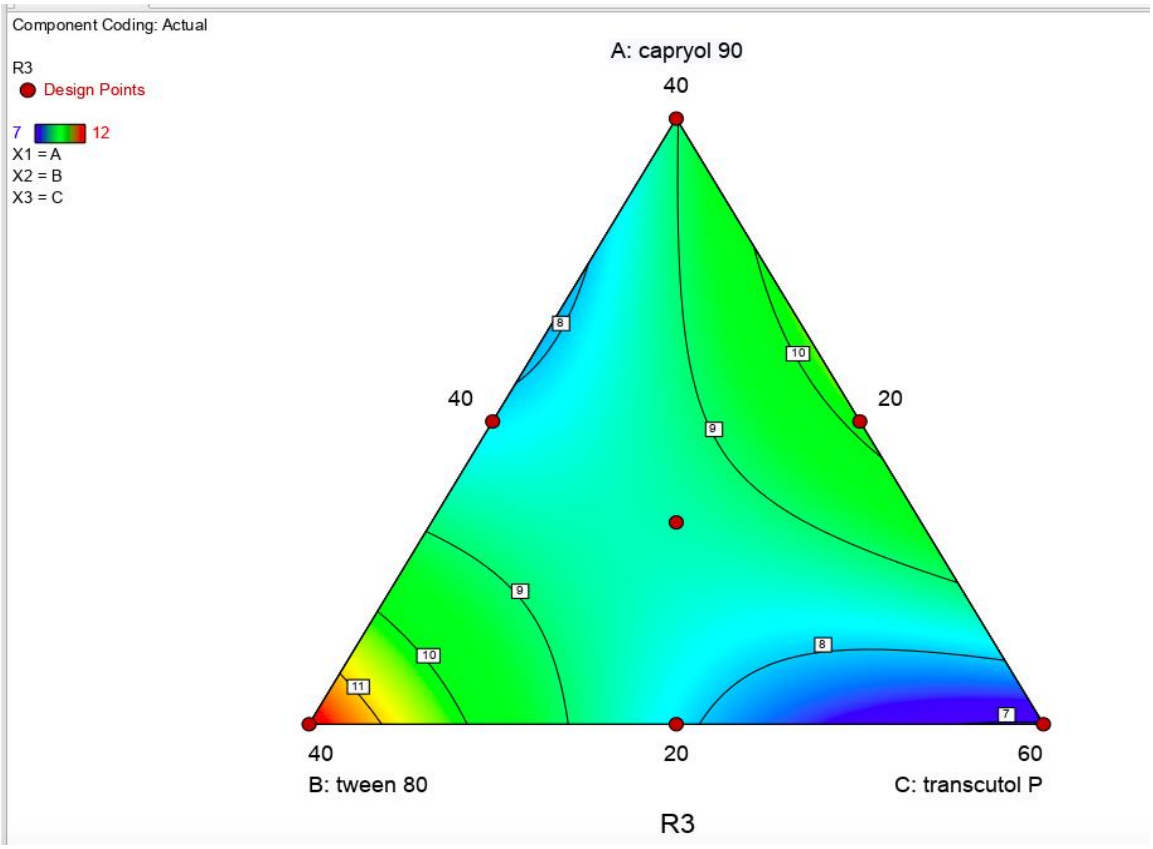

Figure 5.17 Contour plot for Self-emulsification time

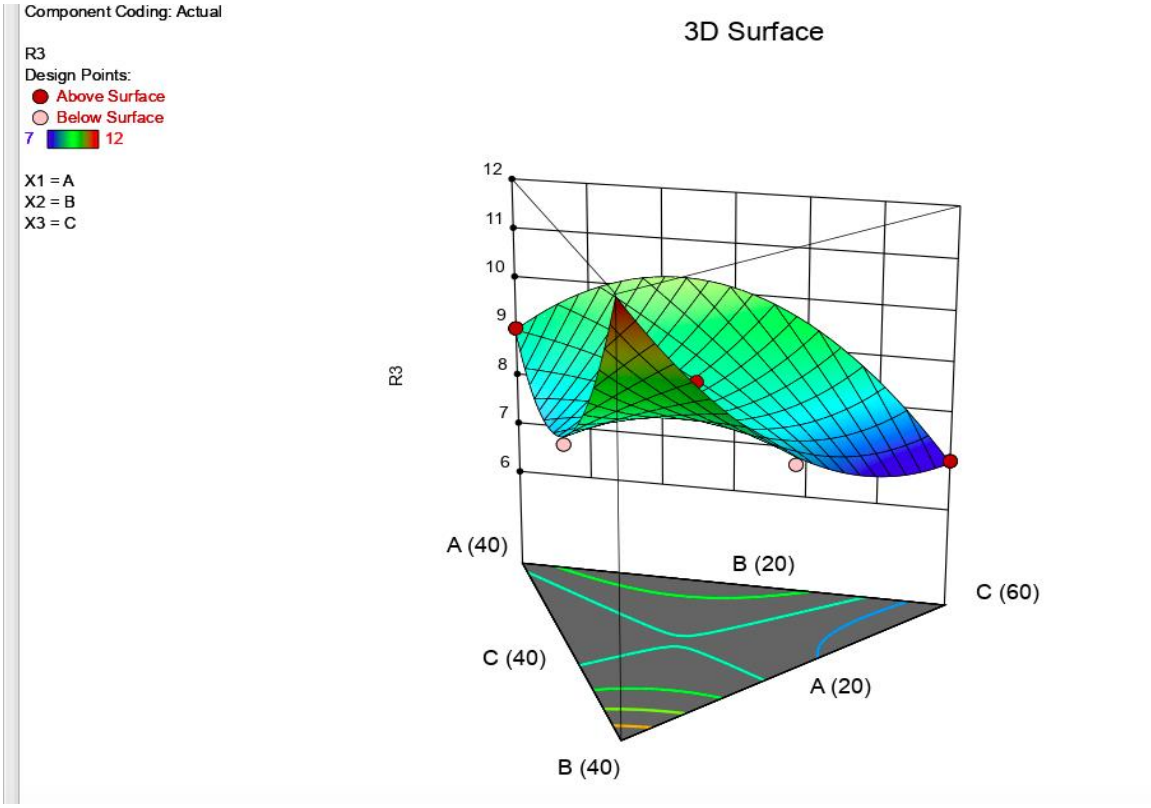

Figure 5.18 3D surface plot for Self-emulsification time

Polynomial equation:

Y(ST)=8.96*A+11.96*B+6.96*C-9.24*AB+8.76*AC-5.24*BC

Result and Discussion:

As per graph and equation it can be observed that, term B has significant effect in this case. While all the variables have positive effect except interaction term AB & BC, from which AB has comparatively more effect. It can be also concluded that as amount of B (Tween 80) increases, self-emulsification time increases more significantly.

Overlay Graph and prediction

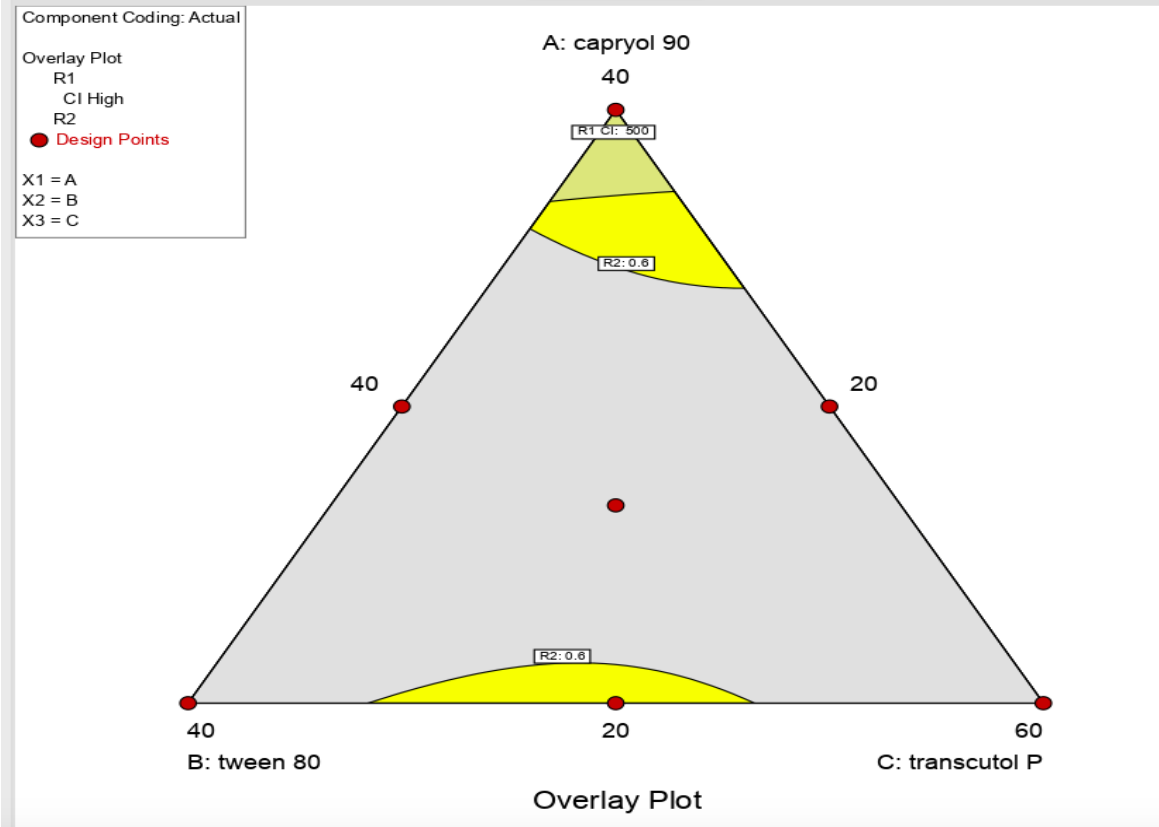

Figure 5.19 Overlay Graph for optimization formula

Result and Discussion:

By keeping the desirable criteria for each response, two solution are obtained where in first solution droplet size is 497.905 nm, PI is 0.4 and ST is 8.9 seconds while in second solution droplet size is 165.84, PI is 0.5 From this and ST is 8.4 seconds, second solution is better in terms of droplet size with

PI of 0.5 because as droplet size decreases, solubility profile will be improved which can give improved bioavailability.

5.10 Future Procedure to be followed:

The optimized batch was to be formulated and evaluated including droplet size, dispersibility studies, in-vitro drug release, Self-emulsification time and cloud point determination.

TABLE 5.20 FORMULA FOR OPTIMIZED BATCH:

Sr.no	Component	Amount	Response	Predicted Result
1.	Capryol 90	20	R1	165.84
2.	Tween 80	31.025	R2	0.5
3.	Transcutol P	48.975	R3	8.4

The predicted result obtained from Design Expert® is given in the table, where globule size is 165.264 nm, PI is 0.55 and Self-emulsification time is 8.4 seconds. If the predicted result is true than, optimized batch will form SEDDS having property of enhanced oral bioavailability by reduced droplet size and increased solubility.

6. CONCLUSION

In this research we investigated potential of Lipid-based formulation Type III i.e. SEDDS for BCS class II drug named ezetimibe. SEDDS are isotropic mixture consist of oil phase, surfactant and co-surfactant/co-solvent. These type of system form o/w type emulsion in gastric environment by gentle agitation. They are homogenous transparent and thermodynamically stable dispersion.

The purpose of study was to select appropriate components and understand effect of each component using ternary diagram to prepare optimized formulation. This type of SEDDS formulation further increase drug's solubility which ultimately improve dissolution profile and result into bioavailability enhancement with decrease intersubject variation.

From preliminary studies such as UV, DSC, FTIR it was concluded that the drug was pure to use for formulation and characterisation. From solubility studies, ternary phase diagrams Capryol 90 was chosen as oil phase, Tween 80 as surfactant and Transcutol P as Co-surfactant.

From preliminary trials, formulation composition was finalised which further optimized using DOE Mixture design and evaluated. The globule size, Polydispersity index, dispersibility studies, self-emulsification time was evaluated for all seven batches obtained from mixture design.

The minimum globule size obtained by using 20 % of oil phase and 20 % of surfactant and 60% of co-surfactant. It was concluded that Transcutol P was responsible for decreasing the globule size in SEDDS formulation. This is because Transcutol P act as co-surfactant which decreases the interfacial tension between oil and water phase ultimately resulting into formation of small droplet of emulsion.

Increase in oil phase increases the particle size significantly due to interfacial tension created between both phases. Other parameters such as self-emulsification time, dispersibility studies also decreases with increase in concentration of co-surfactants.

It was concluded that amount of each component and ratio of Smix majorly affects the emulsification area and oil phase plays major role in formation of emulsion. The range of oil phase between 20-30% was found to be good while as increase towards 40%, it affects the size of droplets significantly.

The result obtained till now concludes that SEDDS is potential formulation strategy for poorly soluble drugs to improve their solubility profile which can ultimately result in better bioavailability profile.

7. SUMMARY

Ezetimibe is poorly soluble drug belong to BCS class II, and first agent of cholestrol inhibitor. It inhibits biliary and circulatory cholestrol which help in treatment of hyperlipidaemia and hypercholesteremia. Improvement in NAFLD is also seen by use of ezetimibe, for which various clinical trials are ongoing. It can be used alone or in combination with statins for high cholestrol.

In this research, we investigated a novel formulation known as SEDDS for ezetimibe to increase its solubility and improve its dissolution profile. By formulating SEDDS, which is lipid based we also tried to decrease variation in bioavailability and lack of dose proportionality.

First of all we have done a literature survey for choosing all the excipient which have good safety profile and used them within IIG limit. Solubility of ezetimibe was checked in various oils, surfactants and co-surfactants. The components which have high solubility of ezetimibe were selected.

Capryol 90 was selected as an oil phase, Tween 80 was selected as surfactant, Labrasol and Transcutol P was selected as Co-surfactants. These were further examined using Pseudo ternary phase diagram.

Pseudo ternary diagram was made using water titration method for selecting the proper co-surfactant and ratio of Smix. Transcutol P was selected as co-surfactant and 1:1 ratio in Smix was selected for further studies. The isotropic region or emulsification region was identified from phase diagram to which mixture design was applied.

Mixture design is part of DOE used for optimization, simplex centroid design was selected in mixture design which consist of seven runs with three components whose total always remain constant.

All seven batches were formulated and evaluated for different parameters such as globule size, PI, dispersibility studies and self-emulsification time. The data was analysed using MS Excel and DOE.

The data was analysed using ANOVA in DOE and contour graph, 3D surface plot was obtained for interpretation of batches. The desired value of each response was selected in optimization of DOE, which gave the formula of optimized batch with predicted results.

8. FUTURE PERSPECTIVES

8.1. Future perspective for SEDDS:

SEDDS have provided novel strategy in pharma industry for drugs which are poorly soluble. SEDDS are easy to manufacture and scale up which makes it most beneficial system in lipid-based formulation. By use of numerous option in lipid, surfactant and co-surfactant it provide numerous opportunities for many insoluble drugs.

8.2. Future perspective for this research:

The prospects includes:

- Determination of Pharmacokinetic studies of drug after oral administration
- Invitro drug release studies
- In vivo studies
- Cell line studies
- Stability Studies.

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