FORMULATION & EVALUATION OF SELF NANOEMULSIFYING DRUG DELIVERY SYSTEMS OF POORLY WATER SOLUBLE ANTILIPIDEMIC DRUGS

A Thesis submitted to Gujarat Technological University
For the Award of

Doctor of Philosophy
in
Pharmacy
By

Milan Dhirajlal Limbani
Enrollment No. 119997290020

Under supervision of
Dr. L. D. Patel

GUJARAT TECHNOLOGICAL UNIVERSITY
AHMEDABAD

[MAY – 2019]

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DECLARATION

I declare that the thesis entitled **"Formulation & Evaluation of Self Nanoemulsifying Drug Delivery Systems of Poorly Water Soluble Antilipidemic Drugs"** submitted by me for the degree of Doctor of Philosophy is the record of research work carried out by me during the period June 2011 to October 2018 under the supervision of **Dr. L. D. Patel** and this has not formed the basis for the award of any degree, diploma, associateship, fellowship, titles in this or any other University or other institution of higher learning.

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ABSTRACT

In silico prediction of drugs solubility in lipid vehicle remains a difficult task. In the research work, some factors have been identified to be helpful in predicting drug solubility using selected excipients. These factors included the solubility parameter (δ), HLB value, partition coefficient, relative molecular mass (MW), Dielectric constant (ε), dipole moment (μ), fatty acid chain length and saponification value. To the best of our knowledge, no information is available in the literature on the usefulness of solubility parameter, required HLB (RHLB), and required chemical type of emulsifiers or solubilization capacity for solubilizing vehicles as criterion for the selection of surfactant/cosurfactant in the formulation development of SNEDDS using Fenofibrate or Atorvastatin Calcium. The present study showed the importance of selecting a surfactant with the proper HLB for specific oils, as well as the type of surfactant/co-surfactant. The solubility parameter (δ) of Fenofibrate and Atorvastatin Calcium are closest to the solubility parameter (δ) of Capmul MCM. Blend of better surfactant/co-surfactant was obtained when surfactant and co-surfactant at higher and lower HLB level respectively were blended. The greater the difference between the hydrophilic and lipophilic surfactants, the better the coverage by blends at the interface. The study showed the importance of the structural similarities between the lipophilic tails of the surfactant blends. The pseudo ternary phase diagrams for mixtures of Capmul MCM oil with non-ionic surfactant/co-surfactant and water were constructed. The micelles have potential applications, advantages, and usefulness in the pharmaceutical industry as SNEDDS by various routes of administration, as well as in cosmetics and personal care products.

To the best of our knowledge, no information is available in the literature on the improvement of Fenofibrate and Atorvastatin Calcium dissolution and bioavailability using mixture of Capmul MCM oil, Cremophor RH 40 and Transcutol-P by SNEDDS methodology. The present research was aimed to explore SNEDDS formulation development using 3^2 factorial design for dissolution improvement compared to marketed formulation of Fenofibrate and Atorvastatin Calcium. The present work also described the formulation development of stable SNEDDS of Fenofibrate and Atorvastatin Calcium using concentration of oil and surfactant/cosurfactant on the basis of preliminary trials. The 3^2 factorial design was employed using concentration of Capmul MCM oil and Cremophor RH 40: Transcutol-P

(3:1) as independent variables. The Globule size, Polydispersity index, Zeta potential and drug release at 15 minutes for Fenofibrate and Atorvastatin calcium were selected as dependent variables. The optimized batch was selected on the basis of arbitrary criteria using Design Expert software employing overlay plot with desirability approach. The SNEDDS formulations were evaluated for their physico-chemical parameters such as globule size, zeta potential, polydispersity index, drug release profile and physico-chemical stability. The composition of optimized formulation consisted of Capmul MCM Oil as oil, Cremophor RH 40 as surfactant and Transcutol-P as cosurfactant, containing 10mg of Atorvastatin and 45mg of Fenofibrate showing drug release for liquid SNEDDS formulation (>95%), globule size (78.3nm), Zeta potential (-23.13), and infinite dilution capability. *In-vitro* drug release of the optimized batch was highly significant ($p<0.05$) as compared to marketed conventional capsule (Fenostat) of Fenofibrate and Atorvastatin Calcium.

Keywords:

Solubility Parameter (δ), Pseudo ternary phase diagram, Capmul MCM oil, non-ionic surfactant/co-surfactant, SNEDDS, Nanoemulsion.

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This research work is a synergistic product of several individuals. It is not a chronology of events, but an assembly of ideas at work. This thesis had its own set of challenges, therefore this is the time to say sincere thanks to all those who have, in some way or the other helped me to sail through.

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Milan Dhirajlal Limbani

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LIST OF ABBREVIATION

ANOVA	Analysis of Variance
BCS	Biopharmaceutics Classification System
GI	Gastrointestinal
GRAS	Generally Recognized As Safe
HLB	Hydrophilic-Lipophilic Balance
o/w	Oil in Water
PDI	Polydispersity Index
GS	Globule Size
ZP	Zeta Potential
PEG	Polyethylene Glycol
PG	Propylene Glycol
SEDDS	Self-Emulsifying Drug Delivery System
SMEDDS	Self-Microemulsifying Drug Delivery System
SNEDDS	Self-Nanoemulsifying Drug Delivery System
AUC	Area Under Curve
LDL	Low Density Lipid
HDL	High Density Lipid
Apo B	Apo Lipoprotein B
VLDL	Very Low Density Lipid
MW	Molecular weight
BS	Bile Salts
CH	Cholesterol
PL	Phospholipids
CHD	Coronary Heart Disease
MI	Myocardial Infraction
ACS	Acute Coronary Syndrome
FTIR	Fourier transform infrared
DSC	Differential Scanning Calorimetry
XRD	X-ray Diffraction

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CHAPTER – 1

Aim and objectives

1. Aim & Objectives

1.1 Aim

Increasing number of newly discovered chemical entities have poor water solubility and hence it shows low absorption. Technology Catalysts International reported in 2002 that estimates up to 35-40% of all new chemical entities exhibited poor aqueous solubility [1]. One of the many challenges in development of such drugs will be the development of appropriate drug formulations or drug delivery systems with enhanced drug solubility characteristics. The role of solubility enhancement is to attempt to shift the BCS classification of a drug (II→I) in order to eliminate the problems associated with dissolution-limited compounds [2]. Several strategies to improve the solubility and dissolution of poorly water soluble drugs have been developed and described in literature, which were at start based on modifying their physicochemical properties. Particle size reduction and salt formation became frequently taken paths in a quest for dissolution improvement, but both methods revealed limitations [3].

Self Nanoemulsifying Drug Delivery System (SNEDDS) can be used to enhance solubility of poorly water soluble drugs. It provides benefits to BCS Class II and IV drugs, which exhibits poor aqueous solubility and also for drugs having a log P value greater than 2. SNEDDS possess many advantages over other colloidal drug carriers and extensive research relative to their production characterization and efficacy carried out now-a-days excellently and efficiently. These are successful examples of product launched in to market by using SNEDDS formulation.

Fenofibrate decreases elevated serum total and LDL-cholesterol, triglyceride, and Apo lipoprotein B concentrations. It is used to increase HDL-cholesterol concentration in the management of primary hypercholesterolemia and mixed dyslipidemia, including heterozygous familial hypercholesterolemia and other causes of hypercholesterolemia [4]. It exhibits additive antilipidemic effect when used concomitantly with other antilipidemic agents. Fenofibrate shows bioavailability problems due to poor water and physiological fluids solubility (Practically insoluble in water, BCS Class-II drugs). Fenofibrate shows increase in absorption in fed condition of patient compare to fasting condition of patient.

Atorvastatin inhibits HMG-CoA reductase, causing subsequent reduction in hepatic cholesterol synthesis. It reduces serum concentrations of total cholesterol, LDL-cholesterol, VLDL-cholesterol, apo B, and triglycerides [5]. Atorvastatin shows low aqueous solubility and it is rapidly absorbed after oral administration. Food decreases the rate and extent of its absorption about 25% and 9% respectively.

The clinical guideline indicated that not only fibrate therapy, but also a combination therapy with fenofibrate and a statin should be the most effective means of cholesterol and lipid management. The atorvastatin/fenofibrate fixed-combination preparation reported the excellent results rather than use of single drug [6]. Such combination therapy can only be achieved by the use of two separate products, i.e. the patient needs to take one fenofibrate tablet together with another tablet or capsule containing a statin [6]. Hence, SNEDDS formulation were considered for enhance solubility, release rate and oral absorption of poorly soluble antilipidemic drugs. Since the first step in oral absorption process is dissolution of the drug compound in gastrointestinal lumen contents, poor aqueous solubility is rapidly becoming the leading hurdle for formulation scientist working on oral delivery of such drug compounds.

Childhood dyslipidemia is recognized as a vital risk issue for adult cardiovascular disease. American Academy of Paediatrics published a clinical report concerning prevention, screening, diagnosis and treatment of dyslipidemia in children [7]. Difficulty in swallowing – dysphagia – has been diagnosed in 35% of people aged over 50 and frequently appears after stroke and in older people with dementia, Parkinson’s disease and

many other conditions. So, liquid SNEDDS of Fenofibrate and Atorvastatin Calcium seem more convenient to paediatric patients and geriatric patients.

There is a need for developing a formulation of Fenofibrate and Atorvastatin Calcium, which may provide improved drug dissolution with minimum variation in bioavailability of Fenofibrate and Atorvastatin Calcium. SNEDDS formulations are known to reduce inter- and intra-individual variations in bioavailability, which is believed to be caused by a decreased sensitivity of formulation performance to pre-absorptive solubilisation and dietary status [8]. Hence, present research was thought to explore preparation of SNEDDS for combination of Fenofibrate and Atorvastatin Calcium to enhance dissolution which ultimately provides improved bioavailability.

The aim of present work was to prepare stable formulations of Fenofibrate and Atorvastatin Calcium which may improve dissolution profile of drugs and ultimately enhance the bioavailability as compared to conventional marketed formulation.

1.2 Objectives

The objectives of the present work were-

- To develop stable formulations for self-Nanoemulsifying drug delivery system (SNEDDS) in order to enhance solubility and release rate of poorly soluble Antilipidemic drugs (Fenofibrate and Atorvastatin Calcium).
- In silico prediction of drugs solubility in lipid vehicle using several factors like solubility parameter (δ), HLB value, partition coefficient, relative molecular mass (MW), fatty acid chain length, solubilization capacity.
- To select most suitable vehicle and perceive role of lipid vehicle in pseudo ternary phase diagram behaviour to find nanoemulsion area in SNEDDS containing Fenofibrate and Atorvastatin Calcium.
- To study physico-chemical properties of Self Nano Emulsifying Drug Delivery System (SNEDDS).
- To study effect of dilution of SNEDDS formulation that formation of spontaneous curvature of surfactant layer changes.

- To study in-vitro drug release profile by using a suitable in-vitro dissolution model.
- To study in-vitro drug diffusion profile by using a suitable in-vitro diffusion model.
- To carry out stability study of selected SNEDDS formulation as per ICH guidelines.

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CHAPTER – 2

Introduction

2. Introduction

2.1 Introduction

Increasing number of newly discovered chemical entities have poor aqueous solubility and hence it shows low absorption. Technology Catalysts International reported in 2002 that estimates up to 35-40% of all new chemical entities exhibited poor water solubility [1]. The properties of new drug substances shifted towards higher molecular mass and increasing lipophilicity of drug and decreasing the aqueous solubility. Fenofibrate, Atorvastatin & Pitavastatin are example of such a compound suffering from lower aqueous solubility and poor bioavailability [2, 3]. Various methods to enhance the solubility and dissolution of poorly water soluble drugs have been developed and described in literature, which were at start based on modifying their physico-chemical properties. Salt formation and reduction in particle size and became often taken methods in a quest for dissolution improvement, but both methods had limitations [4, 5]. As a result, altering drug solubility or dissolution through formulation approaches has become most popular. This encouraged the development of various alternative formulation strategies including use of lipid formulations. Strategies to enhance drug bioavailability may involve altering of various key factors that determine drug dissolution, as described by Noyes-Whitney equation [6].

The drug dissolution rate can be enhanced by enlarging surface area from where dissolution can take place, and by altering solubility of drug. Lipid formulations and in

particular Self-Nano Emulsifying Drug Delivery Systems can induce increase in drug dissolution rate as these strategies can simultaneously alter several of these factors [7-10]. For drug substances which have low aqueous solubility but sufficient lipophilic properties, it will be beneficial to dose them in a predissolved state, e.g. in a lipid formulation [11-14], thereby reducing the energy associated with a solid-liquid phase transition and overcoming the slow dissolution process after oral intake. Lipid formulations are lipid solution, emulsion, microemulsion, and SNEDDS [15-17]. Pouton proposed the simple classification system for lipid formulations, based on the polarity of the blend as shown in Table 2.1 [18, 19].

This classification helps to better understand fate of various lipid formulation in vivo, it also helps to use a systematic & rational formulation approach avoid "trials and errors" iterations and provide framework to guide regulatory agencies. The lipid formulation classification system briefly classifies lipid based formulation into four types according to their composition and the possible effect of dilution and digestion on their ability to prevent drug precipitation.

Table 2.1: Typical Composition of Lipid-based formulations

Composition	**Type- I**	**Type- II**	**Type- III**		**Type- IV**
			III-A	**III-B**	
	Oils	**SEDDS**	**SEDDS**	**SMEDDS**	**Oil Free**
Mono, di- and tri-glycerides	100%	40-80%	40-0%	< 20%	-
Surfactants (HLB < 12)	-	20-60%	-	-	0-20%
Surfactants (HLB > 12)	-	-	20-40%	20-50%	20-80%
Water soluble surfactants and co-solvents	-	-	0-40%	20-50%	0-80%
Globule size of dispersion (nm)	Coarse	100-250	100-250	50-100	< 50

The lipid formulation is a lipid solution, classified as a Type I formulation. These systems shows poor initial aqueous dispersion and require digestion by pancreatic lipase/co-lipase in the GIT to generate more amphiphilic lipid digestion products and enhance drug transfer into the colloidal aqueous phase. Type I lipid formulations represent a relatively

simple formulation option for potent drugs or highly lipophilic compounds where drug is soluble in oil sufficiently. Nonetheless these formulations are highly dependent on digestion process and suffer from less solvent capacity. Unless the drug is sufficiently lipophilic (log P>4), formulation as an oil solution is limited to highly potent compounds. In case of type II and III formulations, solvent capacity can be enhanced by adding surface active agents.

Lipid formulation comprising hydrophilic surfactants and represented by class III, shows self-emulsifying properties.

Table 2.2: Typical properties of Type I, II, III and IV Lipid formulations

Type	Materials	Characteristics	Advantages	Disadvantages
Type-I	Oils without surfactants (e.g. Mono, di, and triglycerides)	Non dispersing requires digestion	Generally recognized as safe status, simple, capsule compatibility	Formulation has poor solvent capacity unless drug is highly lipophilic
Type- II	Oils and water insoluble surfactants	SEDDS formed without water soluble components	Unlikely to lose solvent capacity on dispersion	Turbid o/w dispersion (Particle size 0.25-2µm)
Type-III	Oils, surfactants, cosolvents (both water insoluble and soluble excipients)	SEDDS/ SMEDDS formed with water soluble components	Clear or almost clear dispersion, Drug absorption without digestion	Possible loss of solvent capacity on dispersion, less easily digested
Type-IV	Water soluble surfactants and cosolvents (No oils)	Formulation disperses typically to form a micellar solution	Formulation has good solvent capacity for many drugs	Likely loss of solvent on dispersion, may not be digestible

Type II lipid formulations constitute SEDDS. Self-emulsification is generally obtained at surfactant contents above 25% (w/w). However at higher surfactant contents (>50–60 %w/w) progress of emulsification may be compromised by formation of viscous liquid

crystalline gels at the oil/water interface. Type II lipid formulations provide benefit of overcoming slow dissolution step typically observed with solid dosage forms and as described above generate large interfacial areas which in turn allows efficient partitioning of drug between oil droplets and aqueous phase from where absorption occurs.

Type III lipid formulations, commonly referred to as self-microemulsifying drug delivery systems, are defined by inclusion of hydrophilic surfactants (HLB>12) and co-solvents. Type III formulations can be further segregated into Type III-A and Type III-B formulations in order to identify more hydrophilic systems (Type III-B) where the content of hydrophilic surfactants and co-solvents increases and lipid content reduces. Type III-B formulations typically achieve greater dispersion rates when compared with Type III-A although risk of drug precipitation on dispersion of formulation is higher given lower lipid content [19].

Type IV formulations do not contain natural lipids and represent most hydrophilic formulations. These formulations commonly offer increased drug payloads when compared to formulations comprising simple glyceride lipids and also produce very fine dispersions when introduced in aqueous media. Little is known however, as to the solubilisation capacity of these systems *in vivo* and in particular whether they are equally capable of maintaining poorly water soluble drug in solution during passage along the GIT when compared with formulations containing natural oils (Type II and Type III). An example of a Type IV formulation is current capsule formulation of the HIV protease inhibitor amprenavir (Agenerase) which contains TPGS as a surfactant and PEG 400 and propylene glycol as co-solvents [20].

Some examples of marketed pharmaceutical SEDDS formulations are as shown in Table 2.3.

Table 2.3: Examples of Marketed SEDDS formulations

Drug Name	Active Compound	Indication	Dosage form	Company
Sandimmune	Cyclosporine A/ II	Immune suppressant	Soft gelatin capsules	Novartis
Neoral	Cyclosporine A/ I	Immune suppressant	Soft gelatin capsules	Novartis
Norvir	Ritonavir	HIV antiviral	Soft gelatin capsules	Abbott Laboratories

Lipirex	Fenofibrate	Antihyperlipoproteinemic	Hard gelatin capsules	Genus
Convulex	Valproic acid	Antiepileptic	Soft gelatin capsules	Pharmacia

2.2 Self-Nanoemulsifying Drug Delivery System (SNEDDS)

SNEDDS is isotropic mixture comprising of oil, surfactant and sometimes co-solvent or co-surfactant. In an aqueous environment a homogeneous, transparent (or at least translucent), isotropic and thermodynamically stable dispersion will result, the formation of which is improved by gentle agitation, *in vivo* provided by gastrointestinal motility [21-23]. When compared with emulsions, which are sensitive and metastable dispersed forms, SNEDDS is physically stable formulations that is easy to manufacture. For lipophilic drug compounds which exhibit dissolution rate-limited absorption, SNEDDS may offer an improvement in rate and extent of absorption and result in more reproducible blood-time profiles. SNEDDS formulations are known to reduce inter- and intra-individual variations in bioavailability, which is believed to be caused by a decreased sensitivity of formulation performance to pre-absorptive solubilisation and dietary status.

There are several reasons why SNEDDS is not common in use at present, contrary to the fact that popularity of SNEDDS formulations has increased over the last years. Some relate the lack of commercial formulation to the custom of pharmaceutical development laboratories while others hold the limited knowledge of its physico-chemical property. Knowledge of emulsification process, efficiency of emulsification and susceptibility of the formulation to digestion is desirable for formulation of SMEDDS and lipid formulation [18].

2.2.1 Advantages of SMEDDS/SNEDDS

(i) Improvement in oral bioavailability

Dissolution rate dependant absorption is a major factor that limits the bioavailability of several poorly water soluble drugs. The ability of SNEDDS to present the drug to GIT in solubilised and nano emulsified form (droplet size between 1-100 nm) and subsequent increase in specific surface area enable more efficient drug transport through intestinal

aqueous boundary layer and through absorptive membrane leading to improved bioavailability [24].

(ii) Decreased in inter- subject and intra-subject variability and food effects

There are various drugs which show large inter-subject and intra-subject variation in absorption lead to decreased performance of drug and patient non-compliance. Food is a major factor affecting therapeutic performance of drug in body. SNEDDS are a boon for such drugs. Several research papers specifying that, performance of SNEDDS is independent of food and SNEDDS offer reproducibility of plasma profile are available [25].

(iii) Ability to deliver peptides that are prone to enzymatic hydrolysis in GIT

One unique property that makes SNEDDS superior as compared to other drug delivery systems is their ability to deliver macromolecules like hormones, peptides, enzyme substrates and inhibitors and their ability to offer protection from enzymatic hydrolysis. The intestinal hydrolysis of prodrug by cholinesterase can be protected if polysorbate-20 is emulsifier in microemulsion formulation [26]. These systems are formed spontaneously without aid of energy or heating thus suitable for thermo labile drugs such as peptides [27].

(iv) Ease of manufacture and scale-up

Ease of manufacture and scale-up is one of most important advantage that makes SNEDDS unique when compared to other drug delivery systems like solid dispersions, liposomes, nanoparticles, etc., dealing with improvement of bio-availability. SNEDDS require very simple and economical manufacturing facilities like simple mixer with agitator and volumetric liquid filling equipment for large-scale manufacturing. [24]

(v) Increased drug loading capacity

SMEDDS provides advantage of increased drug loading capacity when compared with conventional lipid solution as solubility of poorly water soluble drugs with intermediate partition coefficient (2<log P>4) are typically low in natural lipids and much greater in amphilic surfactants, co surfactants and co-solvents [27].

(vi) No influence of lipid digestion process

Unlike the other lipid-based drug delivery systems, the performance of SNEDDS is not influenced by lipolysis, emulsification by bile salts, action of pancreatic lipases and mixed micelle formation [27].

2.2.2 The emulsification process

Self-emulsification is a phenomenon which has been widely exploited commercially in formulations of emulsifiable concentrates of pesticides and herbicides.. In contrast, SNEDDS, using excipients acceptable for oral administration to humans, have not been widely exploited and knowledge about their physico-chemical principles is therefore limited.

(i) Mechanisms of emulsification

According to Reiss, self-emulsification occurs when the entropy change that favours dispersion is greater than the energy required to increase the interfacial area [28, 29]. The work required (W) associated with an increase of the surface area of the dispersion can be determined by

$$W = \Delta A . \gamma$$

Where, ΔA is the increase in the total interfacial area and γ is the interfacial tension. In the case of self-emulsifying systems, the Gibbs free energy associated with the emulsification process is negative given that these systems are characterized by a ultralow oil/water interfacial tension, which greatly facilitates the formation of the large interfacial area and a high entropy of mixing.

(ii) Dilution phases

Upon dilution of a SMEDDS formulation, the spontaneous curvature of the surfactant layer changes via a number of possible liquid crystalline phases. The droplet structure can pass from a reversed spherical droplet to a reversed rod-shaped droplet, cubic phase, lamellar phase, hexagonal phase and several other structures until, after appropriate dilution, a spherical droplet will be formed again (Figure 2.1).

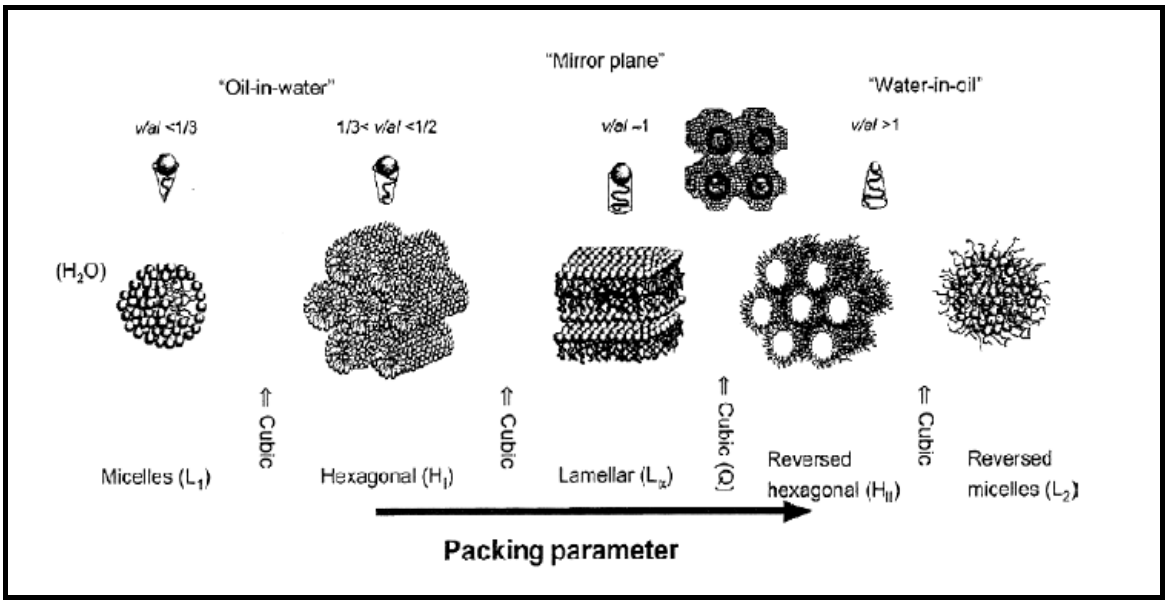

Figure 2.1 Most commonly encountered phases upon addition of water to an oil-surfactant combination

Many roles have been attributed to the occurrence of liquid crystalline phases upon aqueous dilution of lipid formulation.

2.2.3 The efficiency of emulsification

The efficiency of self-emulsification process is correlated to time necessary for a self-emulsifying formulation to form a stable microemulsion upon addition of water [17]. The globule size of a diluted SNEDDS formulation is measured as a means to estimate formulation performance, since it determines the surface area for dissolution and hence rate and extent of drug release. The size of the oil globule should be very small (less than 150nm), which can be facilitated by a low interfacial tension at the oil-water interface whereas the interfacial layer is kept more flexible and fluid [11, 15, 16, 21, 30, 31].

2.2.4 Susceptibility to digestion

The positive effect of food on bioavailability of many poorly water soluble drugs is often ascribed to the ingested lipid and points to the beneficial role of lipids on drug absorption. The presence of lipids in the GIT increases drug solubilisation and thus drug dissolution via a number of potential mechanisms [32-34].

- An increased secretion of bile salts and endogenous biliary lipids

- An intercalation of administered lipids into bile salt structures, directly or after digestion
- A reduced gastric transit time, resulting in an increased dissolution time
- Changes of the physical and biochemical barrier function of the intestinal tract. Several lipid digestion products and surfactants show permeability enhancing properties and/or alternate the activity of intestinal efflux transporters.

2.2.5 Factors Affecting SNEDDS

(i) Polarity of lipophilic phase

The polarity of globule is governed by the HLB, the chain length and degree of unsaturation of the fatty acid, the molecular mass of micronized for their propensity to inhibit crystallization and, thereby, generate and maintain the supersaturated state for prolonged time periods [23]. A super-saturable self-microemulsifying drug delivery system of paclitaxel was developed employing HPMC as a precipitation inhibitor with a conventional SMEDDS formulation. In-vitro dilution of SMEDDS formulation resulted in formation of a microemulsion, followed by slow crystallization of paclitaxel on standing. This result indicated that the system was supersaturated with respect to crystalline paclitaxel, and the supersaturated state was prolonged by HPMC in the formulation. In the absence of HPMC, the SMEDDS formulation underwent rapid precipitation, yielding a low paclitaxel solution concentration. A pharmacokinetic study showed that the paclitaxel SMEDDS formulation produced approximately a 10-fold higher maximum concentration (Cmax) and a 5-fold higher oral bioavailability (F~9.5%) compared with that of orally administered Taxol formulation (F~2.0%) and the SMEDDS formulation without HPMC (F~1%) [23].

(ii) Nature and dose of drug

The very high dose drugs are not suitable for SNEDDS unless they exhibit extremely good solubility in at least one of the components of SNEDDS, preferably lipophilic phase. The drugs which exhibit limited solubility in water and lipids (typically with log P values of approx. 2) are most difficult to deliver by SNEDDS. The ability of SNEDDS to maintain the drug in solubilised form is greatly influenced by the solubility of the drug in oil phase. Pouton‘s study reveal that such formulations can take up to five days to reach equilibrium and that drug can remain in a super-saturated state for up to 24 hours after the initial

emulsification event. It could thus be argued that such products are not likely to cause precipitation of drug in the gut before drug is absorbed, and indeed that super-saturation could actually enhance absorption by increasing the thermodynamic activity of the drug. There is a clear need for practical methods to predict the fate of drugs after the dispersion of lipid systems in GIT [23].

2.2.6 Biopharmaceutical Aspects

The ability of food to enhance the bioavailability of poorly water-soluble drugs is well known. Although incompletely understood, the currently accepted view is that lipids may enhance bioavailability via a number of potential mechanisms, including.

(i) Increases in effective luminal drug solubility. The presence of lipids in the GIT stimulates an increase in secretion of bile salts and endogenous biliary lipids including phospholipids and cholesterol, leading to the formation of BS/PL/CH intestinal mixed micelles and an increase in solubilization capacity of GIT [35].

(ii) Alterations (reduction) in gastric transit, thereby slowing delivery to the absorption site and increasing time available for dissolution [35].

(iii) Changes in the biochemical barrier function of GIT. It is clear that certain lipids and surfactants may attenuate the activity of intestinal efflux transporters, as indicated by the p-glycoprotein efflux pump, and thus reduce the extent of enterocyte-based metabolism.

(iv) Stimulation of intestinal lymphatic transport. For highly lipophilic drugs, lipids may enhance the extent of lymphatic transport and increase bioavailability directly or indirectly via a reduction in first-pass metabolism. A hydrophilic drug is less likely to be absorbed through the lymphatic and instead may diffuse directly in to the portal supply. Hence in this case, increased dissolution from the large surface area afforded by emulsion may be a contributing factor to enhanced absorption of drugs [35].

2.3 Why Combination of drugs is required?

Fenofibrate decreases elevated serum total and LDL-cholesterol, triglyceride, and Apo lipoprotein-B concentrations. It is used to increase HDL-cholesterol concentration in the management of mixed dyslipidemia and primary hypercholesterolemia, including heterozygous familial hypercholesterolemia and other causes of hypercholesterolemia [36]. It exhibits additive antilipidemic effect when used concomitantly with other

antilipidemic agents. Fenofibrate shows bioavailability problems due to poor water and physiological fluids solubility (Practically insoluble in water, BCS Class-II drugs). Fenofibrate shows increase in absorption in fed condition of patient compare to fasting condition of patient [36].

Atorvastatin inhibits HMG-CoA reductase, causing subsequent reduction in hepatic cholesterol synthesis. It reduces serum concentrations of total cholesterol, LDL-cholesterol, VLDL-cholesterol, apo B, and triglycerides [37]. Atorvastatin shows low aqueous solubility and it is rapidly absorbed after oral administration. Food decreases the rate and extent of oral absorption about 25% and 9% respectively.

The clinical guideline indicated that combination therapy with fenofibrate and statin should be the most effective means of cholesterol and lipid management. The atorvastatin/fenofibrate fixed-combination preparation reported the excellent results rather than use of single drug [38]. Atorvastatin/fenofibrate combination therapy can only be achieved by the use of two separate products, i.e. the patient needs to take one Atorvastatin tablet together with another tablet or capsule containing a fibrate [38].

Childhood dyslipidemia is recognized as a vital risk issue for adult cardiovascular disease. Due to increased awareness surrounding this problem, American Academy of Paediatrics published a clinical report concerning prevention, screening, diagnosis, and treatment of dyslipidemia in children [39]. Difficulty in swallowing – dysphagia – has been diagnosed in 35% of people aged over 50 and frequently appears after stroke and in older people with dementia, Parkinson’s disease and many other conditions. So, liquid SNEDDS of Fenofibrate and Atorvastatin Calcium are more convenient to paediatric patients and geriatric patients.

2.4 Need for Formulating SNEDDS

There are several solubility enhancement technology reported in literature and several extensive reviews and books assessing poor water-solubility considerations.
Basically, drug can be poorly soluble in aqueous media or simultaneously in aqueous and organic media. In first case, a number of formulation approaches are available for saturation solubility enhancement such as specific or non-specific complexation (solid

dispersions in polymers or use of cyclodextrin) and solubilization techniques (solvent-mixtures, etc.). However, for drugs those are poorly soluble in both aqueous and organic media, most of these approaches are of limited success, if not unusable, as they rely on the use of solvents for formulation development, and other alternatives have to be used in order to lead to a dissolution rate increase (salt formation, reduction in particle size, prodrug, etc.). According to the Noyes-Whitney equation, out of the many ways to increase the dissolution rate of a poorly water-soluble drug, increasing drug specific surface area by particle size reduction has a long history and has been extensively studied for over 30 years [40].

Many studies had been conducted to signify the influence of drug particle size reduction on several pharmacological parameters (urinary excretion, plasma concentrations, etc.) and a positive influence could be observed for drugs such as procaine, spironolactone, tolbutamide and sulfadiazine. A common method for increasing the dissolution rate of poorly water soluble compounds, and eventually the oral bioavailability (BCS class-II drugs), through reduction in globule size. Micronized drug powders are fine powders with average particle size that is typically in range of 2-5μm and that have an overall particle size distribution ranging from 0.1 to 25μm. Many drugs such as valproic acid, ritonavir, and cyclosporine-A have been shown to have their dissolution and bioavailability enhanced following decreasing globule size (SNEDDS or nanoemulsion) when compared to the bulk drug [41].

Fibrates drugs have generally low aqueous solubility, poorly and variably absorbed after oral administration. Generally they are prescribed to be taken with food in order to enhance the bioavailability. Fenofibrate is insoluble in water. Atorvastatin calcium is very slightly soluble in distilled water, pH 7.4 phosphate buffers, acetonitrile, slightly soluble in ethanol and freely soluble in methanol. Statins are drug substances that generally have low aqueous solubility, rapidly absorbed after oral administration. Food decreases the rate and extent of oral absorption about 25% and 9%, respectively.

Fenofibrate and Atorvastatin calcium are class II compound which means that its oral bioavailability is restricted by the dissolution rate in the GIT. Drug release from the dosage form is the rate limiting step in the absorption process while permeability is sufficient. Class II drugs are considered the best candidates for intervention by formulation

e.g. in self-emulsifying dosage forms. The lipophilicity of Fenofibrate and Atorvastatin Calcium indicates the potential utility of a SNEDDS formulation for enhancing drug dissolution.

There is need for developing a single formulation contains statin and fibrate as active components, which is stable and provides desired biopharmaceuticals properties to the active substances (e.g. suitable bioavailability, less dependency on food intake, etc.), and which can be easily manufactured at commercial scale. Furthermore, there is need for developing formulation comprising fibrate and statin, which can be processed into pharmaceutical dosage form with high degree of flexibility of choosing the particular kind of dosage form. In addition, there is still a need for formulation that has suitable bioavailability that can substantially reduce or overcome the differential between bioavailability of drug (in particular relevant for fenofibrate) in patients. Furthermore, there is also need for formulation that enables reduction on observed side effects.

Although SNEDDS of drugs effectively leads to an increase in surface area. For drugs that present very low water solubility, this increase in surface area and the eventual corresponding dissolution rate enhancement might not lead to a sufficiently high oral bioavailability. A solution for this problem is to further reduction in globule size to the nanometer range i.e. nanosization. SNEDDS have the advantage of having greater surface area, and being characterized, unlike micronized drugs, by an increase in saturation solubility.

The production of SNEDDS shall increase the drug dissolution rate by means of:

(i) Increase of saturation solubility
(ii) Increase of surface area following globule size reduction
(iii) Increase of the time available for dissolution following inherent adherence characteristics of nanoemulsion to the GI wall (high specific surface area being indicative of a high interactive potential with biological surface).

SNEDDS can be used to increasing drugs solubility that are poorly water soluble. These specific advantages make it a unique dosage form. It is also a simple strategy that has several advantages over other techniques. It provides benefits to BCS Class II, III and IV drugs, which exhibit low aqueous and also for drugs having a log P value greater than 2

[42, 43]. SNEDDS have many advantages over other colloidal drug carriers and extensive research relative to their production, characterization and efficacy is carried out now-a-days. Lipidic systems, such as emulsion and liposomes, can be used for compounds that are water insoluble and soluble in oil (with high log P).

Traditional approaches often attempt to solubilize insoluble drugs using an excessive amount of co-solvents, but this may poses toxicity problems. The need to administer very large doses of drugs must then be accomplished without the interference of toxic effects caused by co-solvents. SNEDDS have reveled their potential to tackle the problem associated with the delivery of poorly water-soluble and are unique because of their simplicity and the disadvantages they confer over other strategies. Anti-cancer, immunosuppressant, anti-infective, lipid-lowering agents, anti-emetic, anti-asthmatic drugs, as well as vaccine adjuvants may be formulated as SNEDDS. Thus, there is a need for developing a formulation of Fenofibrate and Atorvastatin Calcium, which may provide improved drug dissolution with minimum variation in bioavailability of Fenofibrate and Atorvastatin Calcium. SNEDDS formulations are known to reduce inter- and intra-individual variations in oral bioavailability, which is believed to be caused by decreased sensitivity of formulation performance to pre-absorptive solubilisation and dietary status [23].

2.5 SNEDDS components

2.5.1 Oil

Lipid edibility is a vital determinant of the ability of a lipid to increase hydrophobic drug absorption. Undigested lipids don’t seem to be solely ineffective at promoting drug absorption but have even been reported to inhibit the method presumably by providing a non-absorbable and lipophilic reservoir from which drug release cannot efficiently occur [44]. An outline of lipid phases used in this research is presented below:

Castor oil is a vegetable oil obtained by pressing the seeds of the castor oil plant (*Ricinus communis*). Castor oil is a colourless to very pale yellow liquid with a distinct taste and odour once first ingested. Castor oil is famous as a source of ricinoleic acid, a

monounsaturated, 18-carbon fatty acid. Its boiling point is 313°C (595°F) and its density is 961 kg/m^3.

Labrafac PG (Propylene glycol dicaprylocaprate) is oily lipid vehicle for use in self-emulsifying formulations to obtain a coarse dispersion i.e. SEDDS or SMEDDS. HLB Value is 1.

Oleic acid is a fatty acid that occurs naturally in various animal and vegetable fats and oils. It is an odourless, colourless oil. Oleic acid is classified as a monounsaturated omega-9 fatty acid. Its boiling point is 360°C and its density is 895 kg/m^3.

Capmul MCM Oil (Glyceryl Caprylate/Caprate) is a mono-diglyceride of medium chain fatty acids (mainly capric and caprylic). It is an excellent solvent for many organic compounds, including steroids. It is also a useful emulsifier for w/o emulsion. Capmul MCM have an HLB value of about 5.5 to 6.

Light Liquid Paraffin Oil is highly purified mixture of liquid saturated hydrocarbons obtained from petroleum and is highly paraffinic in nature. Light Liquid Paraffin oil is transparent, and free from fluorescence in daylight. It is colourless, tasteless, and odourless when cold. Light liquid paraffin oil highly refined hydro-treated oil has excellent thermal & chemical stability, having high flash point and is soluble in Chloroform and solvent Ether.

2.5.2 Surface active agent (Surfactant)

Surfactants have ability to penetrate biological membranes (so referred as hydrophobic surfactant) or solubilise membrane components (hydrophilic surfactants). In general, toxicity of surfactants decreases from cationic to anionic surfactants, which successively are less tolerated than non-ionic surface active agents. For immediate formation of o/w emulsion and/or rapid spreading of the SNEDDS formulations in an aqueous environment, high HLB surfactants are preferred. Moreover, the solubilising capacity of surfactants is of prime importance for preventing precipitation of the drug within the GI lumen which is vital for effective absorption. An outline of surfactants used in this research is described below:

In this research project, Tween-80, Span-20, Labrafac Lipophile WL 1349 (triglyceride of caprylic/capric acid), Cremophor EL (Polyoxyl 35 castor oil), Labrasol (saturated C8-C10 polyglycolysed glycerides), Kolliphor RH 40 (polyoxyl 40 Castor Oil), Capmul GMO-50 (Glyceryl Monooleate), Captex 355 (Glycerol Tricaprylate/Caprate) are used as surfactant for SMEDDS/SNEDDS formulations.

Tween 80 or polyoxyethylene 20 sorbitan monooleate is an ester of sorbitan polyethoxylate. It belongs to a series of surface active compounds, which differ mainly in the fatty acid chain length. The HLB value of Tween 80 is about 15.

Span 20 or Sorbitan monododecanoate is an ester of sorbitan (a sorbitol derivative) and stearic acid. It is amber to brown oily liquid. Non-toxic and odourless. Span 20 is slightly soluble in isopropanol, xylene, cotton seed oil and mineral oil, slightly soluble in liquid paraffin, The HLB value is about 8.6.

Labrafac Lipophile WL1349 (triglyceride of caprylic/capric acid) is medium chain fatty acid triglyceride. It is oily lipid vehicle or oily phase for use SEDDS or SMEDDS. The HLB value is about 1-2.

Labrasol (Caprylocaproyl macrogol-8 glycerides) is non-ionic water dispersible surfactant composed of well-characterised polyethylene glycol esters, a small glyceride fraction and free PEG. It also able to self-emulsify on contact with aqueous media forming SMEDDS. The HLB value is about 12.

Cremophor RH 40 is non-ionic water dispersible surfactant composed of macroglycerol hydroxystearate 40. It also able to self-emulsify on contact with aqueous media forming SMEDDS. The HLB value is about 14-16.

Capmul GMO-50 (Glyceryl Monooleate) is a mixture of monoglycerides, mainly glyceryl monooleate, together with variable quantities of diglycerides and triglycerides. It is obtained by esterification of glycerol with food-grade oleic acid of vegetable origin, or by partial glycerolysis of vegetable oil that consists mainly of triglycerides of oleic acid. The HLB value is about 3-4.

Captex 355 is manufactured by the esterification of caprylic/capric fatty acids and glycerin. It contains not less than 95% of saturated fatty acids with 8 and 10 carbon atoms. The HLB value is about 3-4.

2.5.3 Co-solvent (Co-surfactant)

Organic solvent, appropriate for oral administration may help to solubilize large amounts of surfactants or drug within the lipid base. Furthermore cosolvent increase flexibility of the surfactant layer and enhance the emulsification process given that disruption of the oil-water interface can be caused by diffusion of cosolvent away from the formulation. Nonetheless, the importance of the latter two phenomena *in-vivo* is commonly criticised since these compounds would dissolve quickly into the aqueous medium.

In this research, PEG-400, Propylene Glycol, Transcutol-P, Acconon MCS-2 are used as a co-surfactant for SMEDDS/SNEDDS formulations.

PEG 400 (polyethylene glycol 400) is a low-molecular-weight grade of polyethylene glycol. It is a clear, colourless, viscous liquid. Due in part to its low toxicity, PEG 400 is widely used in a variety of pharmaceutical formulations. The HLB value is about 11-13.

Propylene Glycol is an organic compound. It is a viscous colourless liquid which is nearly odourless but possesses a faintly sweet taste. Chemically it is classed as a diol and is miscible with a broad range of solvents, including water, acetone, and chloroform. The HLB value is about 3.4.

Transcutol-P is clear, colourless, viscous liquid. It have a mild, pleasant odour. It is hydroscopic in nature. It is miscible with water and wide range of solvents. The HLB value is about 4.2.

Acconon MCS-2 is non-ionic surfactant which is useful for bioavailability emulsifier, mild surfactant, solubilizer, dispersant and viscosity controlling agent. It is miscible with water and wide range of solvents. The HLB value is about 12.5.

2.6 General Methods for evaluation of SNEDDS

SNEDDS are evaluated for various parameters like Globule size, Polydispersity index, Zeta potential, Infra-Red (IR) study, estimation of drug content, in vitro dissolution study and stability study.

2.6.1 Mean Globule Size and Polydispersity Index

The mean globule size and width of globule size distribution are important characterization parameters as they govern the saturation solubility, dissolution rate, physical stability and even biological performance of SNEDDS. Globule size and polydispersity index (PDI) of SNEDDS were determined using Zetasizer Nano ZS (Malvern Instruments, UK), which follows principle of LASER high diffraction. SNEDDS was added (after suitable dilution) to sample cell and put into the sample holder unit and measurement was carried out with the help of software of same instrument. The PDI is an important parameter that governs the physical stability of SNEDDS and should be as low as possible for the long-term stability of SNEDDS. A PDI value of 0.1–0.25 indicates a fairly narrow size distribution whereas a PDI value greater than 0.5 indicates a very broad distribution. No logarithmic normal distribution can definitely be attributed to such a high PDI value.

2.6.2 Zeta Potential

The determination of the zeta potential of a SNEDDS is essential as it gives an idea about the physical stability of SNEDDS. The zeta potential of a SNEDDS is governed by both stabilizer and drug itself. In order to obtain a SNEDDS exhibiting good stability, for an electrostatically stabilized SNEDDS a minimum zeta potential of ±30mV is required whereas in the case of a combined electrostatic and steric stabilization, a minimum zeta potential of ±20mV is desirable [43].

2.6.3 Infra-Red (IR) study

The compatibility between drug and excipient can be detected by IR study. The spectra is recorded over the wave number range of 4000 to 500 cm^{-1}.

2.6.4 Estimation of Drug Content

Fenofibrate & Atorvastatin Calcium from pre-weighed SNEDDS was extracted by dissolving in methanol. Then methanolic extract was separated out and Fenofibrate & Atorvastatin Calcium content were analysed HPLC Method at 248nm, against standard solution of Fenofibrate & Atorvastatin Calcium.

2.6.5 In-vitro Dissolution study

In vitro drug release studies were carried out for all formulations according to USP dissolution parameters. USP Type II dissolution test apparatus (Electrolab TDT-06P, India). The dissolution medium (900 ml water) was maintained at 37±0.5°C and stirred at 50 rpm. Aliquots were collected periodically and replaced with fresh dissolution medium. Aliquots, after filtration through 0.45µ PVDF filter paper, were analysed by HPLC at 248nm for Fenofibrate & Atorvastatin Calcium content.

2.6.6 Stability study

The stability study of the formulation is carried out according to ICH guidelines at long term condition (25±2ºC/60±5%RH) and accelerated condition (40±2ºC/75±5%RH) for six months by storing the SNEDDS in respective stability chamber.

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CHAPTER – 3

Literature Review

3. Review of Literature

3.1 About Self Nanoemulsifying Drug Delivery System (SNEDDS)

Holmberg, et al., (2010) described an invention of pharmaceutical composition suitable for oral administration, in form of an emulsion pre-concentrate, comprising of drug, one or more surfactants and an oil or semi-solid fat. It formed in-situ oil-in-water emulsion upon contact with aqueous media such as gastrointestinal fluid. The composition may also consist one or more short-chain alcohols. It is useful in the treatment of pain and inflammation [1].

Usmani A. et al., (2019) prepared doxorubicin self nanoemulsifying drug delivery system with *Nigella Sativa* oil against human hepatocellular carcinoma. The developed SNEDDS was evaluated for drug release and in vitro anticancer efficacy in liver cancer (HepG2) cell line. The optimized formulation showed mean particle size of 79.7 nm with PDI 0.098 and the minimum viscosity of 16.42 cps with % transmittance of 1.332 with maximum drug release of 96.9% in 32 hours as compared to DOX alone. Stability data showed stable emulsion in both 25°C and -4°C. The optimized formulation showed improved efficacy in HepG2 cells by cytotoxicity, showed significant results $p<0.05$ with 2.5µg/ml of (inhibitory concentration) IC50 [2].

Vivek Borhade et al., (2012) prepared Clotrimazole nanoemulsion for malaria chemotherapy. Clotrimazole was formulated in nanoemulsion based system for improving

its solubility and dissolution. Preformulation studies were performed to evaluate drug-excipient compatibility, solution state pH stability, and pH solubility profile. Influence of Clotrimazole and pH of dilution medium on phase behaviour were assessed. Drug-excipient chemical compatibility study facilitated to anticipate acid catalysed degradation of Clotrimazole. The pH of nanoemulsion was adjusted to 7.5 for stabilization of Clotrimazole. Nanoemulsion composed of Capryol 90, Solutol HS 15 and Gelucire 44/14 and enhanced solubility of Clotrimazole was achieved up to 25 mg/ml. The nanoemulsion exhibited mean globule size <25 nm, which was not affected by pH of dilution medium. Dissolution profile of Clotrimazole nanoemulsion in various media showed 100% drug release within 15 min irrespective of pH of medium [3].

Sahoo S. et al., (2019) formulated self-nanoemulsifying oil formulation (SNEOFs) of Efavirenz for increasing its solubility as well as in vitro dissolution rate for improvisation of bioavailability. In vitro release profiles of SNEOFs compared with Efavirenz, percent dissolution efficiency (DE) and dissolution half-life (t_{50}) were evaluated. A low percent DE (30.12%) and high t_{50} was obtained for Efavirenz whereas all SNEOFs showed a DE of greater than 78.48% and less than 9 minutes t_{50}. The optimized SNEOFs (F8) demonstrated a significant ($p<0.05$) increase in bioavailability over Efavirenz [4].

Liu C. et al., (2018) prepared self-nanoemulsifying drug delivery systems to improve the oral bioavailability of Tetrandrine. The optimized SNEDDS formulation had average droplet size 19.75±0.37nm and zeta-potential 1.87±0.26mv. The dissolute rate of Tetrandrine SNEDDS in various dissolution media was remarkably faster than Tetrandrine commercial tablet. Moreover, in vivo pharmacokinetic study results showed that significant increase ($p\leq 0.05$) in the peak concentration (Cmax) and the area under the curve (AUC) of Tetrandrine was observed after the oral administration of Tetrandrine SNEDDS and the absorption of Tetrandrine from SNEDDS resulted in approximately 2.33-fold increase in oral bioavailability compared with the commercial tablet [5].

Stuti Vatsraj et al., (2014) prepared a novel nanoemulsion system for enhanced solubility of a sparingly water soluble Clarithromycin. The therapeutically effective concentration of clarithromycin, 5mg/mL, was achieved using polysorbate 80 combined with olive oil as lipophilic counter ion. A three-level three-factorial central composite experimental design was used. The globule size of clarithromycin for optimized formulation was 30 nm. The

morphology of nanoemulsion was explored using transmission electron microscopy. Antibacterial activity was conducted with optimized nanoemulsion and compared with free clarithromycin [6].

Soheila Honary et al., (2013) Prepared finasteride nanoemulsion by chemometric approach which could predict the globule size of nanoemulsion under different conditions. The organic phase was a homogeneous solution of oil (finasteride as a lipophilic drug) and water–miscible solvent with or without lipophilic surfactant (Span-80), while the aqueous phase consisted of water with or without hydrophilic surfactant (Tween-80). The experiments were designed according to Box-Behnken experimental design. The factors considered were sonication time (0–5 min), and contents of Span-80 (0–0.16 %) and Tween-80 (0–0.26 %). Nanoemulsion data showed that emulsification evaporation technique is an efficient technique to stabilize the droplet size of nanoemulsions [7].

Tamer Elbayoumi et al., (2011) invented administration of poorly water-soluble emulsified therapeutic agents using microporous devices for controlled local intraluminal delivery. A nanoemulsion and corresponding methods of making and using systems of single or blended high HLB value surfactant(s) for the emulsification of single or blended oils and vitamin E components in an aqueous phase were provided. The resulting nanoemulsion was provided the intraluminal delivery of nanoemulsions to a patient [8].

Fanzhu Li et al., (2012) formulated supersaturable self-emulsifying drug delivery system for enhanced oral bioavailability of silybin. The S-SEDDS formulation consisted of silybin, Labrafac CC, Cremophor RH40, Labrasol, and 5% HPMC. The pseudo-ternary phase diagrams were constructed to identify the self-emulsifying region. In vitro dilution of the S-SEDDS formulation resulted in formation of microemulsion, followed by a slow precipitation of silybin, while the conventional SEDDS formulation undergone rapid precipitation, yielding a low silybin solution concentration. The results showed that the presence of HPMC effectively sustained the supersaturated state by retarding the precipitation kinetics [9].

Timothy James Wooster et al., (2010) invented oil-in-water nanoemulsions, processes for their preparation and their use as delivery vehicles for active components for use in ophthalmological, dermatological, food, cosmetic, pharmaceutical, agrichemical, textile,

polymer and chemical applications. The oil-in-water nanoemulsion comprised up to 40 volume % of an oil phase having at least 50 volume % of a triglyceride having a fatty acid chain length of 12 carbon atoms or greater and a hydrophilic non-ionic surfactant having a hydrophilic-lipophilic balance (HLB) greater than 7 and cosolvent and an aqueous phase. The average size of oil droplets was less than 100 nm. The ratio of surfactant to oil is less than 1:1, more preferably 0.2 to 0.8:1 [10].

3.2 Drug

3.2.1 Atorvastatin Calcium

Sanjeev Gubbi et al., (2010) prepared the formulation and characterization of Atorvastatin Calcium liquisolid compact. The dissolution rate of poorly soluble, highly permeable (BCS-II) drug, such as atorvastatin calcium, can be improved by application of the liquisolid (LS) technique. Different liquisolid compacts were prepared using a mathematical model for calculating required quantities of powder and liquid ingredients to produce an acceptably flowable and compressible admixture. Avicel PH 102, Aerosil 200 and Explotab were employed as carrier, coating material and disintegrate, respectively. The release rate of liquisolid compact was markedly higher compared with directly compressed tablet, due to increased wetting property and surface area of the drug. The results of pharmacokinetic parameters, such as the AUC, Tmax and Cmax of liquisolid compact demonstrated better bioavailability compared with their conventional formulation [11].

Ali Ka et al., (2013) prepared solidified self-microemulsion in form of tablet containing atorvastatin calcium. Self-microemulsifying atorvastatin calcium tablet was formulated mainly by using self-emulsifying base, solidifying agent silicon dioxide and sodium starch glycolate as tablet disintegrant. Self-emulsifying base containing Transcutol P, Gelucire 44/14, and Lutrol F68 with their different ratios in the formulation, were best selected by solubility study and ternary phase diagram. Globule size of microemulsion from tablet, physical parameters of the tablet, and drug content were checked. In vitro drug release rate was carried out in phosphate buffer medium (pH 6.8). Average globule diameter of the emulsions formed from the tablet was found to be below 100 nm in optimized of

formulation, which indicated microemulsions were formed. In vitro drug release from the optimized formulation was found to be >90%, which indicated the enhancement of solubility of atorvastatin calcium. Differential thermal analysis (DTA), Powder X-ray Diffraction (X-RD) and Fourier transform infrared (FTIR) study proved the identity of the drug in the optimized formulation [12].

Kiran Bhise et al., (2012) prepared of self-micro emulsifying drug delivery system of low solubility drug for enhanced solubility and dissolution. The solubility of atorvastatin in microemulsion components viz. oil and surfactants was determined. The surfactants were screened for emulsification ability. Based on the solubility and emulsification property sunflower oil and surfactants Cremophor RH 40 and Capmul MCM C8 were selected for further study. The solubility of atorvastatin in different ratios of selected oil and surfactants was determined. The composition of oil: surfactants with maximum solubility for atorvastatin were used for SMEDDS formulation. Pseudo ternary phase diagrams were used to evaluate the micro emulsification existence area. The microemulsions were evaluated for emulsion droplet size, self-emulsification and phase separation, *In vitro* dissolution and stability. The SMEDDS formulation showed complete release in 30 min. as compared with the drug [13].

Lakshmi Narasaiah V et al., (2010) studied the improved dissolution rate of Atorvastatin calcium using solid dispersions with PEG-4000. The study was to improve the physicochemical properties of Atorvastatin calcium like solubility, dissolution property and stability of drug by forming dispersion with PEG 4000 as water soluble carrier. Atorvastatin calcium was formulated by physical mixtures and solid dispersions (dropping method) using 1:1, 1:2 and 1:3 ratios of drug and carrier (PEG 4000). The results showed that PEG 4000 was found to be effective in increasing the saturation solubility and dissolution rate of ATC than that of pure drug. The dispersion with PEG 4000 (1:3) by dropping method showed faster dissolution rate (85.0%) as compared to other dispersions with PEG 4000 (1:1 and 1:2) whichever prepared by physical mixture and dropping method [14].

Wenxiang Dong et al., (2018) prepared solid dispersion of atorvastatin calcium through the solvent evaporation method, using Poloxamer-188. Solid dispersion was characterized by scanning electron microscopy, DSC, XRD and FTIR. The drug solubility studies and

dissolution rates were compared with bulk drug and marketed tablets (Lipitor). The study investigated the pharmacokinetics after oral administration of Lipitor and solid dispersion. The AUC_{0-8h} and C_{max} increased after taking Atorvastatin calcium-P188 solid dispersion orally compared with that of Lipitor [15].

Shamsuddin et al., (2016) studied to enhance the solubility and dissolution profile of the atorvastatin calcium. Solid dispersion (SD) is a technique which enhances the solubility and a dissolution profile of poorly soluble drug. The solubility of pure drug, physical mixture using PEG 4000 (1:3), and SD in phosphate buffer solutions (pH 6.8) was found to be 55.33 ± 0.66, 81.89 ± 2.35, and 93.66 ± 1.35, respectively. FTIR and DSC study showed the significant peak shift of drug in SD. The dissolution rate was significantly increased when the drug polyethylene glycol 4000 ratio was 1:3. The drugs release from pure drug, physical mixture, marketed tablet, and SD at 1 h was 28.92 ±1.66%, 55.26 ± 0.95%, 72.16 ± 1.33%, and 91.66 ± 1.65%, respectively [16].

3.2.2 Fenofibrate

Patel Tejas et al., (2011) prepared the solid dispersion to enhance the solubility of fenofibrate using a water-soluble carrier, Poloxamer 407. The lyophilization technique was used to prepare solid dispersions. A 3^2 full factorial design was used for optimization, wherein the amount of Poloxamer 407 (mg) (X1) and the Lyophilization Temperature (°C) (X2) were selected as independent variables and the T100% (min) and angle of repose was selected as the dependent variable. The DSC and XRD studies demonstrated that enhanced dissolution of fenofibrate from solid dispersion might be due to a decrease in the crystallinity of fenofibrate in lyophilized POL during solid dispersion preparation. Dissolution enhancement of fenofibrate was obtained by preparing its solid dispersions in POL using Lyophilization technique [17].

Jagdale Sachin et al., (2019) studied enhancement of dissolution of fenofibrate using complexation with hydroxy propyl β-cyclodextrin. Complexes of fenofibrate with HPβCD were prepared in 1:1 ratio by kneading and co-precipitation. These complexes were evaluated by dissolution studies, FTIR, and DSC studies. The complexation of fenofibrate with HPβCD exhibited an enhanced dissolution rate. FTIR studies showed the formation

of intermolecular hydrogen bonding between fenofibrate and HPβCD. DSC studies indicated a loss in crystalline state of fenofibrate in complexes [18].

Shelake S et al., (2018) prepared Fenofibrate nanoparticles using precipitation method to enhance solubility and bioavailability. Formulation was characterized using FTIR, DSC, powder XRD, scanning electron microscopy, zeta potential and in vitro drug release studies. Data obtained from the DSC, XRD and FTIR showed no interaction between drug and polymers. Scanning electron microscopy images indicated that nanoparticles were spherical in shape. Water solubility of drug-loaded nanoparticles was increased as compared to the pure drug and showed improved dissolution profile [19].

Amrit B. Karmarkar et al., (2009) described the improvement of Fenofibrate dissolution through its formulation into liquisolid tablets and investigate in vitro performance of formulated liquisolid systems. By use of this technique, liquid medications such as solutions or suspensions of water insoluble drugs in suitable non-volatile liquid vehicles can be easily converted into powders with acceptable flow property. X-ray powder diffraction and Differential Scanning Calorimetry were used for evaluation of physicochemical properties of Fenofibrate in liquisolid tablets. Stereomicroscopy was used to assess morphological characteristics of liquisolid formulation. Enhanced drug release profiles due to increased wetting properties and surface of drug available for dissolution was obtained in case of liquisolid tablets [20].

Yadav VB et al., (2009) described a melt granulation technique to improve the solubility and dissolution characteristics of a poorly water-soluble drug Fenofibrate. The advantage of this technique compared to a conventional granulation was that no water or organic solvents is needed. Because of bypassing drying step, the process is less time consuming and uses less energy than wet granulation. Granules were prepared by using hydrophilic polymer PEG (polyethylene glycol-6000) and surfactant (poloxomers-407). The prepared granules were characterized using powder XRD, DSC and FTIR techniques. A significant enhancement in the solubility and vitro dissolution profile of the melt granules was observed compared to the pure drug and drug excipient physical mixtures. The results of the work suggested that melt granulation was a useful technique to enhance the solubility and dissolution rate of poorly water-soluble drug like, fenofibrate [21].

Sucheta D. Bhise et al., (2009) described bioavailability of a poorly water soluble drug fenofibrate using ternary solid dispersion comprising a novel surfactant. Different methods like melt method, solvent evaporation, and spray drying were used to prepare the solid dispersions. Solid dispersions were characterized by FT-IR, XRPD and DSC. Solubility study indicated that poloxamer 188 along with TPGS significantly increased the solubility as well as the bioavailability of fenofibrate. The solid dispersion prepared by spray drying resulted in maximum increase in solubility, dissolution rate as well as bioavailability of the fenofibrate. The cumulative release of fenofibrate from solid dispersions within 60 min was 2.93 times higher than the pure drug in distilled water. The dissolution of fenofibrate from solid dispersions in Poloxamer 188 along with TPGS reached a satisfactory level (above 90%) within 100 min in water, 0.1N HCl and 0.1M SLS [22].

3.2.3 Fenofibrate and Atorvastatin Combination

Per Holm et al., (2007) described that solid dosage forms consisting of combination of fenofibrate and atorvastatin or its active salt. The solid compositions were manufactured without any need of addition of aqueous medium. Atorvastatin was provided as controlled release or a delayed release formulation resulting in a maintained LDL-lowering effect at a reduced dosage, and fenofibrate provided an increased bioavailability and reduced food effect [23].

P. Sobhita Rani et al., (2014) investigated the solid dispersions of Atorvastatin and Fenofibrate combination, to enhance the solubility and bioavailability. When used in combination, these drugs showed additive beneficial effect and comparatively fewer side effects, in the treatment of Hyperlipidemia. The solubility of the above drug combination was increased by means of solid dispersions with PEG 4000 and PEG 6000 in different concentrations, using solvent evaporation method. The *In-vitro* release profiles of prepared solid dispersions were found to exhibit better dissolution characteristics compared to that of a branded market formulation [24].

Kwang Kon Koh et al., (2005) compared the vascular and metabolic responses (and adverse responses) to statin and fibrate therapies alone or in combination in patients with combined hyperlipidemia. Fifty-six patients were given atorvastatin 10 mg and placebo,

atorvastatin 10 mg and fenofibrate 200 mg, or fenofibrate 200 mg and placebo daily during two-month treatment period of a randomized, double-blind, placebo-controlled crossover trial with two washout periods for two months. Lipoproteins were changed to a greater extent with combined therapy when compared with atorvastatin or fenofibrate alone. Flow-mediated dilator response to hyperemia and plasma high-sensitivity C-reactive protein and fibrinogen levels were changed to a greater extent with combined therapy when compared with atorvastatin or fenofibrate alone [25].

Vasilios G. Athyros et al., (2002) evaluated the effect of atorvastatin-fenofibrate combination on lipid profile, in comparison to each drug alone, in patients with type 2 diabetes and combined hyperlipidemia (CHL). No patient was withdrawn from the study because of side effects. The atorvastatin- fenofibrate combination reduced total cholesterol by 37%, LDL cholesterol by 46%, TGs by 50%, and PF by 20%, whereas it increased HDL cholesterol by 22% ($P < 0.0001$ for all). These changes were significantly better than those of individual therapy. Patients on drug combination, 97.5% reached the LDL cholesterol treatment goal of <100 mg/dl, 100% reached the desirable TG levels of <200 mg/dl, and 60% reached the optimal HDL cholesterol levels of >45 mg/dl. These rates were significantly higher than those of individual therapy. Combined treatment reduced the 10-year probability for myocardial infarction from 21.6 to 4.2% [26].

Mahmut Bilgic, (2012), studied the pharmaceutical formulations designed based on the synergistic effect that use of fenofibrate and/or fibric acid with statin would induce. It used in the treatment of hyperlipoproteinemia, hypertriglyceridemia, hypercholesterolemia, myocardial infarction and stroke and/or related diseases. Formulation presented as multiple dosage forms produced by formulating the two active agents separately. The excipients used in the formulation were selected such that the interaction between them and the active agents is minimized and the formulations are prepared accordingly. Multiple dosage forms prepared this way can be produced such that the two active agents can have different and/or the same release characteristics [27].

3.3 Pseudo Ternary Phase Diagram

Rashmin B. Patel et al., (2013) prepared carbamazepine loaded microemulsion and mucoadhesive microemulsion drug delivery system for its intranasal administration. Carbamazepine microemulsion and mucoadhesive microemulsion were prepared by titration method. The drug-loaded microemulsions were successfully prepared which contain 6% Labrafil M1944 CS as an oily phase, 32% surfactant mixture of Cremophor RH 40: Transcutol P (4:1) and 62% (wt/wt) aqueous phase. Microemulsion formulation which displayed an optical transparency of 99.95%, globule size of 34.32 ± 1.09 nm, and polydispersity index of 0.127 ±0.012 was selected for the incorporation of mucoadhesive component. The drug-loaded mucoadhesive microemulsion that contains 0.5% wt/wt of polycarbophil displayed higher in vitro mucoadhesive potential (21.0± 3.0 min) and diffusion coefficient (0.3172 ± 0.03) than microemulsion [28].

Packiaraj Jeyachandran Manohari et al., (2013) formulated the self-micro emulsifying drug delivery system (SMEDDS) of Pimozide. It contained Capmul MCM NF as oily phase, Cremophor RH 40 as Surfactant and PEG- 8000 as Co-Surfactant. Pimozide loaded SMEDDS were characterized with respect to Visual Assessment, Phase Separation, Emulsion Droplet Size, Pseudoternary Phase Diagram, HLB Determination, Assessment of Self-Emulsification Efficiency, Drug content and In- vitro dissolution study in comparison with ORAP® 2 mg tablet manufactured by TEVA Pharmaceuticals USA. Pimozide loaded SMEDDS showed excellent self-emulsification efficiency and released more than 90% of the drug in 45 minutes whereas ORAP® showed about 45% drug release. The mean globule size of optimized Pimozide SMEDDS was 29.39 nm [29].

Maria Saifee et al., (2013) developed the solid self-micro emulsifying drug delivery system (S-SEDDS) with Aerosil 200 for enhancement of dissolution rate of model drug Glibenclamide (GBM). SEDDS was prepared using Capmul MCM C8, Cremophor RH 40, and Transcutol P as oil, surfactant and cosurfactant respectively. For formulation of stable SEDDS, microemulsion region was identified by constructing pseudo ternary phase diagram containing different proportion of surfactant: co-surfactant (1:1, 2:1 and 3:1), oil and water. The SEDDS was evaluated for turbidity measurement, globule size and zeta potential, viscosity determination and % transmittance. S-SEDDS was prepared by adsorption technique using Aerosil 200 as solid carrier. Globule size was found to be

142.8 nm with PDI 0.396. In-vitro dissolution studies showed that there was enhancement of dissolution rate of GBM as compared with that of plain drug and marketed formulation [30].

Shruti G. Shahu et al., (2013) designed the microemulsion of valsartan for enhancing its solubility and oral bioavailability. Solubility of valsartan was determined in various vehicles and maximum solubility was found in Capmul MCM (oil), Cremophor EL (surfactant) and Transcutol HP (co-surfactant). These components were used to construct pseudo-ternary phase diagrams to identify the micro emulsion existing zone. Optimized microemulsion was characterized for its transparency, particle size, drug content, viscosity, and stability study etc. Particle size of optimized microemulsion was found to be 51.32 nm. Drug content of the microemulsion formulation was 98.29% ± 0.91%. The viscosity data indicated the microemulsion to be O/W type. 78.49% and 71.53% of the drug was found to be released in 4hrs in the in-vitro and in-vivo intestinal permeability studies respectively. By formulating into microemulsion, the solubility of valsartan was significantly enhanced which may increase its bioavailability [31].

Kishor Sagar et al., (2014) studied the solubility and dissolution of poorly soluble drug, Nateglinide, by formulating self-nanoemulsifying drug delivery systems (SNEDDS). Phase solubility of Nateglinide was evaluated in various non-aqueous carriers, oils, surfactants, and co-surfactants. Pseudo ternary phase diagrams were constructed to identify the optimized self-nanoemulsification region. Nateglinide SNEDDS was prepared by using Capmul MCM C-8 (oil), Cremophor EL (surfactant), and Transcutol HP (co-surfactant). Two different adsorbents with high specific surface areas were used i.e. NeusilinUS2, NeusilinUFL2 (magnesium aluminometasilicate). The formulations were characterized for self-emulsification assessment, globule size, polydispersity index, zeta potential, % transmittance, drug content, thermodynamic stability and in-vitro dissolution study. The optimized Nateglinide SNEDDS composed of 20.23% Capmul MCM C-8, 55.77% Cremophor EL and 23 % Transcutol HP. The rate of dissolution of optimized SNDDES showed better result (91.12%) in 35 min when compared with marketed tablet 72.3% and pure drug (32.54%) [32].

3.4 Patents

Sr. No.	Approaches	Patent Number	Claim
1	Self-emulsifying drug delivery system [1]	US7736666	Pharmaceutical composition suitable for oral administration, in form of an emulsion pre-concentrate, comprising (i) active compound; (ii) one or more surfactants; (iii) optionally an oil or semi-solid fat; said composition forming an in-situ oil-in-water emulsion upon contact with aqueous media such as gastrointestinal fluids.
2	Pharmaceutical composition comprising Fenofibrate and Atorvastatin [23]	US2007/0014846 A1	Pharmaceutical Composition in particulate form or in solid dosage forms comprising a combination of fenofibrate and atorvastatin or a pharmaceutically active salt thereof, which upon oral administration provides a relative AUC_{0-24} value ($AUC_{fibric\ acid}/AUC_{atorvastatin}$) of between about 250 and about 10,000.
3	Nanoemulsion formulations for direct delivery [8]	US2011/0045050 A1	Nanoemulsion and corresponding methods of making and using systems of single or blended high HLB value surfactant(s) for the emulsification of single or blended oils and vitamin E components in an aqueous phase are provided.
4	oil-in-water nanoemulsions [10]	US2010/0305218 A1	oil-in-water nanoemulsion comprises up to 40% of an oil phase comprising at least 50% of triglyceride having a fatty acid chain length of 12 carbon atoms or greater and hydrophilic non-ionic surfactant having HLB > 7; and an aqueous phase, in which oil droplets have an intensity average size < 100 nm and the ratio of surfactant to oil is less than 1:1, more preferably 0.2 to 0.8:1.
5	Multiple dosage forms comprising Fenofibrate or Fenofibric acid in combination with HMG CoA reductase inhibitors such as	WO 2012002921 A1	Formulations comprising a HMG-CoA reductase enzyme inhibitor and a fibric acid derivative in order to be used in treatment of hyperlipoproteinemia, hypertriglyceridemia, hypercholesterolemia, myocardial infarction and stroke and/or related diseases

	statins [27]		
6	Self-emulsifying and self microemulsifying formulations for oral administration of toxoids [33]	EP1648517 B1	Development of self microemulsifying formulation for oral administration of toxoids using Cremophor EL as surfactant and at least one oil and surfactant
7	Novel capsule SMEDDS formulations of Etoposide for oral use [34]	US20050220866	The present invention relates to self microemulsifying pharmaceutical compositions comprising Etoposide that are encapsulated comprising a drug phase comprising Etoposide, and a solvent; a co-solvent and an emulsifying base comprising a lipid, a surfactant and a stabilizer
8	Self-nanoemulsifying oily formulation for the administration of poorly water-soluble drugs [35]	CA 2536466 A1	self-nanoemulsifying oily formulation comprising: one or more therapeutic agent(s) which have low solubility in water or are water-insoluble, vitamin E, one co-solvent selected from propylene glycol and ethanol and mixture thereof one surfactant selected from tyloxapol and from mixture of tyloxapol and TPGS, and optionally, a bio enhancer.
9	Method and formulation for increasing the bioavailability of poorly water-soluble drugs [36]	US5993858	A self-microemulsifying excipient formulation for increasing the bioavailability of a drug which includes an emulsion including oil or other lipid material, a surfactant, and a hydrophilic co-surfactant.
10	Self microemulsifying dosage forms of low solubility active ingredients such as co-enzyme Q10 [37]	US20060275358	The present invention includes a SMEDDS comprising a combination of a pair of hydrophilic and lipophilic surfactant. It also contains a lipophilic solvents. The formulations exhibited excellent dissolution properties and storage stability.

3.5 References

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CHAPTER – 4

Materials and Methods

4. Materials and Methods

4.1 Materials used in present work

Sr. No.	Name of Material	Obtained From
1	Fenofibrate	Cadila healthcare Ltd., Ahmedabad, India
2	Atorvastatin Calcium	Cadila healthcare Ltd., Ahmedabad, India
3	Acetonitrile gradient HPLC	Merck, Mumbai, India
4	Castor Oil	Rankem, Mumbai, India
5	Ortho Phosphoric Acid	Rankem, Mumbai, India
6	Hydrochloric acid	Rankem, Mumbai, India
7	Labrafac PG	Gattefose, France
8	Oleic acid	S D Fine Chem Limited, Mumbai, India
9	Methanol HPLC grade	Merck, Mumbai, India
10	Capmul MCM Oil	Abitech, Mumbai, India
11	Propylene glycol AR	S D Fine Chem Limited, Mumbai, India
12	Poly Ethylene Glycol-400	S D Fine Chem Limited, Mumbai, India
13	Labrafac Lipophile WL 1349	Gattefose, France
14	Cremophor EL	BASF, Mumbai, India
15	Light Liquid Paraffin	Rankem, Mumbai, India
16	Span 20	LOBA Chem, Mumbai, India
17	Labrasol	Gattefose, France

18	Capmul GMO 50	S D Fine Chem Limited, Mumbai, India
19	Captex 355	Abitech, Mumbai, India
20	Transcutol-P	Gattefose, France
21	Cremophor RH 40	BASF, Mumbai, India
22	Tween-80	Merck, Mumbai, India
23	Acconon MCS-2	Abitech Mumbai, India
24	Chloroform	Merck, Mumbai, India
25	Sodium Lauryl Sulfate	Merck, Mumbai, India

4.2 Instruments and Apparatus used in present work

Sr. No.	**Instruments/Apparatus**	**Company**
1	Laboratory Centrifuge	REMI motors, Mumbai, India
2	Micropipettes	HiMedia, Mumbai, India
3	Abbe's Refractometer	Krishna scientific, Haryana, India
4	Digital balance	Sartorius Balance, Bangalore, India
5	Electronic balance	Mettler Toledo, Mumbai, India
6	pH meter	Lab India, Mumbai, India
7	Dissolution test apparatus	Electrolab Dissolution Tester TDT-06P, USP, Mumbai, India
8	Environmental shaker	Tempo instruments and equipment Pvt. Ltd., Mumbai, India
9	UV-Visible Spectrophotometer	UV-1700 Shimadzu Co., Japan
10	HPLC	Agilent Technologies, Mumbai, India and Dionex, Mumbai, India
11	Brookfield viscometer	Brookfield, USA
12	Dialysis membrane	HiMedia, Mumbai, India
13	Sonicator	Equitron, Mumbai, India
14	Stability chamber	Thermolab, Mumbai, India
15	Magnetic stirrer	Remi equipment Pvt. Ltd., Mumbai, India
16	Fourier Transform Infrared	Shimadzu Corporation, Japan

	Spectroscopy	
17	Cyclomixer	Remi equipment Pvt. Ltd., Mumbai, India
18	Rotary Shaker	Remi equipment Pvt. Ltd., Mumbai, India
19	Zetasizer Nano ZS	Malvern Instrument, UK

4.3 Drug Profile

4.3.1 Fenofibrate

Appearance

White to almost white crystalline powder.

Chemical Name

2-(4-(4-Chlorobenzoyl) phenoxy)-2-methylpropanoic acid 1-methylethyl ester

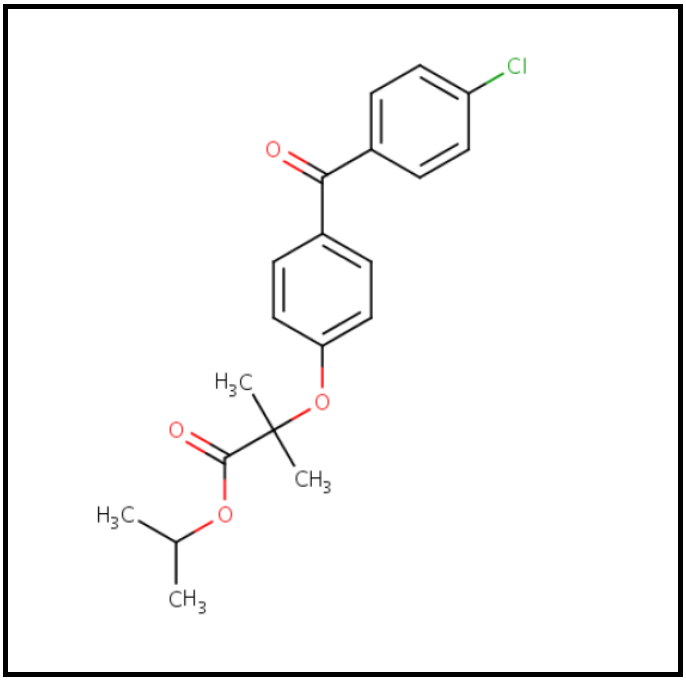
Molecular Formula

$C_{20}H_{21}ClO_4$

Molecular weight

- 360.831

Structure



pKa

Strongest Basic: -4.9

logP

- 5.3

Solubility

Insoluble in water. Very soluble in Methylene Chloride. Slightly soluble in Alcohol.

Melting Point

- 80.5 °C

Indication

For use as adjunctive therapy to diet to reduce elevated LDL-C, Total-C, Triglycerides and Apo B, and to increase HDL-C in adult patients with primary hypercholesterolemia or mixed dyslipidemia (Fredrickson Types IIa and IIb) [1].

Pharmacodynamics

Fenofibrate is a lipid regulating agent indicated as adjunctive therapy to diet to reduce elevated LDL-C, Total-C, Triglycerides and Apo B, and to increase HDL-C in adult patients with primary hypercholesterolemia or mixed dyslipidemia (Fredrickson Types IIa and IIb). Fenofibrate is also indicated as adjunctive therapy to diet for treatment of adult patients with hypertriglyceridemia (Fredrickson Types IV and V hyperlipidemia). Fenofibric acid, the active metabolite of Fenofibrate, produces reductions in total cholesterol, LDL cholesterol, Apo lipoprotein-B, total triglycerides and triglyceride rich lipoprotein (VLDL) in treated patients. In addition, treatment with fenofibrate results in increases in high density lipoprotein (HDL) and Apo proteins APO-AI and APO-AII [1].

Mechanism of Action

Fenofibrate exerts its therapeutic effects through activation of peroxisome proliferator activated receptor a (PPARa). This increases lipolysis and elimination of triglyceride-rich particles from plasma by activating lipoprotein lipase and reducing production of Apoprotein C-III. The resulting fall in triglycerides produces an alteration in the size and composition of LDL from small, dense particles, to large buoyant particles. These larger particles have a greater affinity for cholesterol receptors and are catabolized rapidly [1].

Absorption

Fenofibrate is well absorbed from the gastrointestinal tract. Peak plasma concentrations of fenofibric acid achieved within 3–8 hours and steady-state plasma levels achieved within 5–7 days. After absorption, Fenofibrate is mainly excreted in the urine in the form of metabolites, primarily fenofibric acid and fenofibric acid glucuronide.

Food

Bioavailability of fenofibric acid was not altered following administration of Antara micronized capsules with a low-fat meal; however, administration with a high-fat meal

resulted in substantial increases in peak plasma concentration and AUC of fenofibric acid compared with administration under fasting conditions.

Absorption was increased by approximately 35% following administration of micronized capsules or tablets (e.g., Lofibra) with food [2].

Volume of distribution

- 95 L [moderate renal impairment (creatinine clearance of 50 to 90 mL/min)]
- 30 L [healthy adults]

Protein binding

- ~99% (Serum protein binding)

Metabolism

Fenofibrate is rapidly hydrolysed by esterase to the fenofibric acid (active metabolite), which is principally conjugated with glucuronic acid. Neither fenofibrate nor fenofibric acid undergoes oxidative metabolism (e.g., CYP450).

Route of elimination

Fenofibric acid is primarily conjugated with glucuronic acid and then excreted in urine. Following oral administration in healthy volunteers, approximately 60% of a single dose of radiolabelled fenofibrate appeared in urine, primarily as fenofibric acid and its glucuronate conjugate and 25% was excreted in the feces.

Half life

Approximately 16–23 hours (fenofibric acid).

Clearance

- 1.2 Liter/hours [Elderly]

Toxicity

LD_{50}=1600 mg/kg (Oral, in mice); Investigated as a teratogen and reproductive hazard.

Marketed Products

- Fenofibrate Tablet 48mg by Teva Pharmaceuticals USA Inc.
- Antara Capsule 43 mg by Lupin Pharma

4.3.2 Atorvastatin Calcium

Appearance

White to off white crystalline powder.

Chemical Name

7-[2-(4-fluorophenyl)-3-phenyl-4-(phenylcarbamoyl)-5-(propan-2-yl)-1H-pyrrol-1-yl]-3, 5-dihydroxyheptanoate

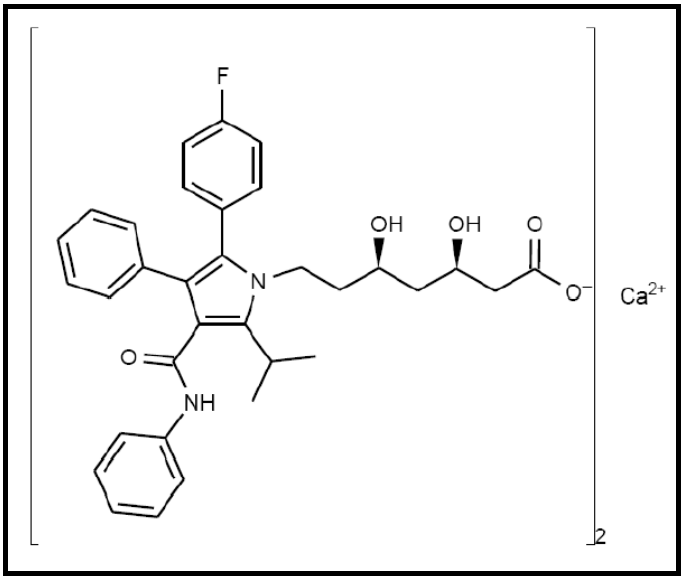
Molecular Formula

$(C_{33}H_{34}FN_2O_5)_2$. Ca

Molecular weight

- 1155.34

Structure



pKa

Strongest Acidic: 4.33

Strongest Basic: -2.7

logP

- 5.7

Solubility

Insoluble in aqueous solutions of pH 4 and below. Atorvastatin Calcium is very slightly soluble in distil water, pH 7.4 phosphate buffer and acetonitrile; slightly soluble in ethanol; and freely soluble in methanol [3].

Melting Point

- 159.2-160.7 °C

Indication

May be used as primary prevention in individuals with multiple risk factors for coronary heart disease and as secondary prevention in individuals with CHD to reduce the risk of

myocardial infarction (MI), stroke, angina, and revascularization procedures. May be used to reduce the risk of cardiovascular events in patients with Acute Coronary Syndrome (ACS). May be used in the treatment of primary hypercholesterolemia and mixed dyslipidemia, primary dysbetalipoproteinemia, homozygous familial hypercholesterolemia, and/or hypertriglyceridemia as an adjunct to dietary therapy to decrease serum total and low-density lipoprotein cholesterol (LDL-C), Apo lipoprotein B, and triglyceride concentrations, while increasing high-density lipoprotein cholesterol (HDL-C) levels. May use atorvastatin/fenofibrate fixed-combination preparation when treatment with both atorvastatin (for prevention of cardiovascular events) and fenofibrate (decrease elevated serum total and LDL-cholesterol, triglyceride, and Apo lipoprotein B concentrations, and to increase HDL-cholesterol concentrations in the management of mixed dyslipidemia and primary hypercholesterolemia) is appropriate [4].

Pharmacodynamics

Atorvastatin, a selective, competitive HMG-CoA reductase inhibitor, is used to decrease serum total and LDL cholesterol, triglyceride, and Apo-B levels while increasing HDL cholesterol. Low HDL-C, high LDL-C and TG concentrations in the plasma are associated with increased risk of atherosclerosis and cardiovascular disease. The total cholesterol to HDL-C ratio is a strong predictor of coronary artery disease and high ratios are associated with higher risk of disease. Increased levels of HDL-C are associated with lower cardiovascular risk. By decreasing LDL-C and TG and increasing HDL-C, atorvastatin reduces the risk of cardiovascular morbidity and mortality. Atorvastatin has a long half-life, and hepatic selectivity, explaining its greater LDL-lowering potency compared to other HMG-CoA reductase inhibitors [3].

Mechanism of Action

Atorvastatin selectively and competitively inhibits the hepatic enzyme HMG-CoA reductase. As HMG-CoA reductase is responsible for converting HMG-CoA to mevalonate in cholesterol biosynthesis pathway, this results in a subsequent decrease in hepatic cholesterol levels. Decreased hepatic cholesterol levels stimulates up regulation of hepatic LDL-C receptors which increases hepatic uptake of LDL-C and reduces serum LDL-C concentrations [3].

Absorption

Atorvastatin is rapidly absorbed after oral administration with maximum plasma concentrations attained in 1 to 2 hours. The absolute bioavailability of atorvastatin (parent drug) is about 14% and the systemic availability of HMG-CoA reductase inhibitory

activity is about 30%. The low systemic bioavailability is due to pre-systemic clearance by gastrointestinal mucosa and first-pass metabolism in the liver.

Food

Food decreases rate and extent of absorption of atorvastatin but does not alter antilipidemic effects.

Volume of distribution

- 381 L

Protein binding

- >98% bound to plasma proteins (principally albumin)

Metabolism

Atorvastatin is extensively metabolized to ortho and para hydroxylated derivatives and various beta-oxidation products. In vitro inhibition of HMG-CoA reductase by ortho and para hydroxylated metabolites is equivalent to that of atorvastatin. Approximately 70% of circulating inhibitory activity for HMG-CoA reductase is attributed to active metabolites. CYP3A4 is also involved in the metabolism of atorvastatin.

Route of elimination

Eliminated primarily in bile after hepatic and/or extra hepatic metabolism. Does not appear to undergo significant enterohepatic recirculation. Less than 2% of the orally administered dose is recovered in urine.

Half life

- 14 hours, but half-life of HMG-CoA inhibitor activity is 20-30 hours due to longer-lived active metabolites.

Toxicity

Generally well-tolerated. Side effects may include abdominal pain, myalgia, constipation, asthenia, and nausea. Other possible side effects include myotoxicity (myositis, myopathy, and rhabdomyolysis) and hepatotoxicity. To avoid toxicity in Asian patients, lower doses should be considered.

Marketed Products

- Atorvastatin Calcium Tablet 10mg by Watson Laboratories, Inc.
- Atorvastatin Calcium Tablet 10mg by Sanis Health Inc.

4.4 Excipient Profile

4.4.1 Capmul MCM Oil [5]

Synonyms

Medium Chain Mono- & Diglyceride of caprylic acid

Product Type

Capmul MCM is a mono-diglyceride of medium chain fatty acids (mainly caprylic and capric). It is an excellent solvent for many organic compounds including steroids. It is also a useful emulsifier for water-oil systems.

Specifications

Specification	**Limit**
Appearance/ Form	Liquid/ Semi-solid
Acid Value	2.5 max
Moisture, Karl Fischer	0.5% max.
Alpha Monocaprylate	48% min.
Free Glycerol	2.5% max.
HLB Value	5.8

Pharmaceutical and Nutritional Applications

· Carrier (vehicle)

· Solubilizer

· Emulsifier/ Co-emulsifier

· Bioavailability enhancer

· Penetration enhancer (dermatological applications)

Storage

Store in a dry place at 68-77ºF.

4.4.2 Labrasol [6]

Synonyms

Caprylocaproyl macrogol glycerides.

Product type

Labrasol is composed of a well-defined mixture of mono - di- and tri-glycerides and mono – and di- fatty acid esters of polyethylene glycol. It is soluble in ethanol, chloroform, methylene chloride, water and insoluble in mineral oils.

Specifications

Specification limits

Appearance	Oily liquid
Odour	faint
Colour (Gardner scale)	< 2.5
Specific gravity at 20°C (d20/4)	1.060 to 1.070
Water content	< 1.00 %
HLB Value	12.0

Uses

High HLB non-ionic amphiphilic excipient for pharmaceutical preparations, used as solubilizing agent and bioavailability enhancer for poorly soluble drugs in oral liquid and capsule formulations, permeation enhancer in topical preparations, surfactant in nanoemulsion.

Storage

Preserve in its original container and prevent exposure to air, light, heat and moisture.

4.4.3 Cremophor RH 40 [6]

Synonyms

Polyoxyl 40 hydrogenated castor oil.

Composition

Polyoxy1 35 hydrogenated castor oil is a non-ionic solubilizer and emulsifier made by reacting hydrogenated castor oil with ethylene oxide in a molar ratio of 1: 40. The main component of Cremophor RH 40 is glycerol polyethylene glycol hydroxy- stearate, which, together with fatty acid glycerol polyglycol esters, forms the hydrophobic part of the product. The hydrophilic part consists of polyethylene glycols and glycerol ethoxylate.

Product type

Cremophor RH 40 forms clear solutions in water. It is also soluble in many organic solvents, e.g. ethanol, n-propranol, isopropyl alcohol, ethyl acetate, chloroform, carbon tetrachloride, trichloroethylene, toluene and xylene. In contrast to anionic emulsifying agents, Cremophor RH 40 becomes less soluble in water at higher temperatures. The aqueous solutions become turbid at a certain temperature. Cremophor RH 40 is miscible with all the other Cremophor grades and, on heating, also with fatty acids, fatty alcohols and certain animal and vegetable oils. It is thus miscible with oleic and stearic acids, dodecyl and octa-decyl alcohols, castor oil, and a number of lipid-soluble substances.

Specification

Specification Limits

Appearance	white to pale yellow oily liquid
Acid value	Less than Equal to 1
Water	Less than Equal to 2.0%
pH (10% in water)	6–7
HLB Value	Between 14 to 16

Storage

Cremophor RH 40 should be stored in tightly closed containers protected from light. Prolonged storage is not recommended unless the containers are completely full.

4.4.4 Transcutol-P [6]

Synonyms

Diethylene glycol monoethyl ether (Highly purified)

Product type

Diethylene glycol monoethyl ether soluble in ethanol and water. Partially soluble in vegetable oils and insoluble in mineral oils.

Specifications

Specification Limits

Appearance	colorless limpid liquid
Odour	Faint
Specific gravity at 20°C (d20/4)	0.985 to 0.991
Acid value	< 0.10
Water content	< 0.10 %
HLB Value	4.2

Uses

Solubilizer of many active ingredients (i.e. indomethacin nifedipine, hormones, sterols). Absorption enhancer. Transcutol P can be used in topical, transdermal and oral pharmaceutical preparations. It can be associated to labrafils and vegetable oils.

Storage

Stored in its original hermetically closed container. The product is packed under nitrogen atmosphere and must be used shortly after opening.

4.5 Estimation of Fenofibrate and Atorvastatin Calcium

4.5.1 Estimation using UV-Visible Spectrophotometer

Fenofibrate and Atorvastatin Calcium were quantitatively analyzed using UV-Visible spectrophotometer (UV-1700 Shimadzu Co., Japan). Standard curve of Fenofibrate and Atorvastatin Calcium were generated in methanol [7, 8].

Determination of λmax of Fenofibrate

A 10µg/ml solution of Fenofibrate in methanol was scanned in UV range of 200 to 400nm. Fenofibrate showed maximum absorbance at 287nm (Figure 4.1).

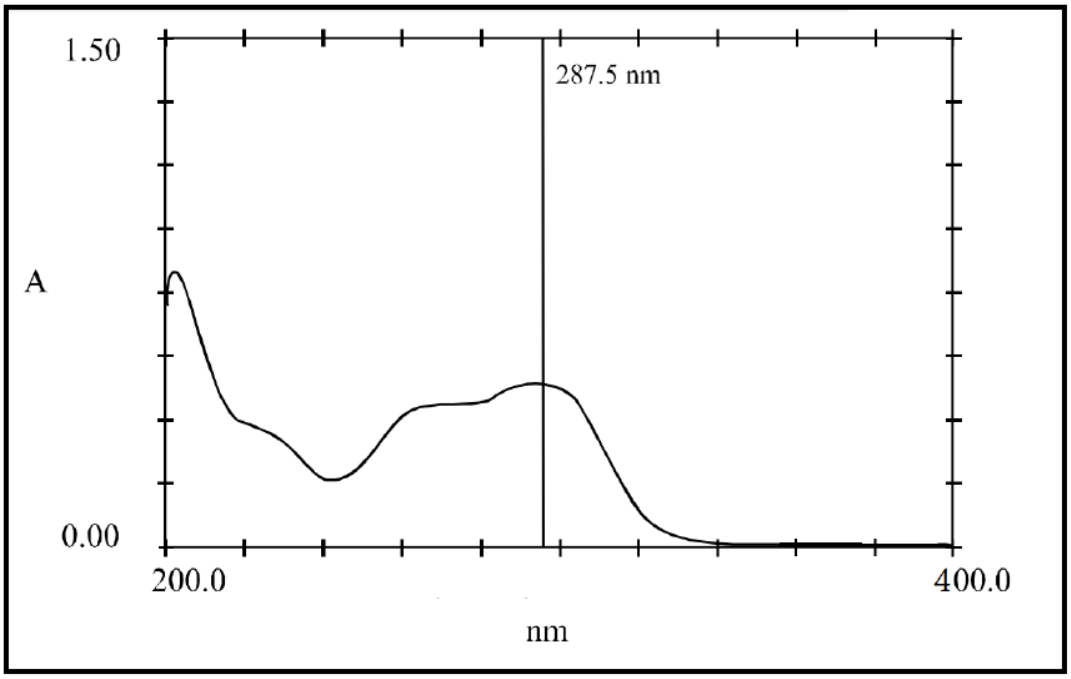


Figure 4.1: UV Spectrum of Fenofibrate in Methanol

Preparation of Standard Curve for Fenofibrate in Methanol

Accurately 25mg of Fenofibrate was weighed and placed in 250ml of volumetric flask and the volume was made up to the mark with methanol. Aliquots from the stock solution and were diluted to prepare 4, 8, 12, 16, 20µg/ml and the absorbance of each solution was taken at 287nm, using methanol as blank solution. Data and Figure of standard curve for Fenofibrate were given in Table 4.1 and Figure 4.2 respectively.

Table 4.1: Standard curve data of Fenofibrate in methanol						
Sr. No.	**Concentration (µg/ml)**	**Absorbance at 287nm**				
		I	**II**	**III**	**Average**	**SD**
1	4	0.2256	0.2244	0.2241	0.2247	0.0008
2	8	0.3987	0.3969	0.3901	0.3952	0.0045
3	12	0.5882	0.5891	0.5807	0.5860	0.0046
4	16	0.7685	0.7694	0.7677	0.7685	0.0009
5	20	0.9475	0.9468	0.9461	0.9468	0.0007

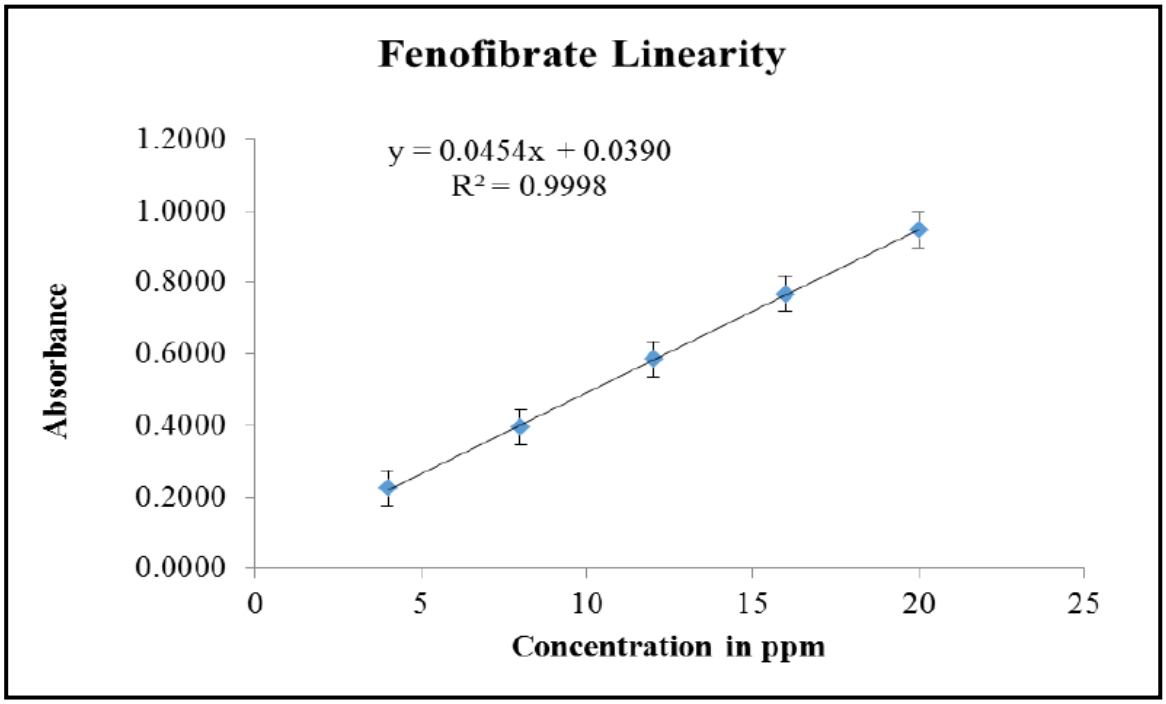


Figure 4.2: Standard curve of Fenofibrate in methanol at 287nm

Results of weighted linear regression analysis

Multiple R	0.9998
R square	0.9998
Slope of regression line	0.0454
Intercept of regression line	0.0390
Equation:	y = 0.0454x + 0.0390

Linearity was observed between 4-20µg/ml concentrations of Fenofibrate, and the drug obeys Beer's law in the range of 4-20µg/ml concentration of drug.

Determination of λmax of Atorvastatin Calcium

A 10µg/ml solution of Atorvastatin Calcium in methanol was scanned in UV range of 200 to 400nm. Atorvastatin Calcium showed maximum absorbance at 246nm (Figure 4.3).

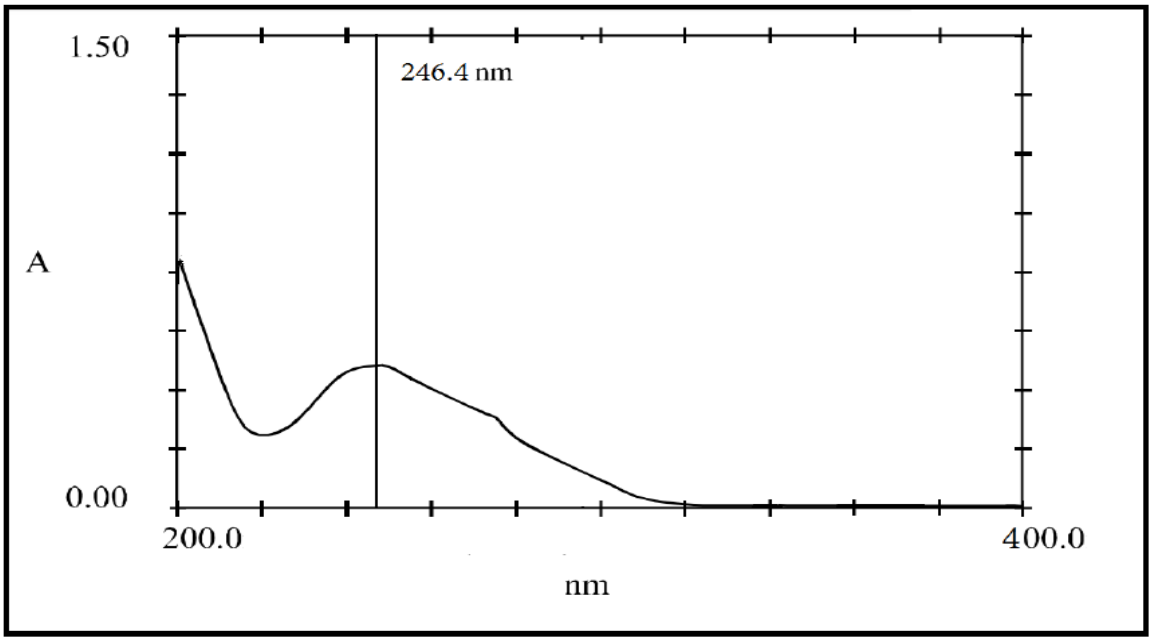


Figure 4.3: UV Spectrum of Atorvastatin Calcium in Methanol

Preparation of Standard Curve for Atorvastatin Calcium in Methanol

Accurately 25mg of Atorvastatin Calcium was weighed and placed in 250ml of volumetric flask and the volume was made up to the mark with methanol. Aliquots from the stock solution were diluted to prepare 4, 8, 12, 16, 20µg/ml and the absorbance of each solution was taken at 246nm using methanol as blank solution. Data and Figure of standard curve for Atorvastatin Calcium were given in Table 4.2 and Figure 4.4 respectively.

Table 4.2: Standard curve data of Atorvastatin Calcium in methanol

Sr. No.	Concentration (µg/ml)	Absorbance at 246nm				
		I	II	III	Average	SD
1	4	0.2209	0.2214	0.2217	0.2213	0.0004
2	8	0.3934	0.3919	0.3911	0.3921	0.0012
3	12	0.5826	0.5801	0.5813	0.5813	0.0013
4	16	0.7616	0.7631	0.7641	0.7629	0.0013
5	20	0.9538	0.9545	0.9551	0.9545	0.0007

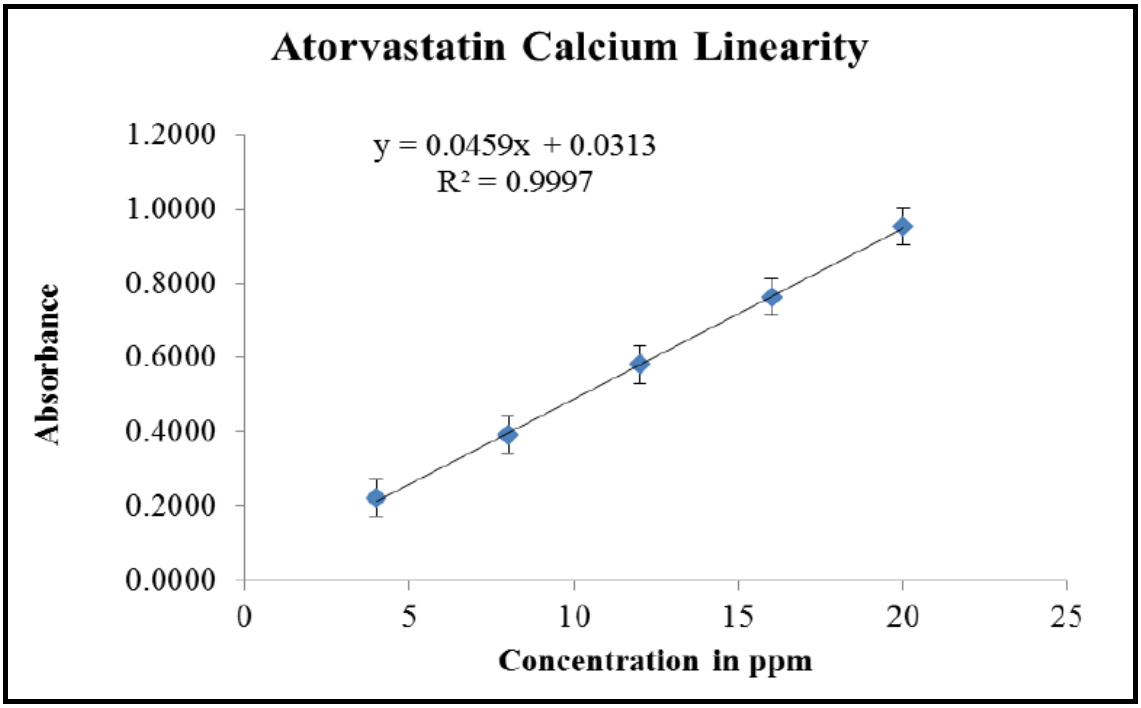


Figure 4.4: Standard curve of Atorvastatin Calcium in methanol at 246nm

Results of weighted linear regression analysis

Multiple R	0.9998
R square	0.9997
Slope of regression line	0.0459
Intercept of regression line	0.0313
Equation:	y = 0.0459x + 0.0313

Linearity was observed between 4-20µg/ml concentrations of Atorvastatin Calcium, and the drug obeys Beer's law in the range of 4-20µg/ml concentration of drug.

4.5.2 HPLC Method for Estimation of Atorvastatin Calcium and Fenofibrate (Dissolution and Assay)

Buffer solution-A: 4.1gm sodium acetate was dissolved in 1000 ml purified water and mixed well. The pH was adjusted to 3.7 with diluted glacial acetic acid [9].

Mobile Phase: Methanol: Buffer solution-A (82:18)

Diluent: Methanol (For Assay)

Chromatographic Condition:

Column: Gemini C18 (250 X 4.6 mm, 5µm)

Flow Rate: 1.0 ml/min

Injection Volume: 10µL

Detector: UV 248nm

Run Time: 12min

Standard Solution: 50ppm (Atorvastatin Calcium) and 240ppm (Fenofibrate)

Retention Time (RT): 3.5 min (Atorvastatin Calcium)

7.9 min (Fenofibrate)

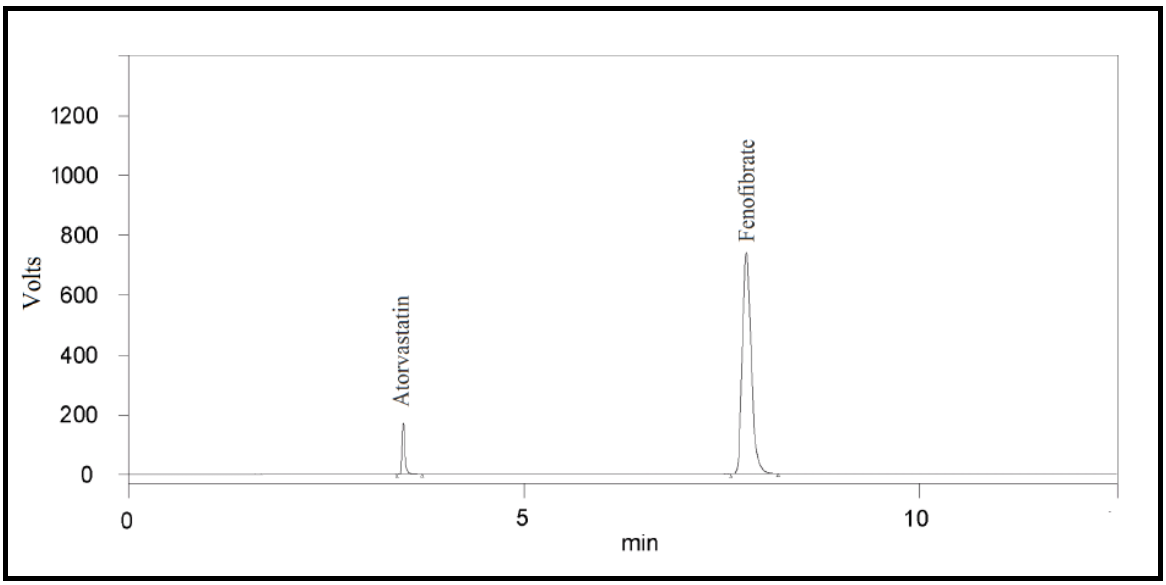


Figure 4.5: HPLC Chromatogram of Fenofibrate and Atorvastatin Calcium

Preparation of Standard Curve for Fenofibrate and Atorvastatin Calcium in Diluent

Weighed accurately 50mg of Fenofibrate and 23.4 mg of Atorvastatin Calcium and transferred into 100 ml of volumetric flask and the volume was made up to the mark with diluent. Aliquots were taken from the stock solution and were appropriately diluted. Linearity was carried out at 20%, 50%, 80%, 100%, 120% and 150% of 240µg/ml concentration of Fenofibrate and 50µg/ml concentration of Atorvastatin Calcium. Data and Figure of standard curve were presented in Table 4.3 - 4.4 and Figure 4.6 - 4.7 respectively.

Table 4.3: Standard curve data of Fenofibrate in diluent						
Sr. No.	**Concentration (µg/ml)**	**Area (mAU)**				
		I	**II**	**III**	**Average**	**SD**
1	48	133.526	132.247	133.359	133.044	0.6953
2	120	330.742	329.498	332.591	330.943	1.5563
3	192	531.347	532.414	532.674	532.145	0.7032
4	240	662.432	661.953	662.968	662.451	0.5078
5	288	795.172	794.831	795.915	795.306	0.5543
6	360	993.501	992.956	993.853	993.436	0.4519

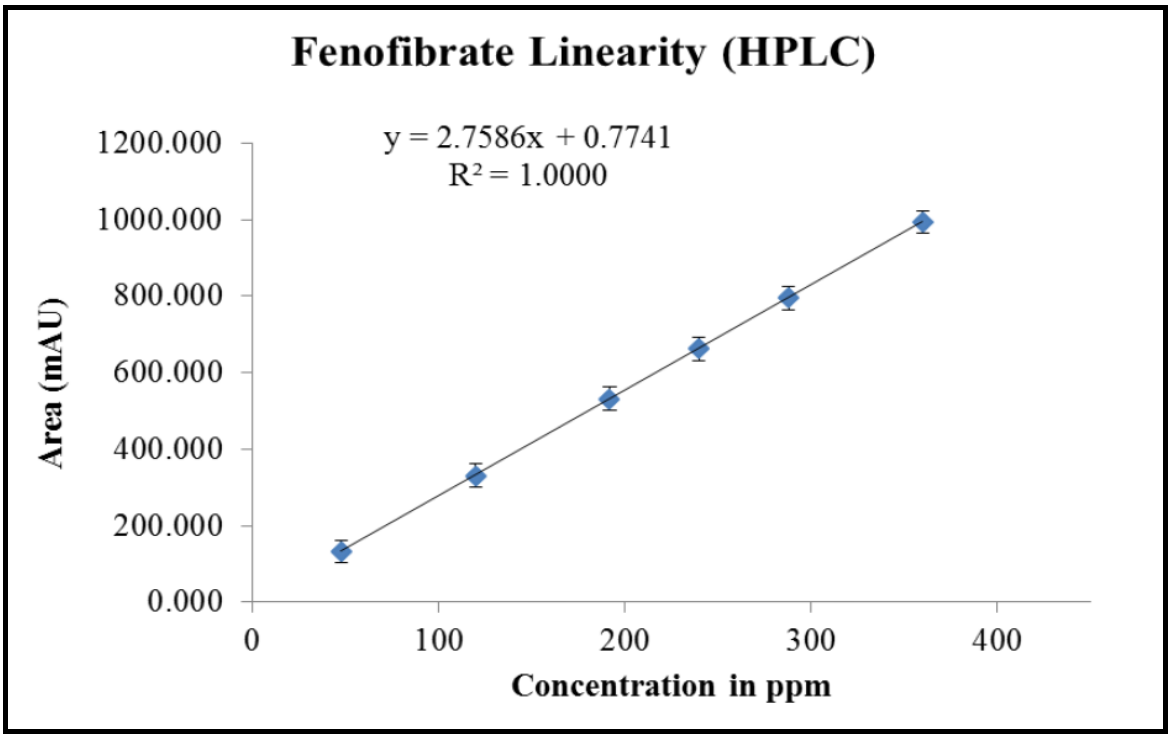


Figure 4.6: Standard curve of Fenofibrate in diluent at 248nm

Results of weighted linear regression analysis

Multiple R	1.0000
R square	1.0000
Slope of regression line	2.7586
Intercept of regression line	0.7741
Equation	y = 2.7586x + 0.7741

Table 4.4: Standard curve data of Atorvastatin Calcium in diluent						
Sr. No.	**Concentration (µg/ml)**	**Area (mAU)**				
		I	**II**	**III**	**Average**	**SD**
1	10	29.543	29.831	30.742	30.008	0.6626
2	25	72.731	72.359	73.024	72.704	0.3333
3	40	117.062	116.794	117.328	117.061	0.2670
4	50	145.634	146.398	147.103	146.378	0.7347
5	60	175.282	175.924	174.169	175.125	0.8880
6	75	218.759	217.235	220.053	218.682	1.4106

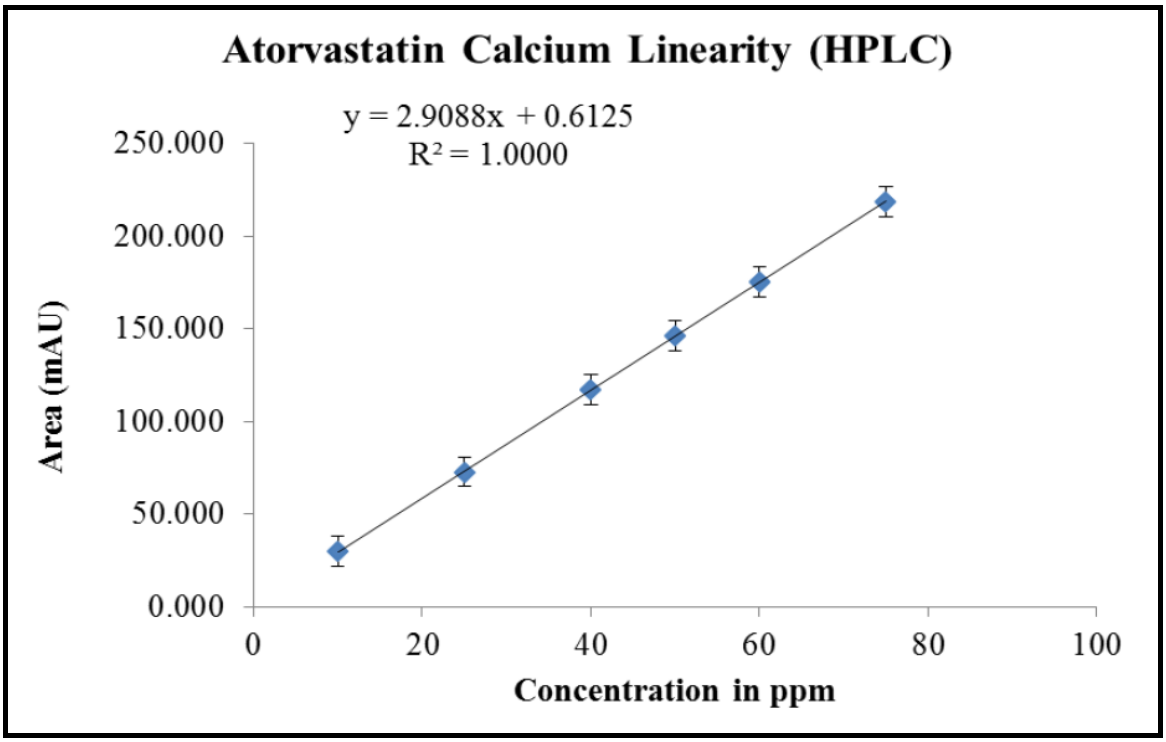


Figure 4.7: Standard curve of Atorvastatin Calcium in diluent at 248nm

Results of weighted linear regression analysis

Multiple R	1.0000
R square	1.0000
Slope of regression line	2.9088
Intercept of regression line	0.6125
Equation	y = 2.9088x + 0.6125

4.6 Solubility Study

Screening of excipients was done by determining the equilibrium solubility of Fenofibrate and Atorvastatin Calcium in various oils, surfactants and co-surfactants [10]. An excess quantity of Fenofibrate and Atorvastatin Calcium were added to the 2ml of excipients. Both components were mixed in a vial for 5 minute using cyclomixer. The mixture in vials was shaken at 25 ± 1.0°C for 48 hrs using controlled temperature rotary shaker. The mixture was centrifuged using laboratory centrifuge at 5000 RPM for 15minute. The supernatant was separated and Fenofibrate/Atorvastatin Calcium was extracted in methanol. The drug content was analyzed using UV-Visible spectrophotometer at 287 and 246nm for Fenofibrate and Atorvastatin Calcium respectively [7, 8].

4.7 Evaluation of effective oil, surfactant and co-surfactant

The selection of surfactant for emulsification ability was done by measuring and comparing the %transmittance of different mixtures of surfactant and selected oils with an objective to explain the bass for selection of components for nanoemulsion formulations from the pseudo ternary phase diagram [11]. Oil selection was done on the basis of their ability to solubilize Atorvastatin Calcium and Fenofibrate. Equal quantity of surfactant was added to the selected oil. The mixture was gently heated at 45-50°C for homogenizing the components. Isotropic mixture was diluted into 250 ml of distilled water to yield a fine emulsion. The time taken for the formation of fine emulsion is called dispersion time and it was noted. The resulting emulsions were observed visually for the relative turbidity. The emulsions were allowed to stand for 2 hr and their % transmittance was assessed at 400-700nm by UV-Visible spectrophotometer using distil water as blank.

The turbidimetric method was used to assess relative efficacy of the co-surfactant to improve the nano-emulsification ability of the surfactants and also to select best co-surfactant from the large pool of co-surfactants available for peroral delivery. Selected surfactant was mixed with co-surfactant i.e. 1:1 Smix mixture. Then, the equal quantity of selected oil was added to this mixture (50:50) and the mixture was homogenized with the aid of the gentle heat (45–50°C). This isotropic mixture was diluted into 250 ml of distil water to yield fine emulsion. The time taken and ease of formation of emulsions was noted by noting the number of flask inversions required to give uniform emulsion. The resulting emulsions were observed visually for relative turbidity.

The emulsions were allowed to stand for 2 hr and their transmittance was measured at 400-700nm by UV-Visible spectrophotometer using distil water as blank. As the ratio of cosurfactants to surfactant is the same in this case, the turbidity of resulting nanoemulsion will help in assessing the relative efficacy of the co-surfactants to improve the nano-emulsification ability of the selected surfactant for the selected oil. Similarly, the nano-emulsification ability and the efficacy of co-surfactant were also determined by varying the surfactant to co-surfactant ratio i.e. from 1:1 to 2:1 and 3:1.

4.8 Drug-Excipient Compatibility of SNEDDS Formulations

Fenofibrate and Excipient were mixed in 1:1 and analyzed by Initial and 40°C/75%RH for 1 month by IR Spectroscopy [12].

Atorvastatin Calcium and Excipient were mixed in 1:1 and analyzed by Initial and 40°C/75%RH for 1 month by IR Spectroscopy [12].

Carrier: Chloroform

Cell: NaCl Cell (Sod. Chloride Cell)

Condition: Initial and 40°C/75%RH for 1 Month

IR Spectra Range: 4000 cm^{-1} to 400 cm^{-1}

Sample Preparation: 10mg of sample in 2ml of Carrier, Mix well.

4.9 Method of Preparation of SNEDDS

Accurately weighed Fenofibrate and Atorvastatin Calcium were placed in a glass vial, and required quantity of oil, surfactant, and co-surfactant were added. The mixture was mixed by gentle stirring and vortex mixing at 40ºC on a magnetic stirrer at 200 rpm, until Fenofibrate and Atorvastatin Calcium were dissolved [13]. The mixture was stored at room temperature in closed container until further use.

4.10 Evaluation Methods for SNEDDS Formulations

4.10.1 Refractive Index and %Transmittance

The Self Nanoemulsifying system (SNEDDS) was added to 250 ml of purified water under continuous stirring (50-60 rpm) on a magnetic stirrer at room temperature. Refractive index of formulation was measured by using an Abbe's Refractometer and %transmittance was measured at 694nm in UV-Visible spectrophotometer using blank [13, 14].

4.10.2 Measurement of Globule Size, Polydispersity Index (PDI) and Zeta Potential

Globule size, Polydispersity index (PDI) and zeta potential of SNEDDS were determined using Zetasizer Nano ZS (Malvern Instruments, UK), which follows principle of LASER light diffraction. Helium–neon gas laser having intensity of 4 mW was the light source. Light scattering was monitored at 25°C at a 90° angle [15, 16]. SNEDDS was added (after suitable dilution) to the sample cell and put into the sample holder unit and measurement was carried out with the help of software of same instrument.

4.10.3 Drug Content

Fenofibrate and Atorvastatin Calcium from pre-weighed SNEDDS was extracted by dissolving in 25ml methanol. The methanolic extract was separated out and Fenofibrate and Atorvastatin Calcium content in methanolic extract were analyzed HPLC Method at 248nm, against standard methanolic solution of Fenofibrate and Atorvastatin Calcium [9].

4.10.4 Effect of Dilution and Aqueous Phase Composition

Robustness of SNEDDS to the dilution and effect of aqueous phase composition were studied using optimized Fenofibrate and Atorvastatin Calcium SNEDDS composition. Fenofibrate and Atorvastatin Calcium SNEDDS were dispersed in 250 ml of aqueous phases (Distil water) with gentle stirring. Resulting Nano emulsions were kept at 25±2°C and evaluated for drug precipitation, phase separation, and changes in size over the period of 24 hours [17, 19].

4.10.5 Measurement of Viscosity of Fenofibrate and Atorvastatin Calcium SNEDDS

Viscosity of SNEDDS comprising Fenofibrate and Atorvastatin Calcium was measured by using Brookfield viscometer at 25°C. It was measured using S-61 spindle at 30rpm before and after dilution with water (250 ml) [14, 15].

4.10.6 Measurement of pH Fenofibrate and Atorvastatin Calcium SNEDDS

pH of SNEDDS comprising Fenofibrate and Atorvastatin Calcium was measured by using pH meter (Lab India) at controlled room temperature. It was measured before and after dilution with water (250 ml) [15, 18].

4.10.7 Self-Emulsification and Precipitation Assessment

Evaluation of the self-emulsifying property of SNEDDS formulations was performed by visual assessment as previously reported. Different compositions were categorized on speed of emulsification, clarity and apparent stability of resultant emulsion. Visual assessment was performed by drop wise addition of pre-concentrate (SNEDDS) into 250 ml of distil water. This was done in a glass beaker at room temperature, and contents were gently stirred magnetically at 50-100rpm [17, 19].

Precipitation was evaluated by visual inspection of resultant emulsion after 24 hours. The formulations were categorized as clear (transparent or transparent with bluish tinge), non-clear (turbid), stable (no precipitation at the end of 24 hours), or unstable (showing precipitation within 24 hrs) [17, 19].

4.10.8 Centrifugation and Freeze– Thaw Cycle

SNEDDS comprising Fenofibrate and Atorvastatin Calcium were diluted with 250 ml and 900 ml distil water and centrifuged at 5000 rpm for 30 minute. In addition, it was subjected to freeze–thaw cycle by storing it at -20°C for 24 hour and then for another 24 hour at 40°C [13, 17, 20].

Nano emulsions were visually observed for phase separation and precipitation, whereas their physical stability was assessed by measuring globule size before and after centrifugation and freeze–thaw cycle [13, 17].

4.10.9 In-Vitro Drug Release Study

In vitro drug release study was carried out for the formulations, marketed product and active drug substance using USP Type II dissolution test apparatus (Electrolab TDT-06P, India). The dissolution medium (900 ml water) was maintained at 37 ± 0.5°C and rotated at 50rpm. Aliquots were collected periodically (10, 15, 20, 30, 45, 60 minutes) and replaced with fresh dissolution medium [21, 22]. Aliquots, after filtration through 0.45µ PVDF filter paper, were analyzed by HPLC at 248nm for Fenofibrate and Atorvastatin Calcium content [9].

4.10.10 In-Vitro Diffusion Study

In vitro diffusion study was carried out for the formulations, marketed product and active drug substance using dialysis technique. One end of pre-treated dialysis membrane tubing

(12 cm in length) was with thread and then diluted of self nanoemulsifying formulation was placed in it. The other end of tubing was also secured with thread and was allow to rotate freely in dissolution vessel of USP Type II dissolution test apparatus that contained 900 ml dialyzing medium (water) maintained at 37 ± 0.5°C and rotated at 50 rpm [23]. Aliquots were collected periodically (30, 60, 120, 180, 240, 360 minutes) and replaced with fresh dissolution medium. Aliquots, after filtration through 0.45µ PVDF filter paper, were analyzed by HPLC at 248nm for Fenofibrate and Atorvastatin Calcium content [9].

4.10.11 Similarity Factor (f_2)

Mathematical comparison of dissolution data to quantify observed differences in the rate and extent of drug release as influenced by formulation and process variables was performed according to the model-independent approach of Moore and Flanner [24]. A similarity factor (f_2) was calculated from mean dissolution data using following equation:

$$f_2 = 50 \log\{[\ 1 + \left(\frac{1}{n}\right)\sum_{t=1}^{n}(R_t - T_t)^2\ ]^{-0.5} \text{ x } 100\} \qquad \text{(Equation 4.1)}$$

where log is logarithm to the base 10, n is the number of time points, $\sum$ is summation over all time points, R_t is the mean dissolution value of the reference profile at time t and T_t is the mean dissolution value of the test profile at the same time point. The US FDA draft guidance document [25] contains more information on similarity factor (f_2). The value of similarity factor (f_2) between 50 and 100 suggests that the two dissolution profiles are similar [25].

4.10.12 Dissolution Efficiency

The dissolution efficiency of the formulation was calculated by the method mentioned by Khan [26] [27]. It is defined as the area under the dissolution curve up to a certain time, t, expressed as a percentage of the area of the rectangle described by 100% dissolution in the same time.

$$\textit{Dissolution Efficiency} = \frac{\int_0^t y.dt}{y_{100}.t}.100\% \qquad \text{(Equation 4.2)}$$

4.10.13 Stability Study of Fenofibrate and Atorvastatin Calcium SNEDDS

Physical and chemical and stability of Fenofibrate and Atorvastatin Calcium SNEDDS was assessed at and 25 ± 3°C/60 ± 5% (room temperature) and 40 ± 2°C/75 ± 5% RH as per ICH guidelines [13, 28]. Fenofibrate and Atorvastatin Calcium SNEDDS were stored in glass vial for 6 months. Samples were withdrawn at 0, 1, 3 and 6 months and assessed for physical change, globule size, zeta potential, drug content, and in-vitro drug release.

4.11 References

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CHAPTER – 5

Study on Drug Solubilization and Role of Lipid Vehicle in Pseudo Ternary Phase Diagram in Formulation Development of SNEDDS Containing Poorly Water Soluble Drugs

5. Study on drug solubilization and Role of lipid vehicle in Pseudo Ternary Phase Diagram in formulation development of SNEDDS containing poorly water soluble drugs

5.1 Introduction

Increasing number of newly discovered drug substances exhibited poor water solubility and hence low absorption after oral administration. An example of such a drug having lower solubility and poor bioavailability are Fenofibrate, Atorvastatin, Pitavastatin, Simvastatin, etc. Various strategies to improve the solubility and dissolution of poorly water soluble drugs have been developed and described in literature like used of surfactant, lipid, permeation enhancer, micronization, salt formation, cyclodextrin complexation, nanoparticles, etc. Among the lipid base system, the self-nanoemulsifying drug delivery system is promising technology to improve the dissolution rate and absorption of poorly water soluble drugs [1, 2].

Nanoemulsion is a clear, isotropic, thermodynamically stable colloidal system which may be formed spontaneously by the chemical energy of surfactants, combinations of surfactants, and co-surfactants upon mixing a suitable oil phase and water without any

mechanical energy input [3, 4]. It has lots of advantages compared with conventional emulsions, including increased drug-loading and enhanced transdermal delivery [4, 5].

In silico prediction of drug solubility in a lipid vehicle is a difficult task. In the research work, some factors have been identified to be helpful in predicting drug solubility using selected excipients. These factors include the solubility parameter (δ), HLB value, partition coefficient, Molecular weight (MW), Dielectric constant (ε), dipole moment (μ), excipient fatty acid chain length, saponification value, surface tension and viscosity.

To the best of our knowledge, no information is available in the literature on the usefulness of solubility parameter, required HLB (RHLB), and required chemical type of emulsifiers or solubilization capacity for solubilising vehicles as criterion for the selection of surfactant/cosurfactant in the formulation development of SNEDDS using Fenofibrate or Atorvastatin Calcium. The purpose of present study was to select appropriate lipid vehicle and to understand role of lipid vehicle in pseudo ternary phase diagram behaviour to find nanoemulsion area in formulation development of self-nanoemulsifying drug delivery system (SNEDDS) containing Fenofibrate or Atorvastatin Calcium.

Solubility parameter (δ), Required HLB (RHLB), required chemical type of emulsifiers and solubilization capacity appeared to be useful criterion for the selection of surfactant/co-surfactant. The present study showed the importance of selecting a surfactant with the proper HLB for specific oil, and also the type of surfactant/co-surfactant. The solubility parameter (δ) of Fenofibrate or Atorvastatin Calcium is closest to the solubility parameter (δ) of Capmul MCM. Blend of better surfactant/co-surfactant was obtained when surfactant and co-surfactant at higher and lower HLB level respectively were blended. The more difference between hydrophilic and lipophilic surfactants, the better the coverage by blends at the interface. The study also showed importance of structural similarities between the lipophilic tails of the surfactant blends. The pseudo ternary phase diagrams for mixtures of Capmul MCM oil with non-ionic surfactant/co-surfactant and water were constructed [6, 7]. The micelles have potential applications, advantages, and usefulness in the pharmaceutical industry as SNEDDS by various routes of administration, as well as in personal care and cosmetics products [8, 9].

5.2 Materials and Methods

5.2.1 Materials used in present work

Sr. No.	Name of Material	Obtained From
1	Fenofibrate	Cadila healthcare Ltd., Ahmedabad, India
2	Atorvastatin Calcium	Cadila healthcare Ltd., Ahmedabad, India
3	Castor Oil	Rankem, Mumbai, India
4	Labrafac PG	Gattefose, France
5	Oleic acid	S D Fine Chem Limited, Mumbai, India
6	Methanol HPLC grade	Merck, Mumbai, India
7	Capmul MCM Oil	Abitech, Mumbai, India
8	Propylene glycol AR	S D Fine Chem Limited, Mumbai, India
9	Poly Ethylene Glycol-400	S D Fine Chem Limited, Mumbai, India
10	Labrafac Lipophile WL 1349	Gattefose, France
11	Cremophor EL	BASF, Mumbai, India
12	Light Liquid Paraffin	Rankem, Mumbai, India
13	Span 20	LOBA Chem, Mumbai, India
14	Labrasol	Gattefose, France
15	Capmul GMO 50	S D Fine Chem Limited, Mumbai, India
16	Captex 355	Abitech, Mumbai, India
17	Transcutol-P	Gattefose, France
18	Cremophor RH 40	BASF, Mumbai, India
19	Tween-80	Merck, Mumbai, India
20	Acconon MCS-2	Abitech Mumbai, India

5.2.2 Instruments and Apparatus used in present work

Sr. No.	Instruments/Apparatus	Company
1	Laboratory Centrifuge	REMI motors, Mumbai, India
2	Micropipettes	HiMedia, Mumbai, India
3	Abbe’s Refractometer	Krishna scientific, Haryana, India

4	Centrifuge	REMI motors, Mumbai, India
5	Digital balance	Sartorius Balance, Bangalore, India
6	Electronic balance	Mettler Toledo, Mumbai, India
7	Environmental shaker	Tempo instruments and equipment Pvt. Ltd., Mumbai, India
8	UV-Visible Spectrophotometer	UV-1700 Shimadzu Co., Japan
9	Cooling centrifuge	REMI, Mumbai, India
10	Sonicator	Equitron, Mumbai, India
11	Cyclomixer	Remi equipment Pvt. Ltd., Mumbai, India
12	Rotary Shaker	Remi equipment Pvt. Ltd., Mumbai, India

5.2.3 Methods

5.2.3.1 Estimation of Fenofibrate and Atorvastatin Calcium

UV spectroscopic method was used for determination of Fenofibrate and Atorvastatin Calcium as described in Section 4.5 of Chapter 4 in details.

5.2.3.2 Solubility Study

Screening of excipients was done by determining the equilibrium solubility of Fenofibrate and Atorvastatin Calcium in various oils, surfactants and co-surfactants [10]. An excess quantity of Fenofibrate or/and Atorvastatin Calcium were added to the 2 ml of the excipients. Both components were mixed in a vial for 5 minutes using cyclomixer (REMI, Mumbai, India). The mixture in vial was shaken at 25 ± 1.0°C for 48 hours using controlled temperature rotary shaker. The mixtures were centrifuged using Laboratory Centrifuge (REMI, Mumbai, India) at 5000 rpm for 15 minutes. The supernatant was separated and Fenofibrate and Atorvastatin Calcium were extracted in methanol. The drug content was analysed using Shimadzu 1700 UV-Visible spectrophotometer at 287nm and 246nm for Fenofibrate and Atorvastatin Calcium, respectively.

5.2.3.3 Selection of blend of surfactant/co-surfactant (Lipid Vehicle)

Selection of surfactant is a critical step in formulating the desired nanoemulsion. Each surfactant or oil has a specific HLB. The corrected HLB of the selected surfactant or blend of surfactant and co-surfactant that match the HLB of the selected oil provides the lowest

interface tension between the oil and water phases. The HLB of the selected surfactant and blend of surfactant and co-surfactant reflects the stability of the system at lower levels, and can be obtained when the HLBs of the surfactant or blend of surfactant: co-surfactant and oil are similar [11].

Capmul MCM is a mono-diglyceride of medium chain fatty acids (mainly caprylic and capric). It is an excellent solvent for many organic compounds including steroids.

Polyoxyl 35 hydrogenated castor oil is a non-ionic solubilizer and emulsifier made by reacting hydrogenated castor oil with ethylene oxide in a molar ratio of 1: 40. It has several uses as a nonionic surfactant, emollient, and thickening agent in various preparations.

Labrasol (Caprylocaproyl polyoxyl-8 glycerides) is a non-ionic solubilizer and emulsifier. It is mixture of monoesters, diesters and triesters of glycerol and monoesters and diesters of polyethylene glycol with a mean relative molecular weight between 200 and 400. They are produced by partial alcoholysis of medium chain triglycerides with polyethylene glycol, by esterification of glycerol and polyethylene glycol with caprylic acid and capric acid, or as a mixture of glycerol esters and ethylene oxide condensate with caprylic acid and capric acid.

Transcutol-P (Diethylene glycol monoethyl ether) is non-ionic solubilizer and emulsifier. Structurally, it is an alcohol and ether. It is a colorless, slightly viscous liquid with a mild pleasant odor.

Capmul MCM oil is composed of mono-diglyceride of medium chain fatty acids (mainly caprylic and capric) in which the side chains match the tail of non-ionic surfactant. Therefore, non-ionic surfactants were chosen to study the phase diagram behaviour of Capmul MCM oil. Non-ionic surfactants are also recognized as being safe and biocompatible, and are not affected by pH changes in media because they are uncharged.
The non-ionic surfactants were chosen for screening to select a suitable blend of surfactant/co-surfactant that would best match Capmul MCM oil. A blend of hydrophilic and lipophilic surfactants is needed to obtain longer stability of the dispersion phase at the

lowest concentration levels [12, 13]. A blend of surfactant/co-surfactant with an HLB that matches that of the oil phase will provide better solubilization and stability of the dispersion system produced. Therefore, the selection of surfactant blends at lower and higher HLB matching the HLB of oil is important in the formulation of a colloidal system.

5.2.3.4 Solubility Parameter

Polarity of a solvent plays an important role in the solubility. Polar solvents are capable of solvating molecules through dipole interaction forces, particularly via hydrogen-bond formation, which is a major mechanism in the solubility of a compound. Polarity of solvents can be defined by dielectric constant (ε), which is an important property related to the solubility and hydrophilic-lipophilic balance [14 – 17]. It was shown that the solubility of a solute decreased as the dielectric constant of solvent decreased [18, 19].

An understanding of cohesive energy between drug and lipid molecules may help to determine how a lipid will behave as a solvent. Cohesion is result of the London forces, polar interactions and specific ones like hydrogen bonding [20, 21]. The commonly used approach in quantifying the cohesion between a solvent and a solute is the solubility parameter (δ), which is defined as the square root of the cohesive energy density, expressed as the energy of vaporization.

$$\delta = (CED)^{1/2} = (\Delta Ev/Vm)^{1/2} \quad \text{…….. (Equation - 5.1)}$$

Where CED is cohesive energy density, ΔEv is the energy of vaporization and Vm is the molar volume.

This parameter may be useful to predict the solvating ability of a lipid or lipid mixture. When solubility parameters of lipid and drug are similar, they are expected to become miscible [22, 23].

According to this calculation, Solubility parameters (δ_F) of lipids and drugs were calculated using the group contribution method devised by Fedor's (Equation - 5.2).

$$\delta_F = [\Sigma \Delta e / \Sigma \Delta v]^{1/2} \quad \text{…….. (Equation - 5.2)}$$

Where Δe = the additive atomic group contributions for the energy of vaporization

Δv = the additive atomic group contributions for the molar volume

In this study, the group contribution method was used to calculate the solubility parameter from knowledge of the structural formula of the selected lipids and drug compounds.
In this mode the contribution of hydrogen bonding is not included. Therefore, hydrogen bonding contribution ($\boldsymbol{\delta_H}$) was calculated as:

$$\boldsymbol{\delta_H = (5000m/V)^{1/2}} \quad \text{…….. (Equation - 5.3)}$$

Where, m is the number of hydrogen donor and acceptors, and V is the molar volume (MW/density).

Total solubility parameter (δ_T) was calculated by adding hydrogen bonding contribution (δ_H) to the Fedor's solubility parameter (δ_F):

$$\boldsymbol{\delta_T = (\delta_F^2 + \delta_H^2)^{1/2}} \quad \text{…….. (Equation - 5.4)}$$

5.2.3.5 Determination of Required HLB (RHLB) of Capmul MCM Oil

To determine RHLB (o/w) for emulsification of Capmul MCM oil, a matched pair of surfactants belonging to same chemical class but having different hydrophilicity i.e. Cremophor RH 40 (non-ionic hydrophilic surfactant) and Transcutol-P (lipophilic surfactant) were selected. The batches of twelve surfactants blends, ranging in HLB from straight Cremophor RH 40 (HLB = 15) to Transcutol-P (HLB = 4.5) were shown in Table 5.1.

Table 5.1: Surfactant/co-surfactant blends of Cremophor RH 40 and Transcutol-P in different weight ratio and different calculated HLB

Blend	Surfactant/co-surfactant blend		Calculated HLB
	Cremophor RH 40	Transcutol-P	
1	100	0	15.00
2	90	10	13.92
3	80	20	12.84
4	75	25	12.38

5	70	30	11.76
6	60	40	10.68
7	50	50	9.60
8	40	60	8.52
9	30	70	7.44
10	20	80	6.36
11	10	90	5.28
12	0	100	4.20

The test formulation containing 25% Capmul MCM (oily phase), 75% water and one of the surfactant/co-surfactant blend showed in Table 5.1 (10% of weight of Capmul MCM) were prepared in test tubes. Test tubes were closed using stopper. Test tubes were shaken once (up and down in a quick, hard motion) and observed for emulsification.

Similarly the test formulations were also prepared in beakers. Further, contents of each beaker were stirred for 1 minute using magnetic stirrer at 600 rpm, transferred in test tubes and observed for separation. The time taken by emulsion for separation of a particular volume of Capmul MCM was recorded. Trials were performed in triplicate. Required HLB for Capmul MCM was determined based on ease of preparation and time for separation. Number of times the test tubes shaken till a homogenous milky emulsion formed and time of separation for Capmul MCM emulsions prepared using emulsifiers of different HLB were shown in Table 5.1.

5.2.3.6 Determination of required chemical type of Emulsifiers

To find appropriate surfactants, one more formulation was prepared using pair of Labrasol and Transcutol-P in such a ratio to give HLB value 12.38 (which is required for Capmul MCM). Ease of preparation and time for separation was determined and compared with the emulsion prepared using Cremophor RH 40 and Transcutol-P mixtures. Number of times the test tubes shaken till a homogenous milky emulsion formed and time of separation for Capmul MCM emulsion prepared using surfactant/co-surfactant blend of same HLB but different chemical type [24].

The individual non-ionic hydrophilic surfactant Labrasol and Cremophor RH 40 was blended with the lipophilic surfactant Transcutol-P in ratios of 1:1, 2:1, 3:1 w/w to produced blends of surfactant/co-surfactant with various HLBs in the range of 8.1–12.5.

5.2.3.7 Measurement of solubilization capacity

The water solubilization capacity, i.e., minimum content of non-ionic surfactant/co-surfactant required to form a nanoemulsion system with Capmul MCM oil, was performed as a criterion for optimization using the water titration method [25, 26]. The results of solubilization capacity were used to select the best emulsifier to study the phase diagram behaviour of Capmul MCM oil. The blend of surfactant/co-surfactant forming a clear system at the minimum concentration was selected as the blend that best matched the HLB of Capmul MCM oil.

Reverse micelle systems have been an interesting area of research in several fields of science and technology, due to their capability to solubilize water in organic solvent in the presence of surfactant [27]. It is known that ethoxylated non-ionic hydrophilic surfactants tend to form reverse micelles in organic media [28]. The results for the reverse micelle systems in this study formed by screening series surfactants/co-surfactant. Cremophor RH 40/Transcutol-P (3:1) showed a high solubilization capacity compared with other S/CoS Ratio. Cremophor RH 40 (Polyoxyl 40 hydrogenated castor oil) is a non-ionic solubilizer and emulsifier made by reacting hydrogenated castor oil with ethylene oxide in a molar ratio of 1: 40.

5.2.4 Construction of Pseudo-Ternary Phase Diagrams

Pseudo ternary phase diagrams were based on the types of mixtures or dispersion systems formed when Capmul MCM oil – surfactant/co-surfactant mixtures were serially titrated with water at ambient temperature. Various weight to weight blends of selected surfactant/co-surfactant in the ratios of 1:1, 2:1 and 3:1 were produced to form surfactant/co-surfactant mixtures with HLB values of 8.1, 9.4, 10.1, 9.6, 11.4 and 12.3, respectively [23]. The Capmul MCM oil and the blend of surfactant/co-surfactant at each HLB value were weighed separately in glass beakers, and were mixed and vortexed thoroughly in specific oil to surfactant/co-surfactant mixture ratios in the range of 0.25:4.75 – 4.5:0.5. Each mixture was slowly titrated with distilled water drop wise using

a pipette. After each addition of water, the system was vortexed for 10–20 seconds, and the final mixture was vortexed for 2–3 minutes at room temperature. Initial visual observation of the resulting mixture was categorized according to the physical characteristics. Microscopic examination was made of the final mixtures to identify the type of emulsion obtained using water-soluble dyes, i.e. Congo red and methylene blue. The details of the visual observation and microscopic identification of the resulting mixture were recorded. The mixture was stored for 24 hours at room temperature to achieve equilibrium. After equilibrium was reached, the final visual observation was recorded. The oil vertex in the triangle phase diagram represented Capmul MCM oil, the S/Cos vertex represented the surfactant/co-surfactant, and the remaining vertex represented the water phase.

To determine effect of drug addition on nanoemulsion boundary, phase diagrams were also constructed using Tri plot v1-4 software in presence of drug using drug-enriched oil as hydrophobic component.

5.3 Results and Discussion

5.3.1 Solubility study

Vehicles should have good solubilizing capacity for the drug substance, which is essential for formulating SNEDDS. The results of solubility of Fenofibrate and Atorvastatin Calcium in various vehicles were shown in Table 5.2 – 5.3 and Figure 5.1 – 5.2. Fenofibrate and Atorvastatin Calcium had excellent solubility in Capmul MCM Oil (Glyceryl Caprylate/Caprate) with comparison to other lipid vehicles. Fenofibrate and Atorvastatin Calcium had excellent solubility in Cremophor RH 40 (Polyoxyl 40 hydrogenated Castor oil) and Transcutol-P as compare to other surfactant and co-surfactant. Capmul MCM Oil as oil, Cremophor RH 40 as surfactant and Transcutol-P as co-surfactant were selected for optimal SNEDDS formulation resulting in improved drug loading capability. Furthermore, with respect to its safety, Capmul MCM Oil (Glyceryl Caprylate/Caprate), Cremophor RH 40 (Polyoxyl 40 hydrogenated Castor oil) and Transcutol-P are included in the FDA Inactive Ingredients Guide.

Table 5.2: Solubility of Fenofibrate in various oils, surfactants and co-surfactants

Material	Solubility (mg/ml)			Average (mg/ml)	SD
	I	II	III		
Castor Oil	72.35	72.05	72.15	72.18	0.1528
Labrafac PG	58.71	58.99	58.84	58.85	0.1401
Oleic Acid	21.47	21.3	21.52	21.43	0.1153
Capmul MCM Oil	**178.52**	**178.99**	**179.28**	**178.93**	**0.3835**
Light Liquid Paraffin	25.60	25.84	25.66	25.70	0.1249
Tween-80	74.61	75.01	74.79	74.80	0.2003
Span-20	47.49	47.15	47.02	47.22	0.2427
Labrafac Lipophile WL 1349	63.68	64.12	63.87	63.89	0.2207
Cremophor RH 40	**112.88**	**112.52**	**113.14**	**112.85**	**0.3113**
Cremophor EL	61.68	61.45	61.32	61.48	0.1823
Labrasol	**120.35**	**119.46**	**119.97**	**119.93**	**0.466**
Capmul GMO-50	36.46	36.24	36.18	36.29	0.1474
Captex 355	25.19	25.11	25.28	25.19	0.0850
PEG-400	36.27	36.5	36.41	36.39	0.1159

Propylene Glycol	34.07	34.14	34.29	34.17	0.1124
Transcutol-P	**176.99**	**177.59**	**176.74**	**177.11**	**0.4368**
Acconon MCS-2	26.02	26.24	25.96	26.07	0.1474

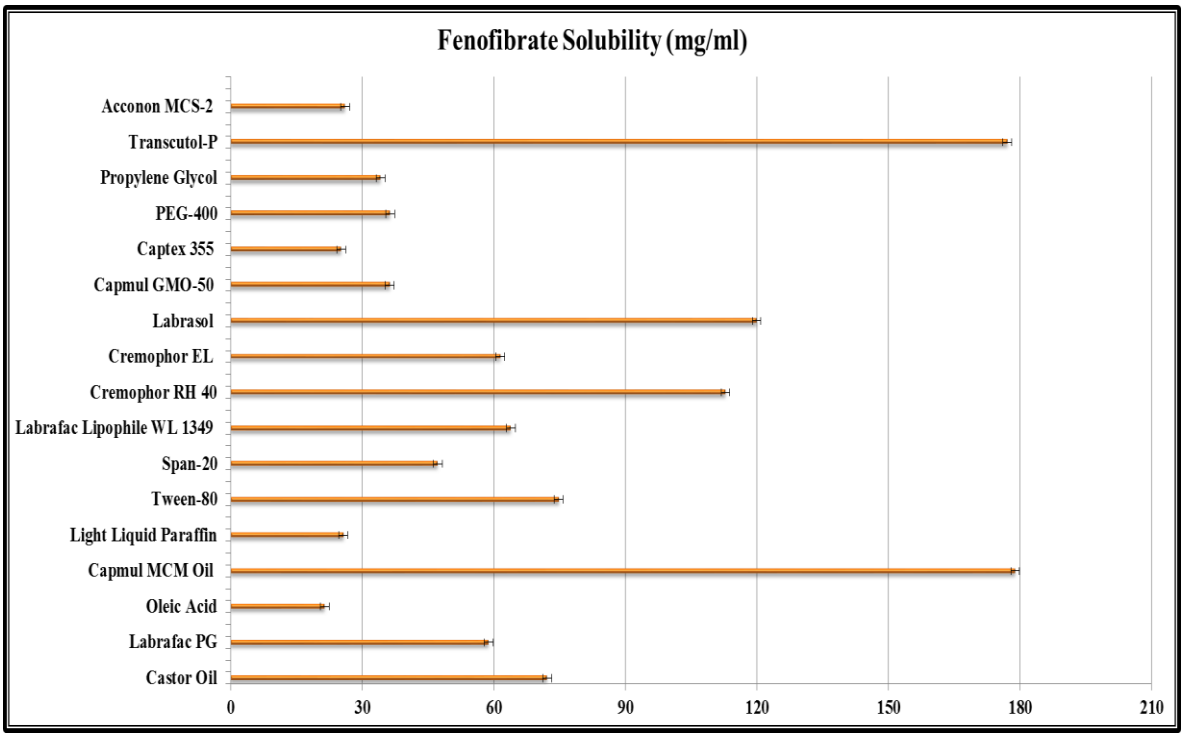

Figure 5.1: Solubility studies of Fenofibrate in various oils, surfactants and co-surfactants. Data expressed as mean ± SD (n = 3).

Table 5.3: Solubility of Atorvastatin Calcium in various oils, surfactants and co-surfactants

Material	Solubility (mg/ml)			Average (mg/ml)	SD
	I	II	III		
Castor Oil	11.59	11.67	11.55	11.60	0.0611
Labrafac PG	28.15	28.18	28.09	28.14	0.0458
Oleic Acid	19.36	19.32	19.52	19.40	0.1058
Capmul MCM Oil	**52.93**	**52.92**	**53.05**	**52.97**	**0.0723**
Light Liquid Paraffin	10.59	10.76	10.73	10.69	0.0907
Tween-80	40.15	40.17	40.08	40.13	0.0473
Span-20	25.99	26.05	26.14	26.06	0.0755

Labrafac Lipophile WL 1349	42.05	41.99	42.02	42.02	0.0300
Cremophor RH 40	**71.59**	**71.02**	**71.36**	**71.32**	**0.2868**
Cremophor EL	30.42	30.49	30.39	30.43	0.0513
Labrasol	**74.46**	**74.41**	**74.57**	**74.48**	**0.0819**
Capmul GMO-50)	26.74	26.66	26.83	26.74	0.0850
Captex 355	14.4	14.24	14.28	14.31	0.0833
PEG-400	38.68	38.73	38.59	38.67	0.0709
Propylene Glycol	10.7	10.84	10.67	10.74	0.0907
Transcutol-P	**82.20**	**82.37**	**82.26**	**82.28**	**0.0862**
Acconon MCS-2	24.18	24.25	24.33	24.25	0.0751

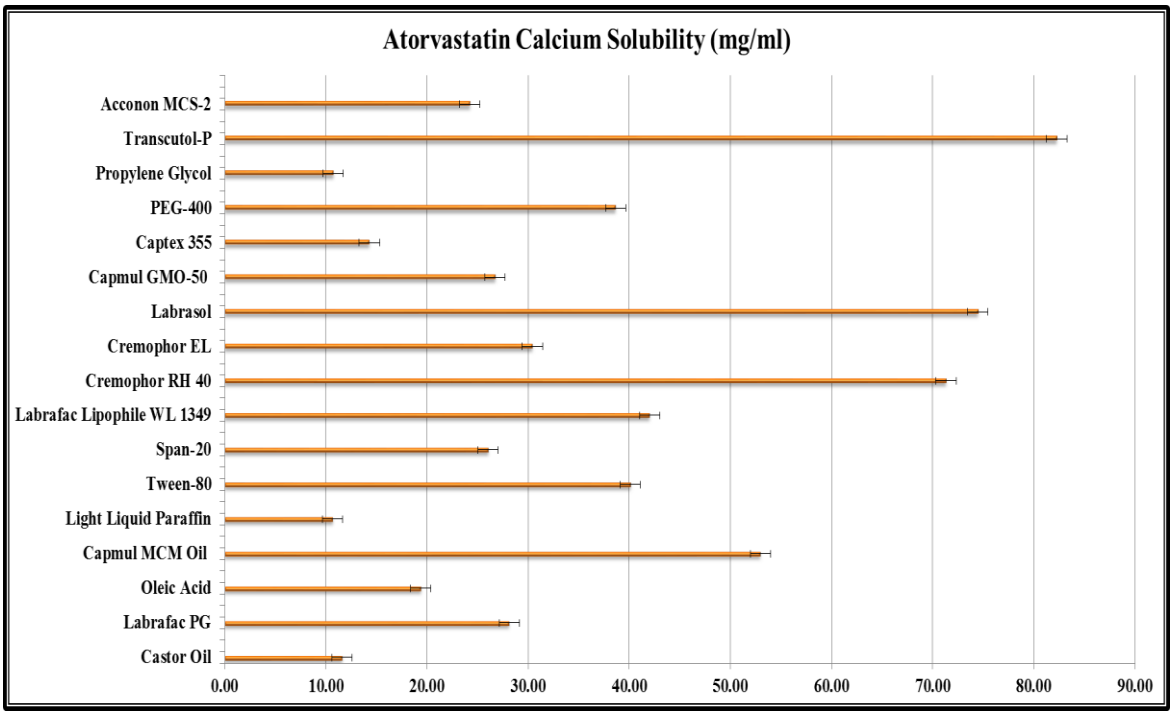

Figure 5.2: Solubility studies of Atorvastatin Calcium in various oils, surfactants and co-surfactants. Data expressed as mean ± SD (n = 3).

5.3.2 Selection of Surfactant/Co-surfactant blend

5.3.2.1 Solubility Parameter (δ)

Lipids used were better solvents for Atorvastatin calcium or Fenofibrate in increasing solubility because Atorvastatin calcium and Fenofibrate has higher lipophilicity with a log P of 5.7 and 5.3 respectively.

Table 5.4: The Solubility Parameter of selected Lipid vehicles				
Materials	**δH** [29-35]	**Δv** [29-35]	**δF**	**δT**
Atorvastatin Calcium	9.79	939.3	11.72	15.27
Fenofibrate	8.08	306.45	14.34	16.46
Capmul MCM Oil	11.74	217.81	12.10	16.86
Light Liquid Paraffin	7.03	405.12	7.62	10.36
Castor oil	7.81	982.56	5.34	9.46
oleic acid	6.87	317.87	10.64	12.67
Labrafac PG	6.95	724.97	9.61	11.86
Tween-80	10.77	560.01	8.73	13.87
Span-20	11.58	335.72	6.34	13.20
Labrafac Lipophile WL 1349	8.19	522.01	9.39	12.46
Cremophor EL	10.15	2233	9.77	14.09
Cremophor RH 40	10.40	2403.8	12.20	16.03
Labrasol	13.69	1094.3	6.68	15.23
Capmul GMO-50	8.88	380.19	12.10	15.01
Captex 355	7.75	499.98	11.51	13.88
PEG-400	12.99	355.56	6.14	14.37
Propylene Glycol	16.53	73.163	6.82	17.88
Transcutol-P	12.15	135.5	9.24	15.26

The solubility parameter calculated for Atorvastatin calcium was $\delta_{T\ (ATR)}$ = 15.27 $(cal/cm^3)^{1/2}$ (Table 5.4). Capmul MCM showed the closest solubility parameter (16.86 $(cal/cm^3)^{1/2}$) to that of Atorvastatin calcium and hence it provided the highest solubility among all lipids used. The same correlation could be observed with the other drug Fenofibrate. The calculated solubility parameter for Fenofibrate was $\delta_{T\ (FENO)}$ = 16.46 $(cal/cm^3)^{1/2}$ and the lipid that has closest solubility parameter is Capmul MCM (Capmul MCM = 16.86 $(cal/cm^3)^{1/2}$ (Table 5.4). Overall, calculated solubility parameter appeared to be a good predictor for the expected solvent effects of the lipids. The predictions are exclusively based on molecular structure of compounds, and no experimental data required.

5.3.2.2 Required HLB (RHLB) of Capmul MCM Oil

Among the surfactant/co-surfactant blends (Cremophor RH 40/Transcutol-P), the composition at 75:25 ratio having HLB 12.38 gave an emulsion that is easy to prepare and take longer time for separation of components than the other eleven mixtures (Table 5.5). These preliminary tests showed that the approximate RHLB for Capmul MCM was 12.38.

Table 5.5: Number of times the test tubes shaken till a homogenous milky emulsion forms and time of separation for Capmul MCM emulsions prepared using emulsifiers of different HLB

Blend	Calculated HLB of Surfactant/co-surfactant blend	Number of times Test tubes shaken till a homogenous milky emulsion forms		Time taken by emulsion for separation (min)	
		Mean	SD	Mean	SD
1	15.00	3.6	0.21	42.7	2.08
2	13.92	3.2	0.15	47.0	2.65
3	12.84	3.0	0.06	58.0	2.00
4	12.38	3.0	0.07	59.0	1.90
5	11.76	5.3	0.26	45.7	1.53
6	10.68	6.1	0.31	43.7	2.52
7	9.60	8.1	0.25	37.0	2.65
8	8.52	9.4	0.35	32.3	2.52
9	7.44	12.0	0.25	28.7	3.06
10	6.36	12.4	0.31	22.7	2.52
11	5.28	16.3	0.36	18.0	2.65
12	4.20	No emulsification		2.3	0.58

Under the HLB system, it was found that the oils, waxes, and other materials likely to be incorporated in to emulsion had an individual required HLB. This means that a surfactant or blend of surfactant/co-surfactant, having desired RHLB will make more stable emulsion than the emulsifier of any other HLB value.

5.3.2.3 Required chemical type of Emulsifiers

Ease of preparation and time for separation was determined and compared with the emulsion prepared using Cremophor RH 40 and Transcutol-P mixtures. Number of times the test tubes shaken till a homogenous milky emulsion formed and time of separation for Capmul MCM emulsion prepared using surfactant/co-surfactant blend of same HLB but different chemical type was shown in Table 5.6.

Table 5.6: Number of times the test tube shaken till a milky emulsion forms and time for separation for Capmul MCM emulsion prepared using surfactant/co-surfactant blend of same HLB but different chemical type

Sr. No.	Surfactant/Co-surfactant blend and HLB	Number of times the test tubes shaken for emulsification		Time taken by emulsion for separation (min)	
		Mean	SD	Mean	SD
1	Cremophor RH 40 and Transcutol-P, 12.38	3.0	0.07	59.0	1.90
2	Labrasol and Transcutol-P, 12.38	4.0	0.25	51.3	1.53

The mixture of Labrasol and Transcutol-P having HLB 12.38 gave similar results for ease of preparation and time for separation (no significant difference) as that of mixture of Cremophor RH 40 and Transcutol-P having similar HLB. The 75:25 mixture of Cremophor RH 40 and Transcutol-P having HLB 12.38 was selected as surfactant/co-surfactant blend for further study.

5.3.2.4 Solubilization Capacity

Reverse micelle systems have been an interesting area of research in several fields of science and technology, due to their capability to solubilize water in organic solvent in the presence of surfactant [27]. It is known that ethoxylated non-ionic hydrophilic surfactants tend to form reverse micelles in organic media [28]. The results for the reverse micelle systems in this study formed by screening series surfactants/co-surfactant were shown in Table 5.7. Cremophor RH 40/Transcutol-P (3:1) showed a high solubilization capacity compared with other S/CoS Ratio. Cremophor RH 40 (Polyoxyl 40 hydrogenated castor

oil) is a non-ionic solubilizer and emulsifier made by reacting hydrogenated castor oil with ethylene oxide in a molar ratio of 1: 40.

Table 5.7: The solubilization capacity of selected surfactants and surfactant/co-surfactant blends

Drug	Surfactant/Co-surfactant	HLB	Solubilization capacity
Fenofibrate	Labrasol/Transcutol-P (1: 1)	8.1	29.688
	Labrasol/Transcutol-P (2: 1)	9.4	23.750
	Labrasol/Transcutol-P (3: 1)	10.1	12.180
	Cremophor RH 40/Transcutol-P (1: 1)	9.6	27.457
	Cremophor RH 40/Transcutol-P (2: 1)	11.4	20.652
	Cremophor RH 40/Transcutol-P (3: 1)	12.3	11.050
Atorvastatin Calcium	Labrasol/Transcutol-P (1: 1)	8.1	12.838
	Labrasol/Transcutol-P (2: 1)	9.4	11.047
	Labrasol/Transcutol-P (3: 1)	10.1	9.694
	Cremophor RH 40/Transcutol-P (1: 1)	9.6	12.179
	Cremophor RH 40/Transcutol-P (2: 1)	11.4	10.106
	Cremophor RH 40/Transcutol-P (3: 1)	12.3	8.796

Labrasol/Transcutol-P (1:1) showed the lowest solubilization capacity (showed highest fraction of surfactant/co-surfactant i.e. 29.688) compared with Cremophor RH 40/Transcutol-P (3:1) (showed lowest fraction of surfactant/co-surfactant i.e. 11.050). This indicated a weak interaction between the oil and surfactant/co-surfactant from the same fatty acid derivative. The results of this study were consistent with the study showing that the maximum solubilization capacity of water depends upon the oxyethylene chain and the configuration of the polar head group and hydrocarbon moiety of non-ionic surfactants and on type of oil [25].

The results for the solubilization capacity of blends of surfactants/co-surfactant showed that Cremophor RH 40/Transcutol-P (3:1) at HLB 12.3 has the highest solubilization capacity compared with the Labrasol/Transcutol-P (3:1) at HLB 10.1. These results indicated the importance of the more lipophilic tail group that is structurally similar to the group on the Capmul MCM oil, which enables the co-surfactants to be well packed at the

interface. Thus, these results reflected the effect of the type of co-surfactant blend on the solubilization capacity. The high solubilization capacity was obtained when surfactant/co-surfactant having the highest and lowest HLB value were mixed together, as shown by the solubilization capacity result for Cremophor RH 40/Transcutol-P (3:1) compared with the Labrasol/Transcutol-P (3:1) blend (Table 5.7). The results of the study indicated the importance of selection of a better surfactant/co-surfactant blend showing strong solubilization capacity, which accordingly gives high stability.

5.3.3 Pseudo ternary phase diagrams

Pseudo Ternary phase diagrams were constructed in presence of Fenofibrate or Atorvastatin Calcium to obtain optimum concentrations of oil, water, surfactant, and co-surfactant. SNEDDS formed fine oil–water emulsions with only gentle agitation, upon its introduction into aqueous media. Phase behaviour investigations of this system demonstrated suitable approach to determining water phase, oil phase, surfactant concentration, and co-surfactant concentration with which transparent, one phase low-viscous nanoemulsion system was formed [36]. Since free energy required to form an emulsion is very low, formation is thermodynamically spontaneous [28]. Surfactants form a layer around emulsion droplets and reduce interfacial energy as well as providing a mechanical barrier to coalescence. The visual test measured the apparent spontaneity of emulsion formation. Microscopic examination was made of the final mixtures to identify the type of emulsion obtained using water-soluble dyes (Congo red). Figure 5.3 showed microscopic images of O/W emulsion using water soluble dyes (Congo red).

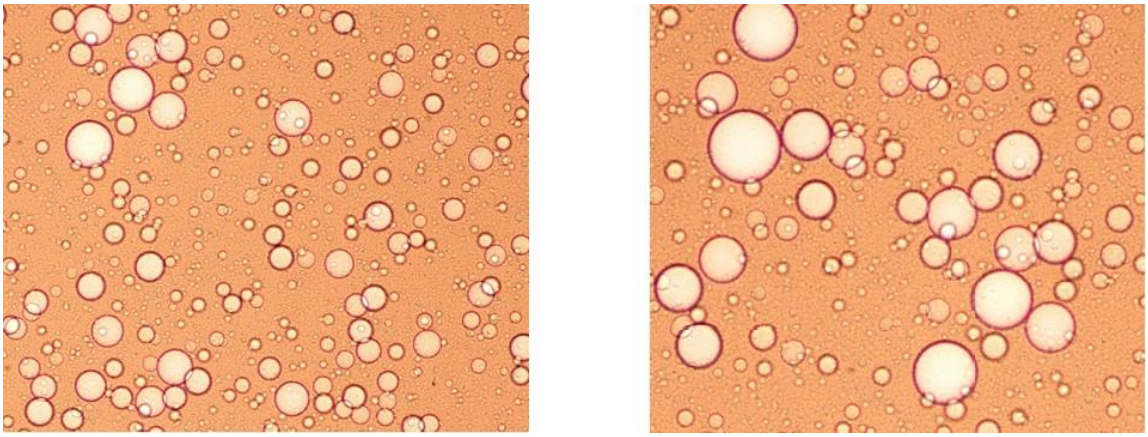

Figure 5.3: Microscopic images of O/W emulsion using water soluble dyes (Congo red)

Figure 5.4-5.15 presented the pseudo ternary phase diagram for mixtures of Capmul MCM oil, S/CoS (Labrasol/Transcutol-P and Cremophor RH 40/Transcutol-P) and water at various component compositions. All types of dispersions, including conventional w/o and o/w emulsions, w/o and o/w microemulsion, can be formed by S/CoS mixtures. A large area of clear isocratic solution (o/w microemulsion/nanoemulsion) was formed at the oil-S/CoS axis in oil-rich regions. The minimum content of Cremophor RH 40/Transcutol-P (3:1) at an HLB of 12.3 formed in an isocratic system was 11.05% (Fenofibrate) and 8.796% (Atorvastatin Calcium). This minimum content of surfactant/co-surfactant in a microemulsion or nanoemulsion system is known as the surfactant solubilization capacity [25]. The smaller the percentage of S/CoS in a microemulsion/nanoemulsion system, the higher the solubilization capacity of the S/CoS, the better the match of the oil and S/CoS HLB, and hence the higher the stability of the product. Based on solubilization capacity, Cremophor RH 40/Transcutol-P (3:1) was selected as the best S/Cos.

The larger area of o/w microemulsion/nanoemulsion formed by Cremophor RH 40/Transcutol-P (3:1) was due to the large molecular packing ratio of Cremophor RH 40/Transcutol-P, which was classified as a strong solubilizer [37, 38]. Recent research has also suggested that the solubilization capacity and formation of o/w microemulsion/nanoemulsion was caused by the extent of packing at the interface and not because of the HLB or the specific hydrophobicity of the surfactants [28].

The main disadvantage of microemulsion/nanoemulsion systems is the lack of biocompatibility due to high surfactant(s) concentrations which might lead to toxicity or skin irritation [39, 40]. Use of Capmul MCM oil that form a reverse micelle system in any formulation can overcome the lack of biocompatibility of such microemulsion/nanoemulsion systems because a low concentration of S/Cos is used.

Figures 5.4-5.15 showed the behaviours of surfactant/co-surfactant blends of Cremophor RH 40/Transcutol-P (with HLB values of 9.6, 11.3, and 12.3), Capmul MCM oil, and water at various concentration levels. The dispersion systems formed by these mixtures had reflected the nature and behaviour of their component compositions. The dispersion systems in the phase diagrams differed geometrically from Labrasol/Transcutol-P phase diagram. They showed much smaller areas of oil-in-water microemulsion/nanoemulsion

compared with Cremophor RH 40/Transcutol-P (HLB 12.3). They also showed variation in area for the microemulsion system and other types of dispersion. Cremophor RH 40/Transcutol-P (3:1) at an HLB of 12.3 formed a large o/w microemulsion/nanoemulsion area. The smaller area of o/w microemulsion/nanoemulsion was due to a lower HLB, which increases the lipophilic character of the surfactant blend [38]. It was also clear from the solubilization capacity results that the Cremophor RH 40/Transcutol-P (3:1) with an HLB of 12.3 was a stronger solubilizer for water in Capmul MCM oil than other blends of Cremophor RH 40/Transcutol-P and Labrasol/Transcutol-P with HLB values in the range of 8.1–12.3. The weak interaction between the oil and S/CoS at lower HLB values for forming a reverse micelle system was due to the weaker solubilization of water at the interface in the presence of high percentages of lipophilic surfactant in the blends. However, excessive amount of co-surfactant will cause system to become less stability for its intrinsic high aqueous solubility and lead to droplet size increasing as a result of expanding interfacial film [41, 42].

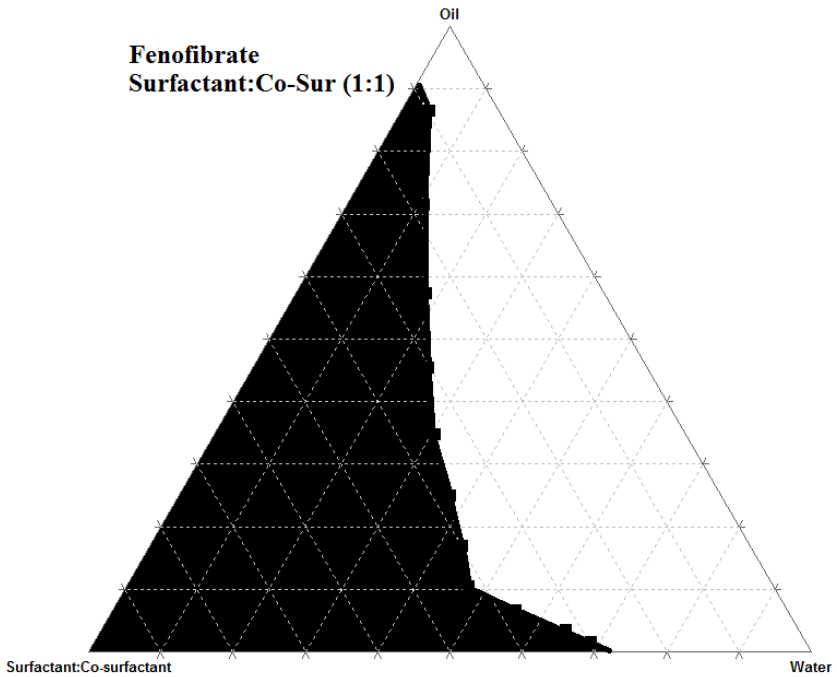

Figure 5.4: S/Cos (Labrasol/Transcutol-P (1:1) at HLB - 8.1)

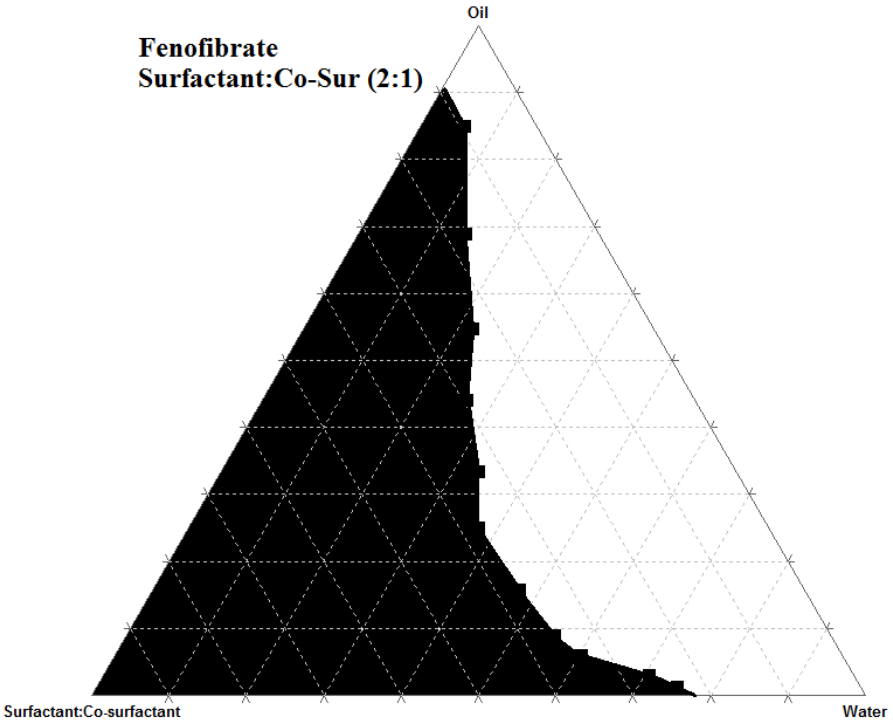

Figure 5.5: S/Cos (Labrasol/Transcutol-P (2:1) at HLB – 9.4)

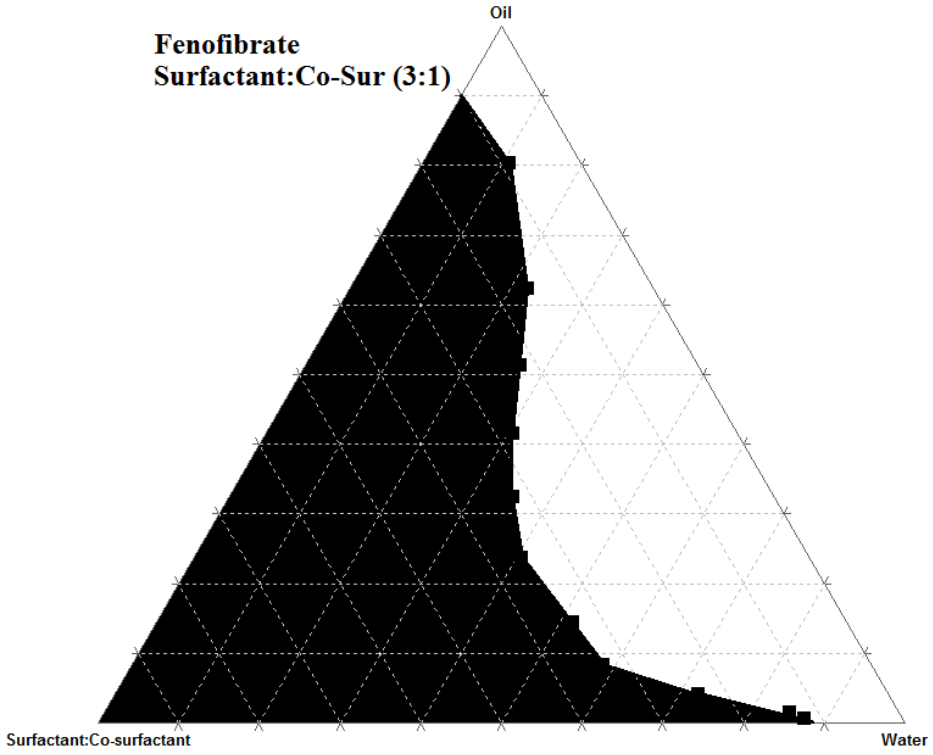

Figure 5.6: S/Cos (Labrasol/Transcutol-P (3:1) at HLB – 10.1)

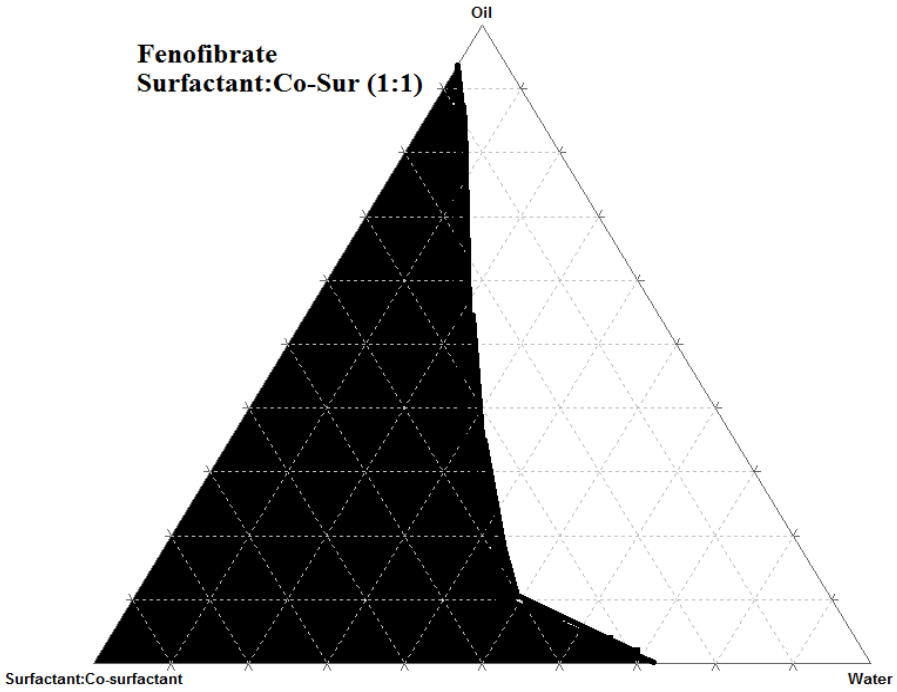

Figure 5.7: S/Cos (Cremophor/Transcutol-P (1:1) at HLB – 9.6)

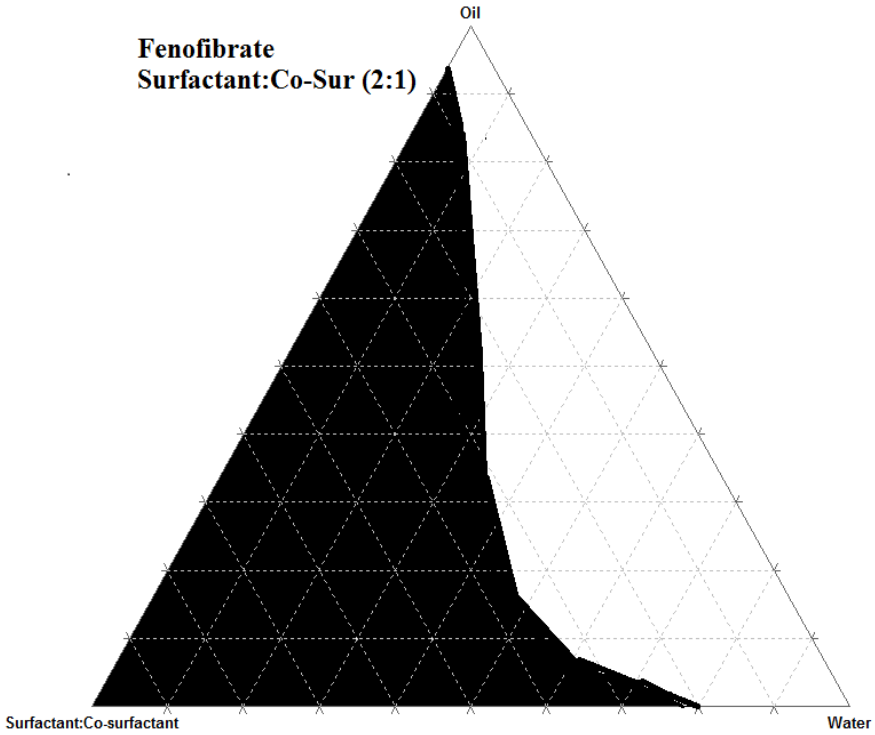

Figure 5.8: S/Cos (Cremophor/Transcutol-P (2:1) at HLB – 11.3)

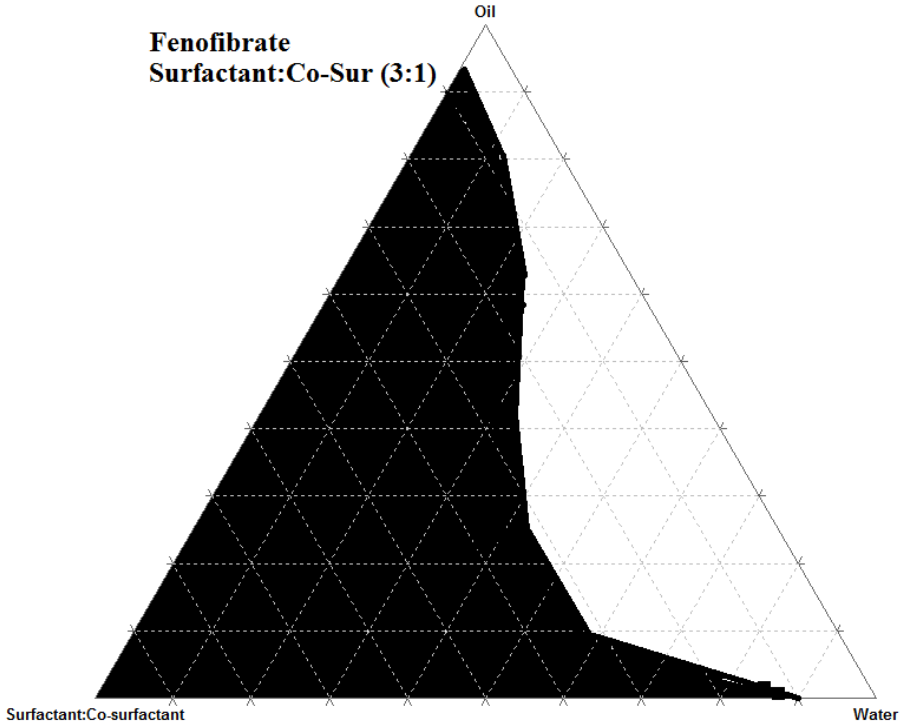

Figure 5.9: S/Cos (Cremophor/Transcutol-P (3:1) at HLB – 12.3)

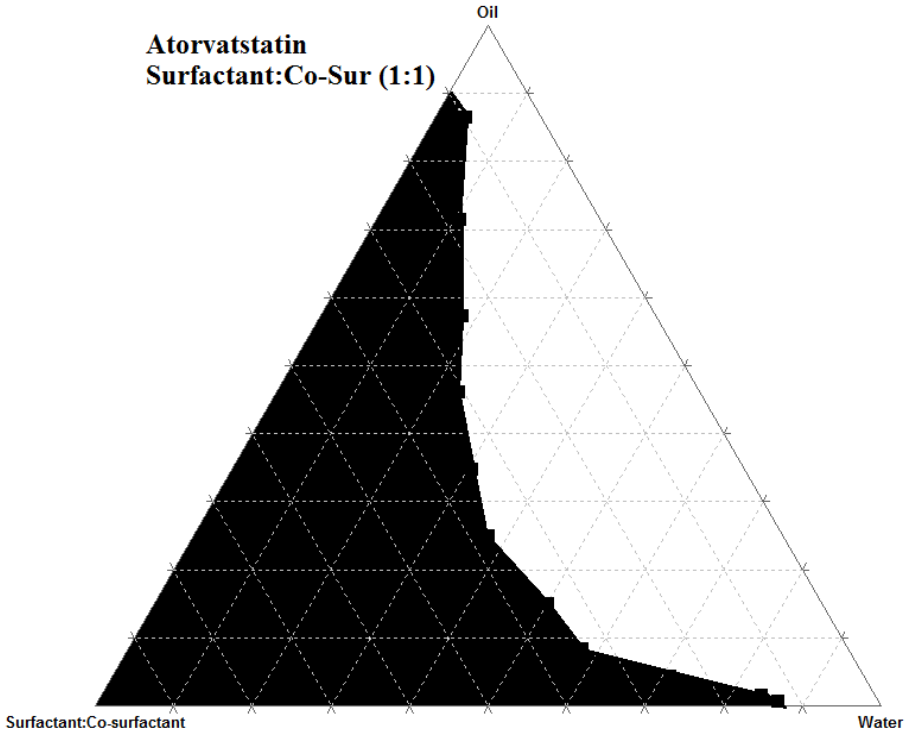

Figure 5.10: S/Cos (Labrasol/Transcutol-P (1:1) at HLB - 8.1)

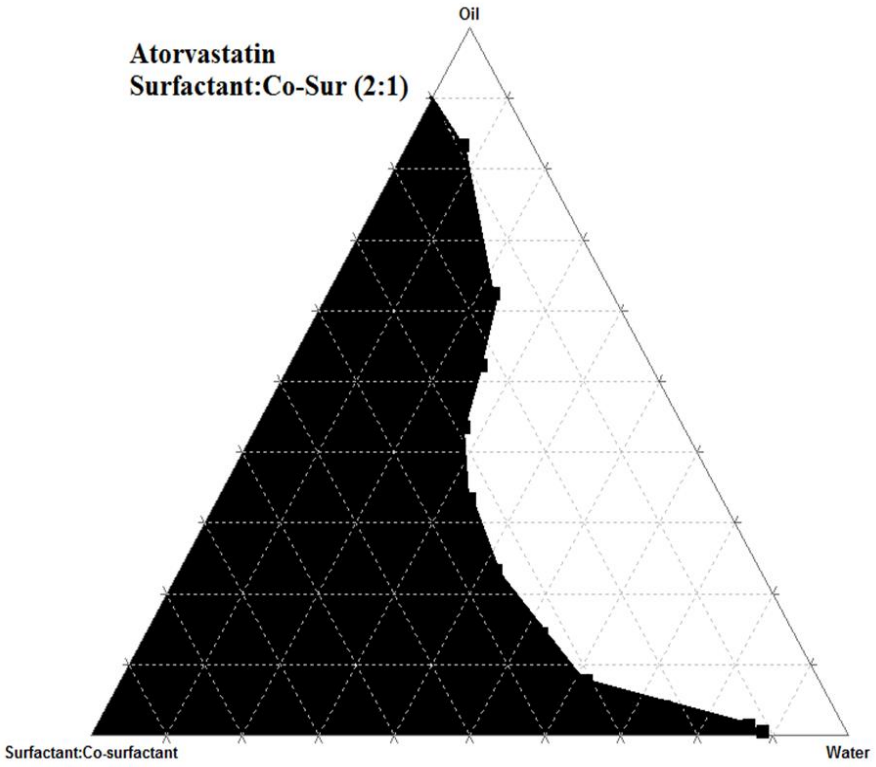

Figure 5.11: S/Cos (Labrasol/Transcutol-P (2:1) at HLB – 9.4)

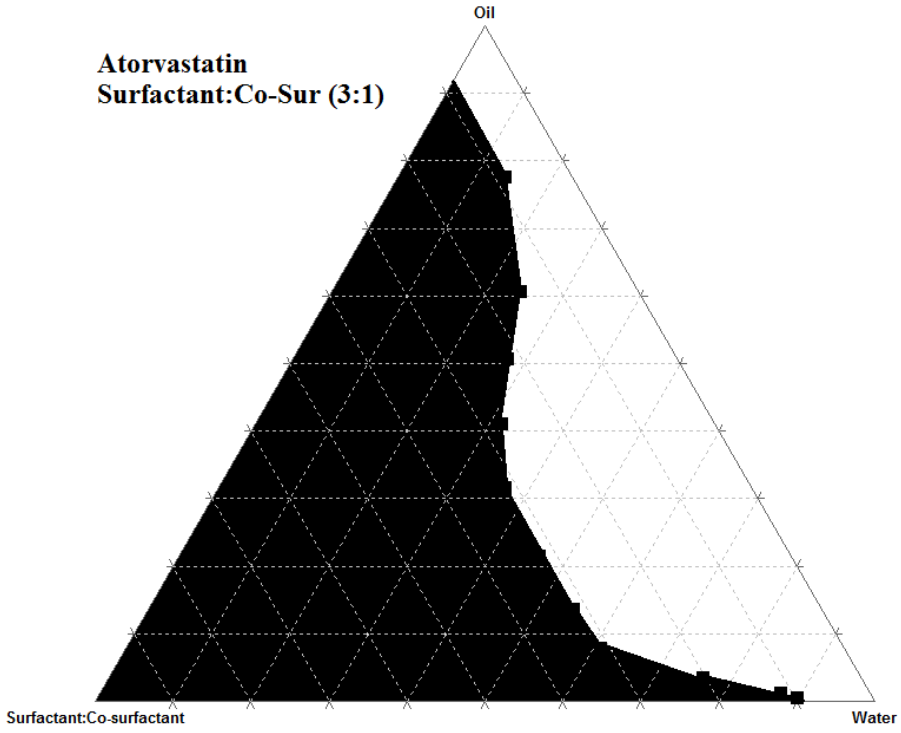

Figure 5.12: S/Cos (Labrasol/Transcutol-P (3:1) at HLB – 10.1)

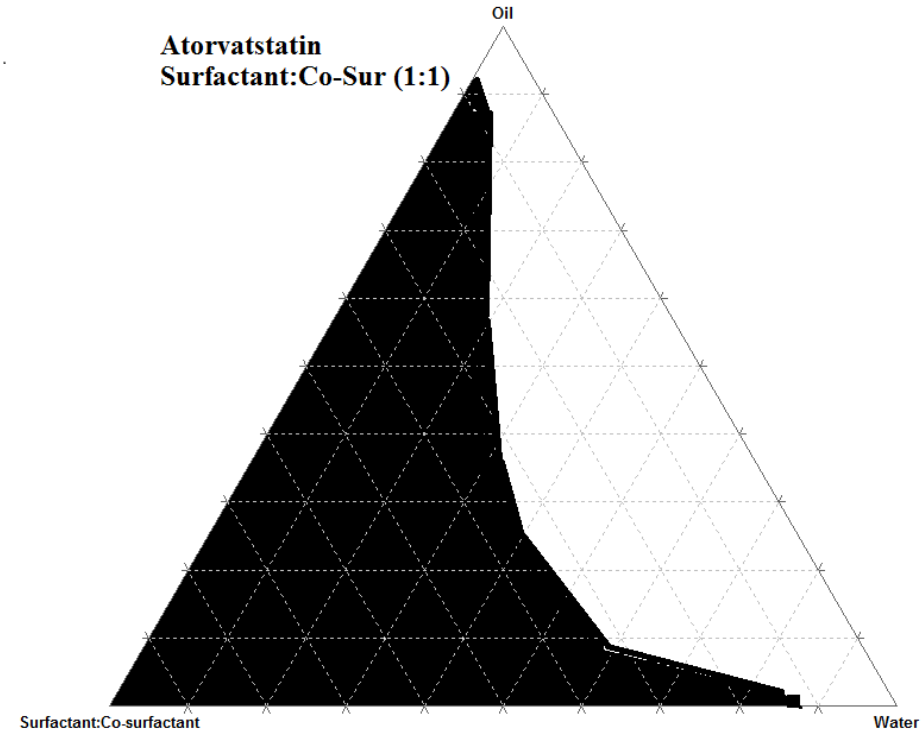

Figure 5.13: S/Cos (Cremophor/Transcutol-P (1:1) at HLB – 9.6)

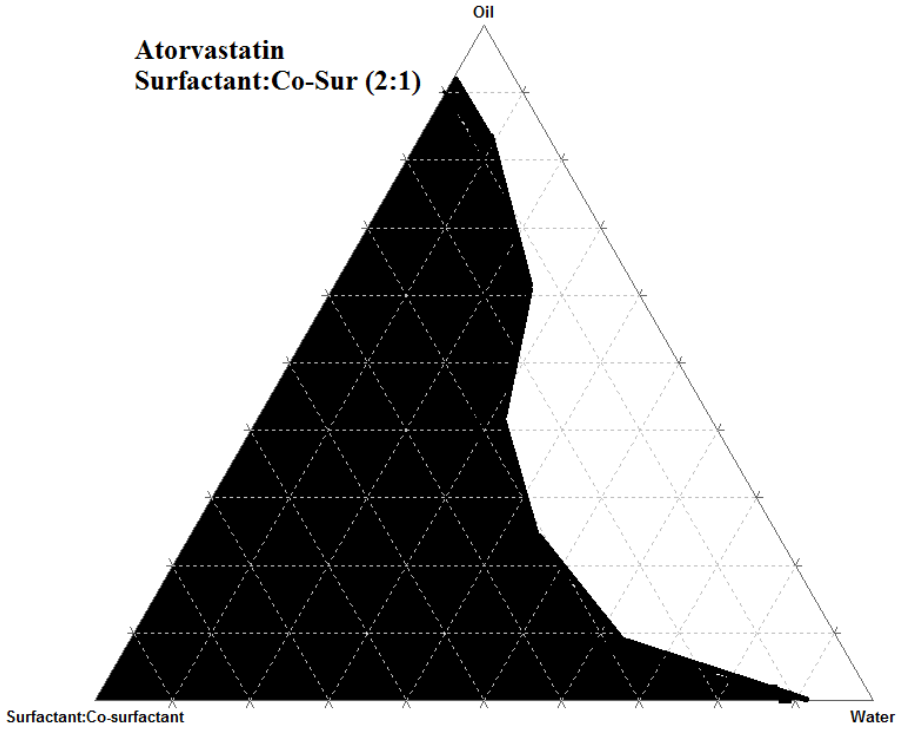

Figure 5.14: S/Cos (Cremophor/Transcutol-P (2:1) at HLB – 11.3)

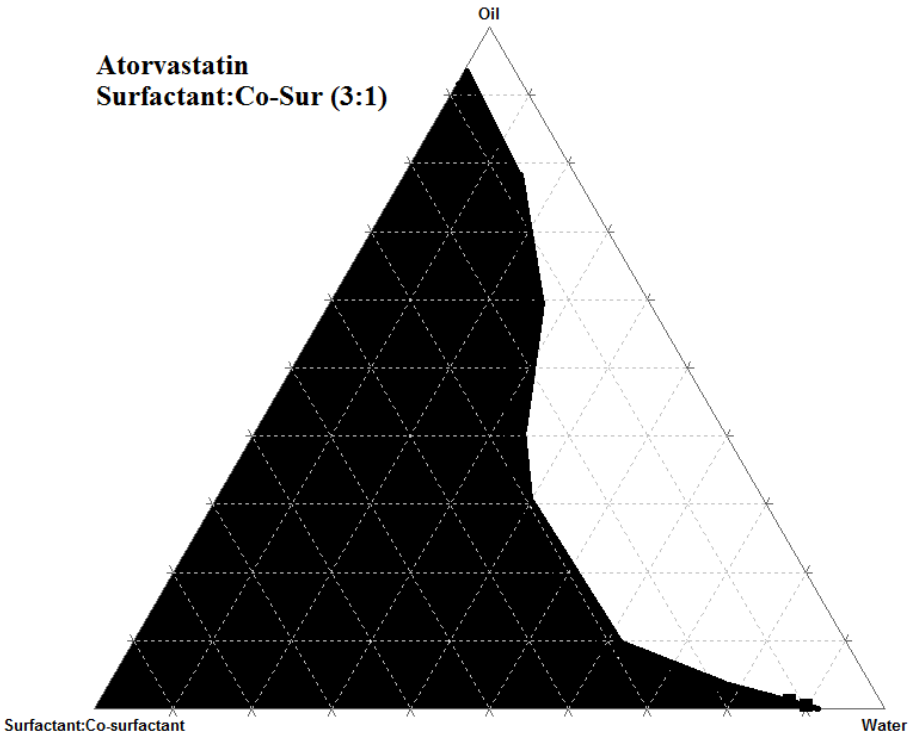

Figure 5.15: S/Cos (Cremophor/Transcutol-P (3:1) at HLB – 12.3)

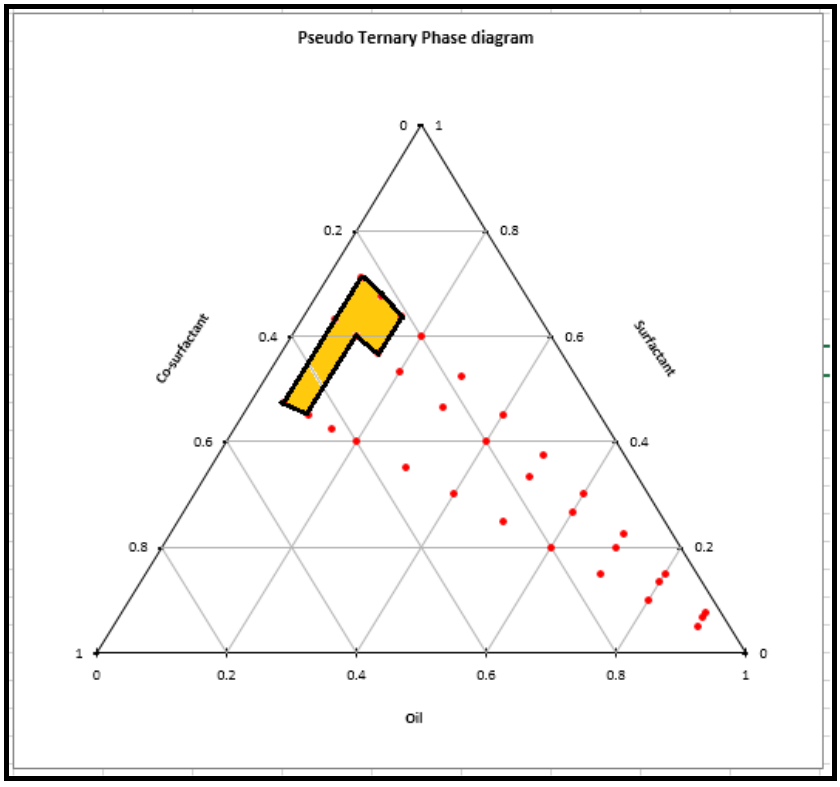

Figure 5.16: Pseudo-ternary phase diagram indicating the efficient self-emulsification region (key, the region of efficient self-emulsification is bound by the solid line)

The pseudo ternary phase diagram of the system comprising the surfactant, co-surfactant and the oily phase was constructed and was given in Figure 5.16. The Area enclosed within the solid line represented the region of self-emulsification. Within this area a ternary mixture formed a fine oil in water emulsion with only gentle agitation. This is possible as surfactants strongly localized to the surface of the emulsion droplet reduces interfacial free energy and provide a mechanical barrier to coalescence resulting in a thermodynamically spontaneous dispersion. The co-surfactants increased interfacial fluidity by penetrating into the surfactant film creating void space among surfactant molecules.

5.4 Conclusion

SNEDDS are isotropic mixtures made up of oil, surfactant and sometimes co-surfactant or co-solvent. In an aqueous environment a homogeneous, transparent (or at least translucent), isotropic and thermodynamically stable dispersion will result up on mild agitation. SNEDDS is best suited for dosage for development of poorly soluble drugs. The solubility parameter (δ), Required HLB (RHLB), required chemical type of emulsifiers and solubilization capacity are useful criterion for the selection of surfactant/co-surfactant in the formulation development of SNEDDS using Fenofibrate and Atorvastatin Calcium.

The purpose of the present study was to select appropriate lipid vehicle and to understand role of lipid vehicle in pseudo ternary phase diagram behaviour to find nanoemulsion area in formulation development of self-nanoemulsifying drug delivery system (SNEDDS) containing Fenofibrate and Atorvastatin Calcium. The solubility parameter (δ), Required HLB (RHLB), required chemical type of emulsifiers and solubilization capacity were determined for selection of blend of surfactant/co-surfactant. The pseudo ternary phase diagrams for mixtures of Capmul MCM oil with non-ionic surfactant/co-surfactant and water were constructed in the study. The study showed the importance of selecting a surfactant with the proper HLB for specific oil, and also the type of surfactant/co-surfactant. The solubility parameter (δ) of Fenofibrate and Atorvastatin Calcium was found closest to the solubility parameter (δ) of Capmul MCM. Blend of better surfactant/co-surfactant was obtained when surfactant and co-surfactant at higher and lower HLB level respectively were blended. The greater the difference between the hydrophilic and lipophilic surfactants, the better the coverage by blends at the interface. The study also showed the importance of the structural similarities between the lipophilic tails of the surfactant blends.

The pseudo ternary phase diagrams of the systems containing the surfactant co-surfactant and the oily phase were constructed. The area enclosed within the solid line represented the region of self-emulsification. Within this area, a ternary mixture formed a fine oil in water emulsion with only gentle agitation. This was possible as surfactants strongly localized to the surface of the emulsion droplet reduced interfacial free energy and provided a mechanical barrier to coalescence resulting in a thermodynamically spontaneous dispersion. The co-surfactants increased interfacial fluidity by penetrating

into the surfactant film creating void space among surfactant molecules. The SNEDDS have potential applications, advantages, and usefulness in the pharmaceutical industry as SNEDDS by different routes of administration, as well as in personal care and cosmetics products. Solubility Parameter (δ), Required HLB (RHLB), required chemical type of emulsifiers and Solubilization capacity were appeared to be useful as a criterion for the selection of surfactant/co-surfactant along with pseudo ternary phase diagrams in formulation development of SNEDDS.

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CHAPTER – 6

Formulation and Development of Fenofibrate and Atorvastatin Calcium SNEDDS Using 3^2 Factorial Design

6. Formulation and Development of Fenofibrate and Atorvastatin Calcium SNEDDS Using 3^2 Factorial Design

6.1 Introduction

SNEDDS are isotropic mixtures made up of oil, surfactant and cosurfactant or cosolvent. In an aqueous environment, a homogeneous, transparent (or at least translucent), isotropic and thermodynamically stable dispersion will result up on mild agitation. SNEDDS is best suited for dosage for development of poorly soluble drugs. According to the Biopharmaceutical Classification System (BCS), drugs with poor solubility fall either in BCS class II or BCS class IV. BCS class II drug showed poor solubility and good permeability and its bioavailability problems can be overcome by improving the solubility. It provides benefits to BCS Class II and IV drugs, which exhibits poor aqueous solubility and also for drugs having a log P value greater than 2 [1]. SNEDDS possess many advantages over other colloidal drug carriers and extensive research relative to their production characterization and efficacy is carried out now-a-days excellently and efficiently. These are successful examples of product launched in to market by using SNEDDS formulation.

Fenofibrate decreased elevated serum total and LDL-cholesterol, triglyceride, and Apo lipoprotein B (Apo B) concentrations, and to increase HDL-cholesterol concentrations in

the management of primary hypercholesterolemia and mixed dyslipidemia, including heterozygous familial hypercholesterolemia and other causes of hypercholesterolemia [2]. It exhibits additive antilipidemic effects when used concomitantly with other antilipidemic agents. Fenofibrate shows bioavailability problems due to poor water and physiological fluids solubility (Practically insoluble in water, BCS Class-II drugs). Fenofibrate shows increase in absorption in fed condition of patient compare to fasting condition of patient.

Atorvastatin inhibits HMG-CoA reductase, causing subsequent reduction in hepatic cholesterol synthesis. It reduces serum concentrations of total cholesterol, LDL-cholesterol, VLDL-cholesterol, apo B, and triglycerides [3]. Atorvastatin shows low aqueous solubility and rapidly absorbed after oral administration. Food decreases the rate and extent of drug absorption by about 25% and 9% respectively.

The clinical guideline indicated that not only fibrate therapy, but also a combination therapy with fenofibrate and a statin should be the most effective means of cholesterol and lipid management. The atorvastatin/fenofibrate fixed-combination preparation reported the excellent results rather than use of single drug [4]. Such a combination therapy can only be achieved by the use of two separate products, i.e. the patient needs to take one fenofibrate tablet together with another tablet or capsule containing a statin [4]. Hence, SNEDDS formulation were considered for enhance solubility, release rate and oral absorption of poorly soluble antilipidemic drugs. Since the first step in oral absorption process is dissolution of the drug compound in gastrointestinal lumen contents, poor aqueous solubility is rapidly becoming the leading hurdle for formulation scientist working on oral delivery of such drug compounds.

Childhood dyslipidemia is recognized as a vital risk issue for adult cardiovascular disease. American Academy of Paediatrics published a clinical report concerning prevention, screening, diagnosis and treatment of dyslipidemia in children [5]. Difficulty in swallowing – dysphagia – has been diagnosed in 35% of people aged over 50 and frequently appears after stroke and in older people with dementia, Parkinson's disease and many other conditions. So, liquid SNEDDS of Fenofibrate and Atorvastatin Calcium seem more convenient to paediatric patients and geriatric patients.

Literature reported Fenofibrate and Atorvastatin Calcium as practically insoluble in water as per US Pharmacopeia definition and both are BCS class II drug [2, 3]. Fenofibrate and Atorvastatin Calcium have low oral bioavailability, fed and fasted absorption variability and differential oral bioavailability based on age.

To the best of our knowledge, no information is available in the literature on the improvement of Fenofibrate and Atorvastatin Calcium dissolution and bioavailability using mixture of Capmul MCM oil, Cremophor RH 40 and Transcutol-P by SNEDDS methodology. The present research was aimed to explore SNEDDS formulation development using 3^2 factorial design for dissolution improvement compared to marketed formulation of Fenofibrate and Atorvastatin Calcium.

The present work described the formulation development of stable SNEDDS of Fenofibrate and Atorvastatin Calcium using concentration of Capmul MCM oil and Cremophor RH 40: Transcutol-P in 3:1 ratio on the basis of preliminary trials. The 3^2 factorial design was employed using concentration of Capmul MCM oil and Cremophor RH 40: Transcutol-P (3:1) as independent variables. The Globule size (GS), Polydispersity index (PDI), Zeta potential (ZP) and drug release at 15 minutes for Fenofibrate and Atorvastatin calcium were selected as dependent variables. The optimized batch was selected on the basis of arbitrary criteria using Design Expert software employing overlay plot with desirability approach. Contour and response surface plots were constructed for the responses.

A check point analysis was performed to validation the evolved polynomial equations in the formulation development of Fenofibrate and Atorvastatin Calcium SENDDS. Difference of theoretically computed values of GS, PDI, ZP and drug release at 15 minutes for Fenofibrate and Atorvastatin calcium and the mean values of experimentally obtained GS, PDI, ZP and drug release at 15 minutes for Fenofibrate and Atorvastatin calcium were compared by using Student’s t-test. Stability study for optimized SNEDDS containing Fenofibrate and Atorvastatin Calcium was performed as per ICH guidelines by keeping in stability chamber at room temperature (25°C) & 60% RH and accelerated condition (40°C) & 75% RH for 6 months [6, 7]. The optimized formulation was subjected to in vitro dissolution to evaluate improvement in drug release as compared to marketed product.

6.2 Materials and Methods

6.2.1 Materials used in Present Work

Sr. No.	Name of Material	Obtained From
1	Fenofibrate	Cadila healthcare Ltd., Ahmedabad, India
2	Atorvastatin Calcium	Cadila healthcare Ltd., Ahmedabad, India
3	Acetonitrile gradient HPLC	Merck, Mumbai, India
4	Castor Oil	Rankem, Mumbai, India
5	Ortho Phosphoric Acid	Rankem, Mumbai, India
6	Hydrochloric acid	Rankem, Mumbai, India
7	Labrafac PG	Gattefose, France
8	Oleic acid	S D Fine Chem Limited, Mumbai, India
9	Methanol HPLC grade	Merck, Mumbai, India
10	Capmul MCM Oil	Abitech, Mumbai, India
11	Propylene glycol AR	S D Fine Chem Limited, Mumbai, India
12	Poly Ethylene Glycol-400	S D Fine Chem Limited, Mumbai, India
13	Labrafac Lipophile WL 1349	Gattefose, France
14	Cremophor EL	BASF, Mumbai, India
15	Light Liquid Paraffin	Rankem, Mumbai, India
16	Span 20	LOBA Chem, Mumbai, India
17	Labrasol	Gattefose, France
18	Capmul GMO 50	S D Fine Chem Limited, Mumbai, India
19	Captex 355	Abitech, Mumbai, India
20	Transcutol-P	Gattefose, France
21	Cremophor RH 40	BASF, Mumbai, India
22	Tween-80	Merck, Mumbai, India
23	Acconon MCS-2	Abitech Mumbai, India
24	Chloroform	Merck, Mumbai, India
25	Sodium Lauryl Sulfate	Merck, Mumbai, India

6.2.2 Instruments and Apparatus used in present work

Sr. No.	Instruments/Apparatus	Company
1	Laboratory Centrifuge	REMI motors, Mumbai, India
2	Micropipettes	HiMedia, Mumbai, India
3	Abbe's Refractometer	Krishna scientific, Haryana, India
4	Digital balance	Sartorius Balance, Bangalore, India
5	Electronic balance	Mettler Toledo, Mumbai, India
6	pH meter	Lab India, Mumbai, India
7	Dissolution test apparatus	Electrolab Dissolution Tester TDT-06P, USP, Mumbai, India
8	Environmental shaker	Tempo instruments and equipment Pvt. Ltd., Mumbai, India
9	UV-Visible Spectrophotometer	UV-1700 Shimadzu Co., Japan
10	HPLC	Agilent Technologies, Mumbai, India and Dionex, Mumbai, India
11	Brookfield viscometer	Brookfield, USA
12	Dialysis membrane	HiMedia, Mumbai, India
13	Sonicator	Equitron, Mumbai, India
14	Stability chamber	Thermolab, Mumbai, India
15	Magnetic stirrer	Remi equipment Pvt. Ltd., Mumbai, India
16	Fourier Transform Infrared Spectroscopy	Shimadzu Corporation, Japan
17	Cyclomixer	Remi equipment Pvt. Ltd., Mumbai, India
18	Rotary Shaker	Remi equipment Pvt. Ltd., Mumbai, India
19	Zetasizer Nano ZS	Malvern Instrument, UK

6.2.3 Methods

6.2.3.1 Estimation of Fenofibrate and Atorvastatin Calcium

UV spectroscopic method was used for determination of Fenofibrate and Atorvastatin Calcium as described in Section 4.5 of Chapter 4 in details.

6.2.3.2 Solubility Study

Screening of excipients was done by determining the equilibrium solubility of Fenofibrate and Atorvastatin Calcium in different oils, surfactants and co-surfactants as described in Section 4.6 of Chapter 4 in details.

6.2.3.3 Drug-Excipient Compatibility of SNEDDS Formulations

Drug-Excipient Compatibility of SNEDDS Formulations was studied as per method described in Section 4.8 of Chapter 4 in details.

6.2.3.4 Method of Preparation of SNEDDS

Accurately weighed Fenofibrate and Atorvastatin Calcium were placed in a glass vial, and required quantity of oil, surfactant, and co-surfactant were added. The mixture was mixed by gentle stirring and vortex mixing at 40ºC on a magnetic stirrer at 200rpm, until Fenofibrate and Atorvastatin Calcium were dissolved. The mixture was stored at room temperature in closed container until further use [6].

6.2.3.5 Method of Optimization of Preliminary Parameters

In the present study, the important preliminary parameters were the selection of oil, surfactant and co-surfactant, concentration of oil, surfactant and cosurfactant, ratio of surfactant/cosurfactant in formulation development of Fenofibrate and Atorvastatin Calcium SNEDDS. Preliminary parameters were optimized by varying one parameter at a time, while keeping others constant, so that the effect of varied parameter could be evaluated. The parameters were optimized to achieve minimum globule size.

Selection of oil, surfactant and cosurfactant

The selection of variable was based on the results of solubility data for Atorvastatin Calcium and Fenofibrate in oils and surfactants/co-surfactants, emulsifying ability of surfactant/co-surfactant, predicting drug solubility factors such as solubility parameter (δ), Required HLB value, Molecular weight, required chemical type of emulsifiers, solubilization capacity, dielectric constant (ε), dipole moment (μ), excipient fatty acid chain length, surface tension, viscosity etc. Capmul MCM was found satisfactory as oil, Cremophor RH 40 and Transcutol-P were found best as surfactant and cosurfactant on

basis of solubility data. Selection of oil, surfactant and cosurfactant described in Section 5.3.2 of Chapter 5 in details.

Ratio of Surfactant to Cosurfactant

Selection of ratio of surfactant to cosurfactant is very important in formulation development of SNEDDS. Selection was based on the results of solubility data for Fenofibrate and Atorvastatin Calcium in surfactants/co-surfactants, emulsifying ability of surfactant/co-surfactant, predicting drug solubility factors such as solubility parameter (δ), Required HLB value, Molecular weight, required chemical type of emulsifiers, solubilization capacity and Pseudo ternary phase diagram. Ratio of Cremophor RH 40: Transcutol-P was selected as 3:1 and described in Section 5.3.3 of Chapter 5 in details.

Preliminary Trials

After selection of appropriate lipid vehicle, Capmul MCM was selected as oil, and Cremophor RH 40: Transcutol-P (3:1) was selected as surfactant/co-surfactant mixture to found suitable concentration of both in formulation development of SNEDDS of Fenofibrate and Atorvastatin Calcium. The efficiency of self-emulsifying systems was measured from the rate of emulsification upon hydration with mild agitation. The time taken for the formation of fine emulsion was noted as dispersion time. Preliminary batches were formulated and their results of dispersion status as clear, turbid or clear and translucent in SNEDDS and dispersion time were recorded. Clear and transparent dispersion status and minimum dispersion time were required.

6.2.3.6 3^2 factorial design for optimization of formulation parameters of Fenofibrate and Atorvastatin Calcium SNEDDS

The preliminary trials were carried out using different concentration of Capmul MCM oil, Cremophor RH 40 and Transcutol-P (3:1). On the basis of results of preliminary trials for selection of lipid vehicle, the concentration of Capmul MCM oil (X_1) and Concentration of Cremophor RH 40: Transcutol-P (3:1) (X_2) were taken as independent variables at three levels. The Globule size (GS) (Y_1), Polydispersity index (PDI) (Y_2), Zeta potential (Y_3), drug release at 15 minutes of Fenofibrate (Y_4) and drug release at 15 minutes of Atorvastatin Calcium (Y_5) were considered to play significant role in the formulation performance of SNEDDS and all the five were taken as dependent parameters in present study. Multiple regression analysis, contour plot and 3D response surface plot were used

to study the main and interaction effects of the variables on the responses. The responses were measured for each trials (n=3) and then either simple linear Equation (6.1), or interactive Equation (6.2) or quadratic Equation (6.3) model was fitted by carrying out multiple regression analysis and F-statistics to identify statistically significant term [8, 9].

$Y = b_0 + b_1X_1 + b_2X_2$ …….. (Equation 6.1)

$Y = b_0 + b_1X_1 + b_2X_2 + b_{12}X_1X_2$ ……...…….. (Equation 6.2)

$Y = b_0 + b_1X_1 + b_2X_2 + b_1^2X_{11} + b_2^2X_{22} + b_{12}X_1X_2$ …….…….. (Equation 6.3)

Where Y is the dependent variable (Globule size, Polydispersity index, Zeta potential or drug release), while b_0 is the intercept, bi (b_1 and b_2) are coefficient of main effects, bi^2 (b_1^2 and b_2^2) are the quadratic terms, and bij (b_{12}) is the interaction terms. Microsoft EXCEL was used to identify non-significant terms. A coefficient is significant if ti>tcrit(v), where v denotes the degrees of freedom of residual variance. The refined model may be used for calculating the residuals or for drawing the contour plot.

Contour Plot

Contour plot is a diagrammatic representation of the values of the response and it is helpful in explaining visually the relationship between independent and dependent variables. The reduced model was used to plot two dimension contour plot using demo version of Design Expert 11 software.

Response Surface Plot

Response surface plot is helpful in understanding the main and the interaction effects of variables in the formulation development. The effect of level of independent variable on the response parameter can be understood from the respective response surface plot.

Optimization of SNEDDS formulation using overlay plot by Design Expert software

The desirability function approach is a technique for the simultaneous determination of optimum settings of input variables that can determine optimum performance levels for one or more responses [10]. The desirability procedure involves two steps:

(1) Finding the levels of the independent variables that simultaneously produce the most desirable predicted responses on the dependent variables.

(2) Maximize the overall desirability with respect to the controllable factors.

A desirability function, D(Y), is typically a (weighted) geometric mean of *n* individual desirability functions, di(yi), one for each element, yi of Y. Each di(yi) value is converted from associated response yi and scaled to be between 0 and 1. With a value of zero indicating unacceptable quality and 1 point out that the quality of associated response is optimal. A general form of mathematical relationship of responses with desirability function is as follows [10];

$$\max D(Y) = (d_1(y_1)^{k1} \times d_2(y_2)^{k2} \times \ldots \times d_n(y_n)^{kn})^{(1/\Sigma i ki)} \quad \text{.....(Equation 6.4)}$$

Where, y_i denote the determined value of response i, $d_i(y_i)$ is the converted desirability value of i'th response and k_i represent the relative importance of response i compared to others. If all responses have the same importance, then D(Y) become a geometric mean of all *n* transformed responses without weights. Overall desirability value can only be close to 1 if all of the responses are close to their optimal values, because D(Y) is a geometric mean of the $d_i(y_i)'$s. Likewise, D(Y) will be small if any of the $d_i(y_i)'$s are sufficiently close to zero. In consequence, to optimize responses simultaneously, one seeks to find values of x to maximize D(Y) [8]. The optimization of SNEDDS formulation was performed using Design Expert software employing overlay plot with desirability approach.

Checkpoint Analysis

A check point analysis was performed to validation the evolved polynomial equations in the formulation development of Fenofibrate and Atorvastatin Calcium SNEDDS. Each batch was prepared three times and mean value of responses was determined. Difference of theoretically computed values of GS, PDI, Zeta potential and drug release at 15 minutes for Fenofibrate and Atorvastatin Calcium and then mean values of experimentally obtained GS, PDI, Zeta potential and drug release at 15 minutes for Fenofibrate and Atorvastatin Calcium were compared by using Student's t-test.

6.2.3.7 Measurement of evaluation parameters of Fenofibrate and Atorvastatin Calcium SNEDDS Formulations

(i) Measurement of Globule Size, Polydispersity Index (PDI) and Zeta Potential

Globule size, Polydispersity index (PDI) and zeta potential of SNEDDS were determined using Zetasizer Nano ZS (Malvern Instruments, UK), which follows principle of LASER

light diffraction. SNEDDS was added (after suitable dilution) to the sample cell and put into the sample holder unit and the measurements were carried out with the help of software of same instrument.

(ii) In-Vitro Drug Release Study

In vitro drug release study was carried out for the formulations, marketed product and active drug substance using USP Type II dissolution test apparatus (Electrolab TDT-06P, India). The dissolution medium (900 ml water) maintained at 37 ± 0.5°C and rotated at 50rpm. Aliquots were collected periodically (10, 15, 20, 30, 45, 60 minutes) and replaced with fresh dissolution medium. Aliquots, after filtration through 0.45µ PVDF filter paper, were analyzed by HPLC at 248nm for Fenofibrate and Atorvastatin Calcium content.

Fenofibrate and Atorvastatin Calcium SNEDDS Formulation was evaluated by other parameters such as %Transmittance, Refractive index, effect of dilution, Viscosity, pH, self-emulsification and precipitation, centrifugation and freeze-thaw cycle, and in-vitro drug diffusion as described in Section 4.10 of Chapter 4.

6.2.3.8 Stability Study of Fenofibrate and Atorvastatin Calcium SNEDDS

Chemical and physical stability of Fenofibrate and Atorvastatin Calcium SNEDDS was assessed at 40 ± 2°C/75 ± 5% RH and 25 ± 3°C/60 ± 5% (room temperature) as per ICH guidelines [6, 7]. Stability study of SNEDDS formulation was carried out as described in Section 4.10 of Chapter 4.

6.2.3.9 Comparison of in vitro drug release between Optimized SNEDDS formulation, pure drug powder and marketed product

In vitro drug release study was performed as method described in Section 4.10 of Chapter 4 for optimized SNEEDS formulations, marketed product and active drug substance to compare the in vitro drug release profile.

Similarity Factor (f_2)

Mathematical comparison of dissolution data to quantify observed differences in the rate and extent of drug release as influenced by formulation and process variables was performed according to the model-independent approach. A similarity factor (f_2) was calculated from mean dissolution data. The value of similarity factor (f_2) between 50 and

100 suggests that the two dissolution profiles are similar. The similarity factor (f2) was described in detailed in Section 4.10.12 of Chapter 4.

Dissolution Efficiency

The dissolution efficiency of the batches was calculated by the method mentioned by Khan. It is defined as the area under the dissolution curve up to a certain time, t, expressed as a percentage of the area of the rectangle described by 100% dissolution in the same time. The dissolution efficiency was described in detailed in Section 4.10.13 of Chapter 4.

6.3 Results and Discussion

6.3.1 Optimization of preliminary parameters

6.3.1.1 Screening of lipid vehicle using solubility method

Screening of SNEDDS formulation involves formulation composition should be simple, safe, non-toxic and compatible. It should possess good solubility and large efficient self-nanoemulsification region which should be found in pseudo-ternary phase diagram, and have efficient droplet size after forming nanoemulsion [11-13]. Vehicles should have good solubilizing capacity of drug substance, which is essential for composing SNEDDS. The results of solubility of Fenofibrate and Atorvastatin Calcium in various vehicles were presented in Table 6.1.

Table 6.1: Solubility of Fenofibrate and Atorvastatin Calcium in various oil, surfactant and co-surfactant

Material	**Solubility (mg/ml) ± SD**	
	Fenofibrate	**Atorvastatin Calcium**
Castor Oil	72.18 ± 0.15	11.60 ± 0.06
Labrafac PG	58.85 ± 0.14	28.14 ± 0.04
Oleic Acid	21.43 ± 0.11	19.40 ± 0.10
Capmul MCM Oil	178.93 ± 0.38	52.97 ± 0.07
Light Liquid Paraffin	25.70 ± 0.12	10.69 ± 0.09
Tween-80	74.80 ± 0.20	40.13 ± 0.04
Span-20	47.22 ± 0.24	26.06 ± 0.07
Labrafac Lipophile WL 1349	63.89 ± 0.22	42.02 ± 0.03
Cremophor RH 40	112.85 ± 0.31	71.32 ± 0.28
Cremophor EL	61.48 ± 0.18	30.43 ± 0.05
Labrasol	119.93 ± 0.46	74.48 ± 0.08
Capmul GMO-50	36.29 ± 0.14	26.74 ± 0.08
Captex 355	25.19 ± 0.08	14.31 ± 0.08
PEG-400	36.39 ± 0.11	38.67 ± 0.07
Propylene Glycol	34.17 ± 0.11	10.74 ± 0.09
Transcutol-P	177.11 ± 0.43	82.28 ± 0.08

Selection of variable was based on the results of solubility data for Atorvastatin Calcium and Fenofibrate in oils, predicting drug solubility factors such as solubility parameter (δ), Required HLB value, Molecular weight, solubilization capacity, dielectric constant (ε), dipole moment (μ), excipient fatty acid chain length, surface tension, viscosity etc. Fenofibrate and Atorvastatin Calcium had excellent solubility in Capmul MCM Oil (Glyceryl Caprylate/Caprate) with comparison to other lipid vehicles. Capmul MCM oil (Glyceryl Caprylate/Caprate) was found satisfactory as oil. Fenofibrate and Atorvastatin Calcium had excellent solubility in Labrasol, Cremophor RH 40 (Polyoxyl 40 hydrogenated Castor oil) and Transcutol-P as compare to other surfactant and co-surfactant. Capmul MCM Oil (Glyceryl Caprylate/Caprate) as oil, Labrasol, Cremophor RH 40 (Polyoxyl 40 hydrogenated Castor oil) as surfactant and Transcutol-P as co-surfactant were selected for optimal SNEDDS formulation for improved drug loading capabilities. Selection of oil described in Section 5.3 of Chapter 5 in details.

6.3.1.2 Evaluation of surfactant and co-surfactant for its emulsifying ability

The percent transmittance values of dispersions were given in Table 6.2. Emulsification study clearly distinguished the ability of various surfactants to emulsify Capmul MCM oil. The study indicated that Cremophor RH 40 (HLB: 15) and Labrasol (HLB: 12) had very good ability to emulsify Capmul MCM oil followed by Tween 80 (HLB: 15), whereas, Cremophor EL (HLB: 13) and Labrafac PG (HLB: 1) appeared to be poor emulsifier for Capmul MCM oil. It indicated that the HLB value of the surfactant used played an important role and there was a great difference in the emulsification ability. This observation was in line with the investigation reported by Malcolmson and Warisnoicharoen who concluded that micro emulsification is also influenced by the structure and chain length of the surfactant [14, 15]. Cremophor RH 40 and Labrasol rendered very good nanoemulsion requiring short time for nanoemulsification and hence both were selected for further investigation.

In addition, surfactants must lower the interfacial tension to facilitate the dispersion process during the formation of nanoemulsion from SNEDDS. They provides a flexible film around the droplet that can readily collapse and also provides a curvature at the interfacial region for the desired different types of nanoemulsion like o/w type, w/o type and/or bicontinuous type, depending upon the lipophilicity of the surfactant.

The turbidimetric method was used to judge emulsification efficacy of the co-surfactant to improve the nanoemulsification ability and also to select best co-surfactant [16]. All the co-surfactants increased the spontaneity of the nanoemulsion formation as it leads to greater penetration of the surfactant monomers, thereby further decreasing the interfacial tension. Interestingly, PEG-400 and propylene glycol as cosurfactants appeared to be equivalent in improving nanoemulsification ability of Cremophor RH 40 and Labrasol. In case of lipophilic co-surfactants such as Transcutol-P, good correlation was observed between the structure i.e. the chain length of co-surfactant and the transmittance values of resulting dispersions. Larger the chain length of the co-surfactant lesser was the transmittance value. This correlation was also applicable to Capmul GMO-50, PEG-400, Propylene Glycol, Captex 355 and Acconon MCS-2 (Table 6.3).

Table 6.2: Emulsification ability of various non-ionic surfactants for Capmul MCM oil

Surfactant	**% Transmittance**
Cremophor RH 40	99.9
Labrafac PG	95.3
Tween-80	91.8
Labrasol	99.0
Cremophor EL	85.5

Table 6.3: Emulsification ability on surfactant/co-surfactant combinations for Capmul MCM oil

Co-Surfactant	**% Transmittance**	
	Cremophor RH 40	**Labrasol**
Transcutol-P	99.8	97.1
Captex 355	89.4	90.1
Capmul GMO-50	91.7	89.8
Acconon MCS 2	84.3	82.3
PEG-400	96.1	94.7
Propylene Glycol	92.6	92.1

Captex 355 was less effective as co-surfactant. This may be attributed to the presence of longer in chain length than Transcutol-P as co-surfactant. This observation was also in line with investigation reported by Malcolmson and Warisnoicharoen [14, 15].

Transcutol-P appeared to be the best among all the hydrophilic co-surfactants which can further be validated with the help of droplet size analysis. Lipophilic co-surfactants despite of their good potential were not investigated further as systems with higher lipid content may cause stability problem. Transcutol-P, a lipophilic co-surfactant having good solubilizing potential for Fenofibrate and Atorvastatin was selected and Cremophor RH-40 – Transcutol-P - Capmul MCM oil and Labrasol – Transcutol-P - Capmul MCM oil systems were developed for further studies.

Selection of variable was based on the results of solubility data for Atorvastatin Calcium and Fenofibrate in oils and surfactants/co-surfactants, emulsifying ability of surfactant/co-surfactant, predicting drug solubility factors such as solubility parameter (δ), Required HLB value, Molecular weight, required chemical type of emulsifiers, solubilization capacity, dielectric constant (ε), dipole moment (μ), excipient fatty acid chain length, surface tension, viscosity etc. Cremophor RH 40 and Transcutol-P were found best as surfactant and cosurfactant on basis of solubility data. Selection of surfactant and cosurfactant described in Section 5.3.2 of Chapter 5 in details.

6.3.2 Drug-Excipient Compatibility of SNEDDS Formulations

Drug-Excipient compatibility study was done to check presence or absence of drug excipients interaction [17]. Atorvastatin Calcium and excipients were mixed in 1:1 ratio. It was analysed at Initial and 40ºC/75% RH for 1 month by IR spectroscopy.

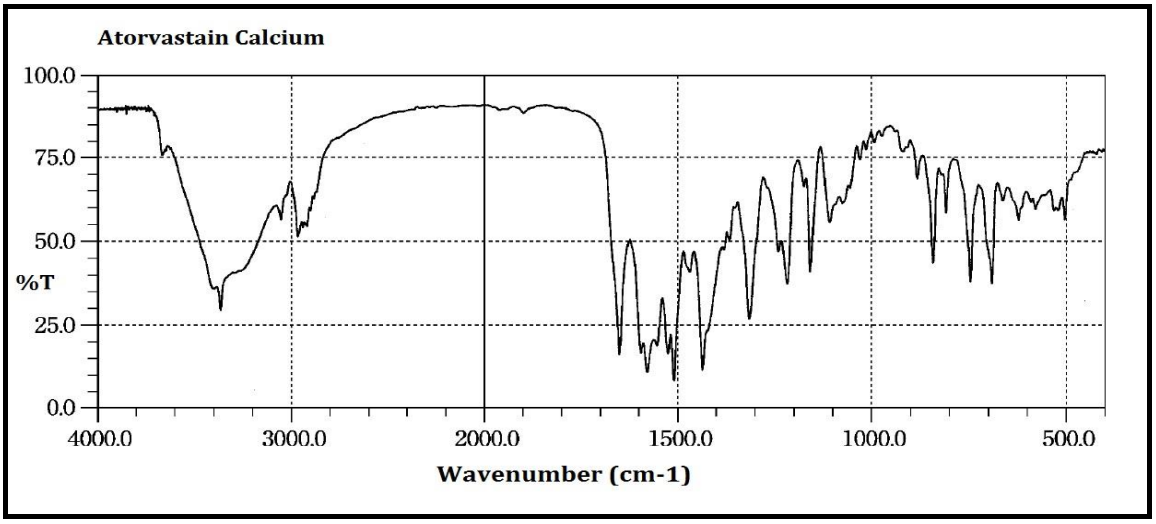

Figure 6.1: IR Spectrum of Atorvastatin Calcium

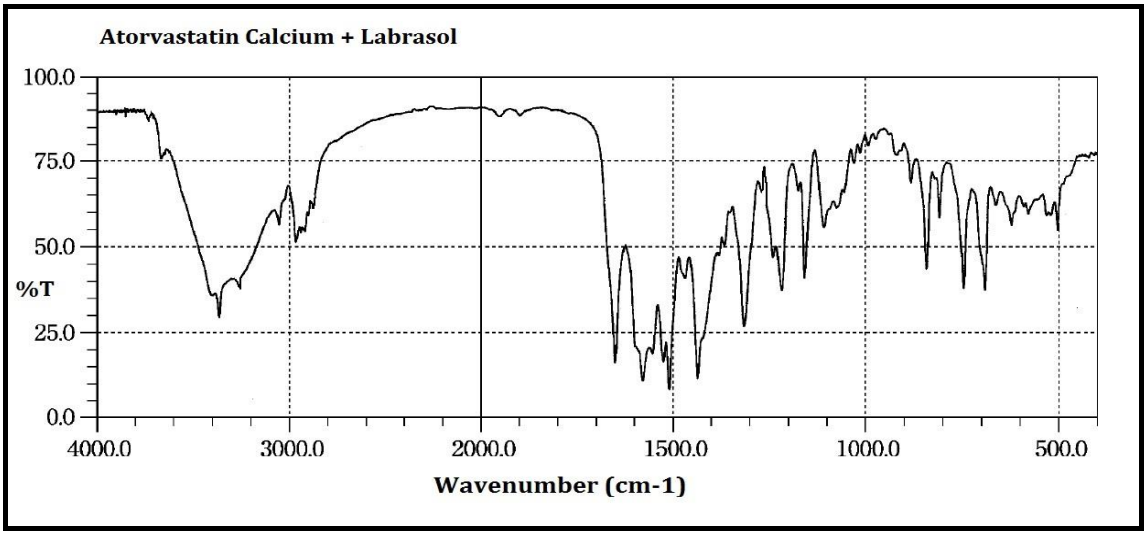

Figure 6.2: IR Spectrum of Atorvastatin Calcium + Labrasol

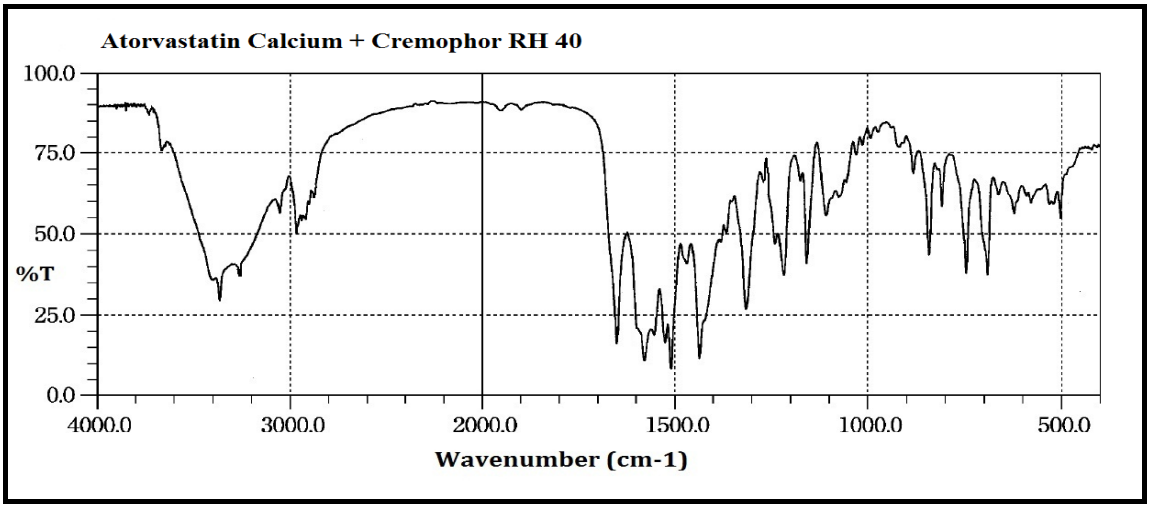

Figure 6.3: IR Spectrum of Atorvastatin Calcium + Cremophor RH 40

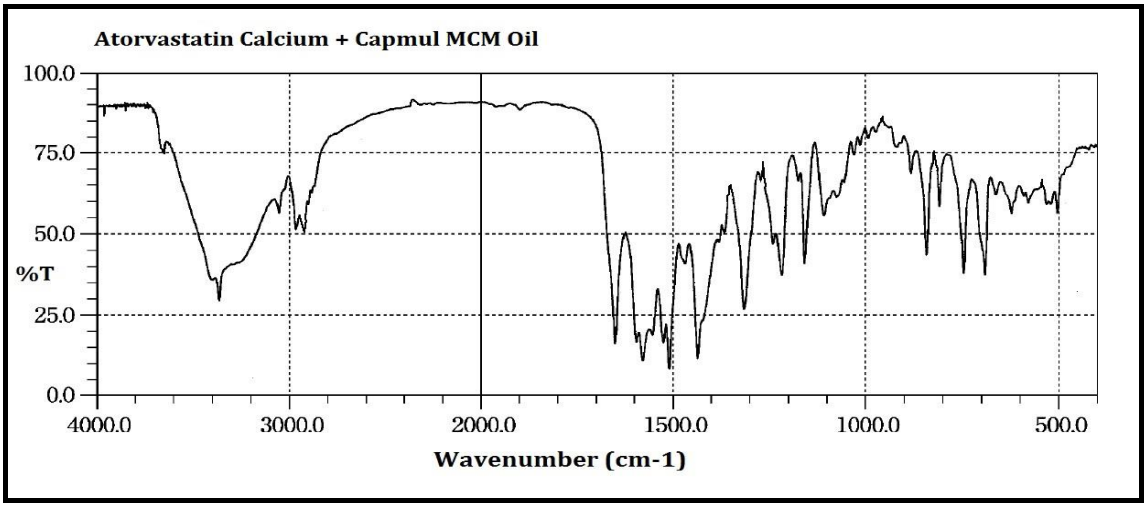

Figure 6.4: IR Spectrum of Atorvastatin Calcium + Capmul MCM oil

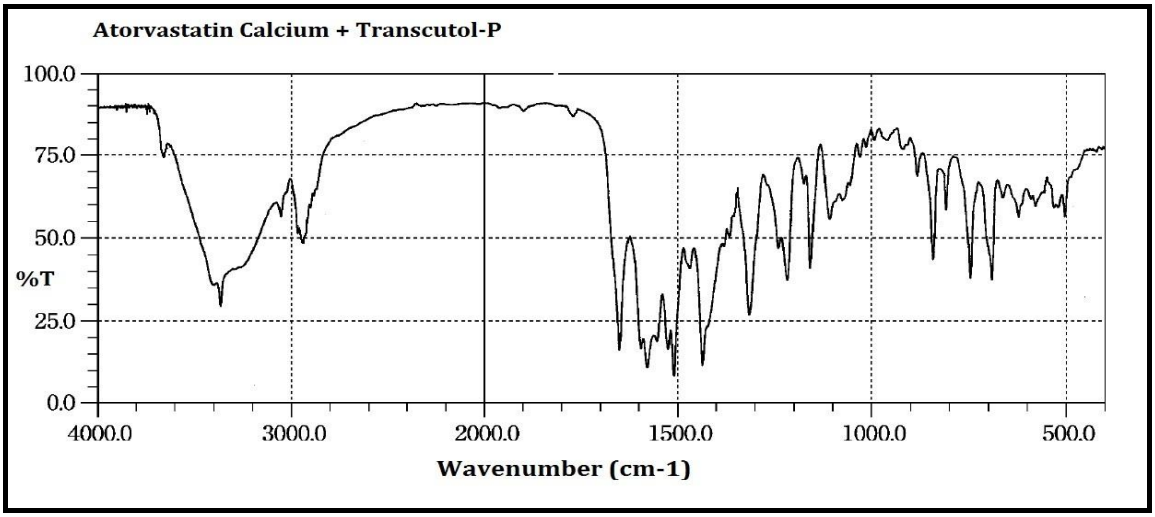

Figure 6.5: IR Spectrum of Atorvastatin Calcium + Transcutol-P

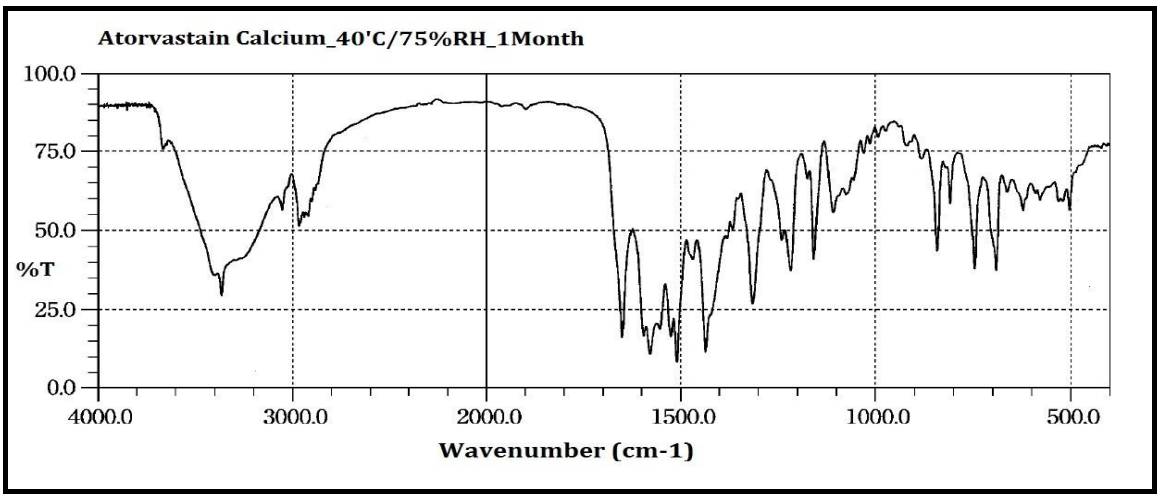

Figure 6.6: IR Spectrum of Atorvastatin Calcium (40°C/75%RH for 1 month)

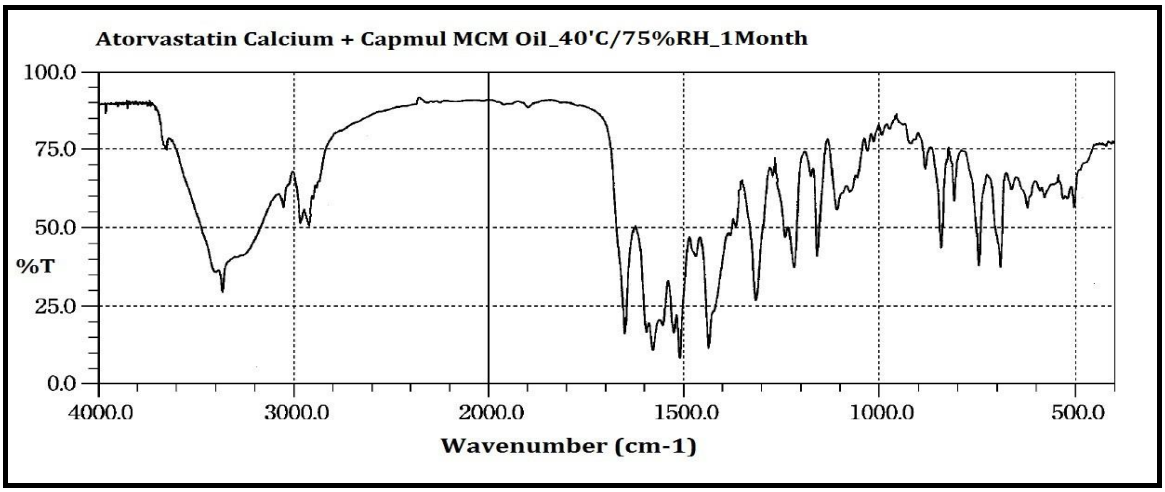

Figure 6.7: IR Spectrum of Atorvastatin Calcium + Capmul MCM oil (40°C/75%RH for 1 month)

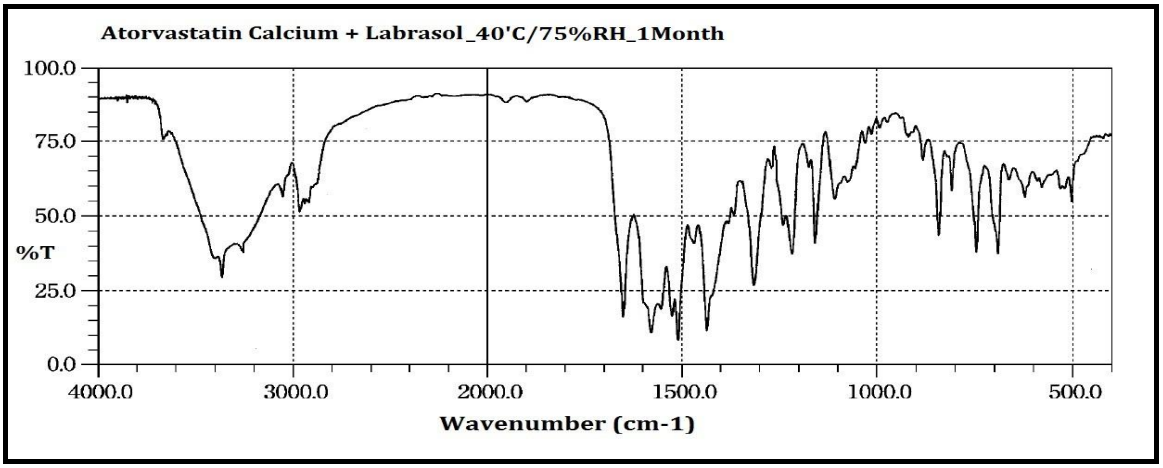

Figure 6.8: IR Spectrum of Atorvastatin Calcium + Labrasol (40°C/75%RH for 1 month)

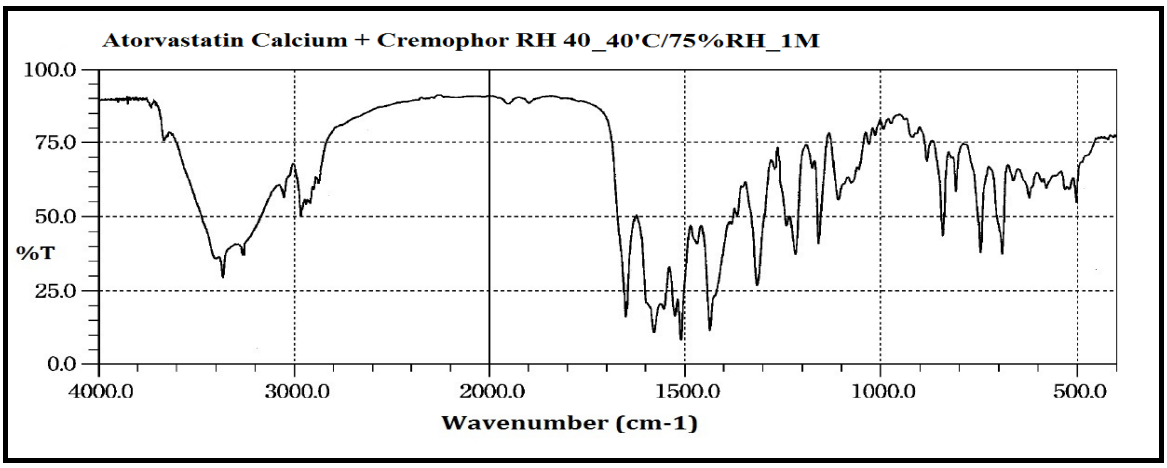

Figure 6.9: IR Spectrum of Atorvastatin Calcium + Cremophor RH 40 (40°C/75%RH for 1 month)

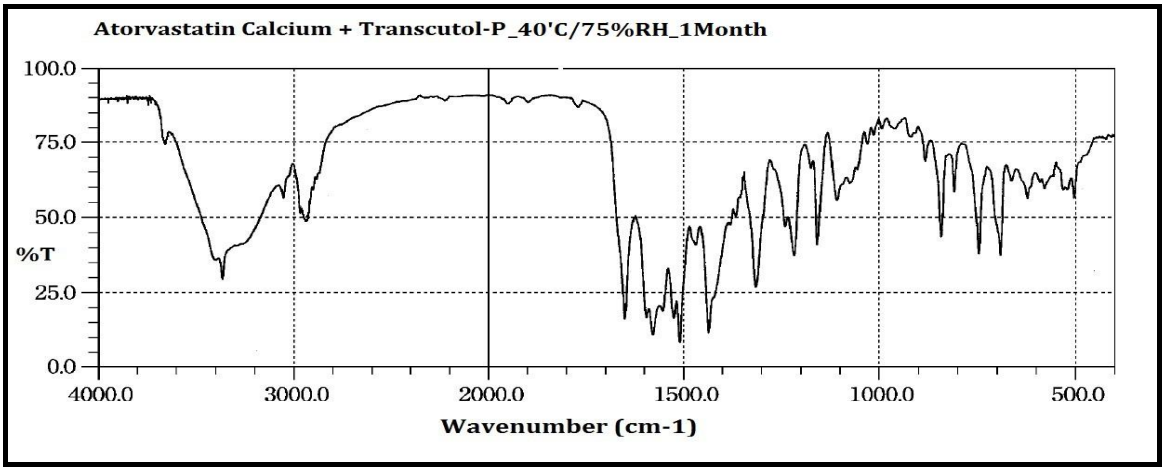

Figure 6.10: IR Spectrum of Atorvastatin Calcium + Transcutol-P (40°C/75%RH for 1 month)

Table 6.4: Drug-Excipient compatibility for Atorvastatin Calcium

Drug + Excipients	**R^2 Value ***	
	Initial	**40ºC/75% RH_1 Month**
Atorvastatin + Capmul MCM Oil	0.9891	0.9799
Atorvastatin + Labrasol	0.9967	0.9883
Atorvastatin + Transcutol-P	0.9836	0.9881
Atorvastatin + Cremophor RH 40	0.9911	0.9827
*R^2 value should be not less than 0.9500 (more than 0.9500 R^2 value shows very less or absence of drug-excipients interaction		

Fenofibrate and Excipients were mixed in 1:1 ratio. It was analysed at 40ºC/75% RH at Initial and 1 month by IR Spectroscopy.

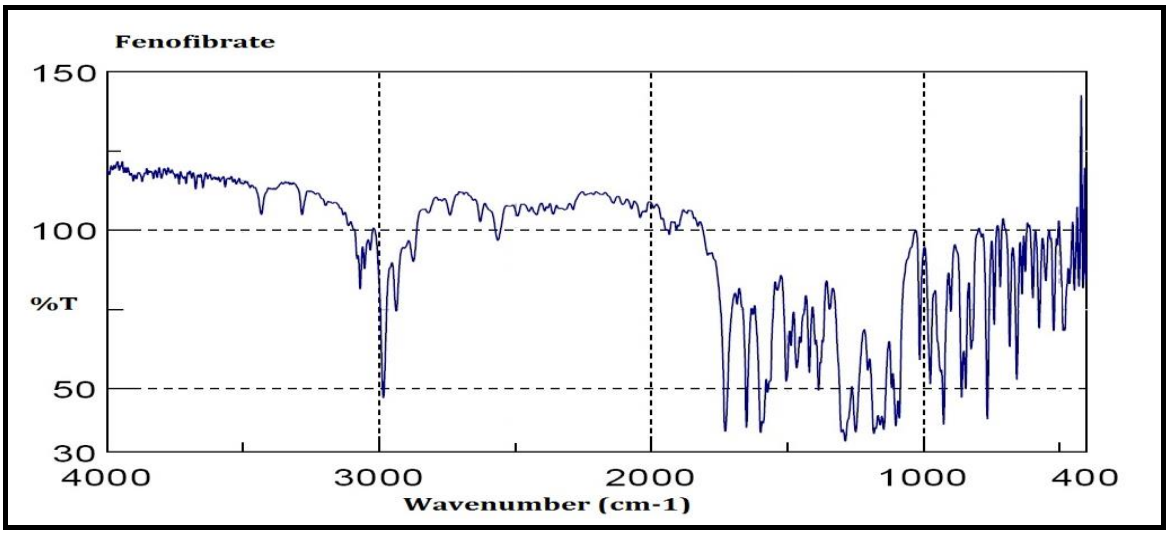

Figure 6.11: IR Spectrum of Fenofibrate

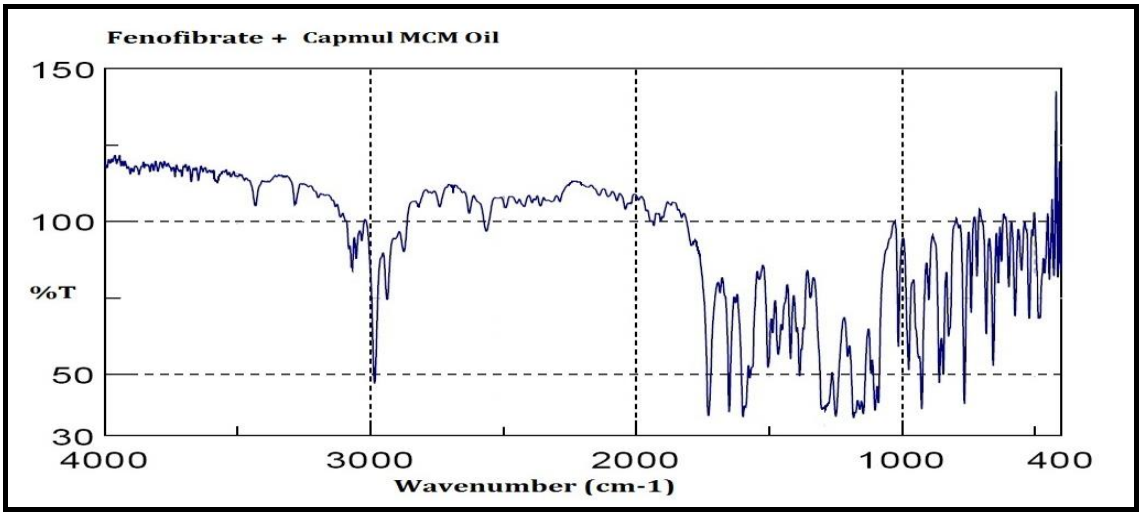

Figure 6.12: IR Spectrum of Fenofibrate + Capmul MCM oil

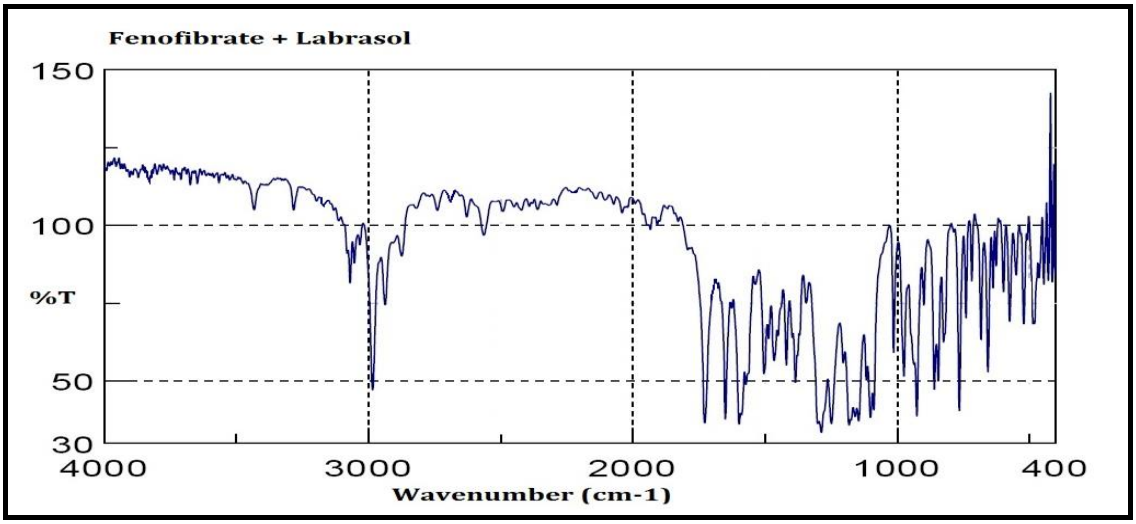

Figure 6.13: IR Spectrum of Fenofibrate + Labrasol

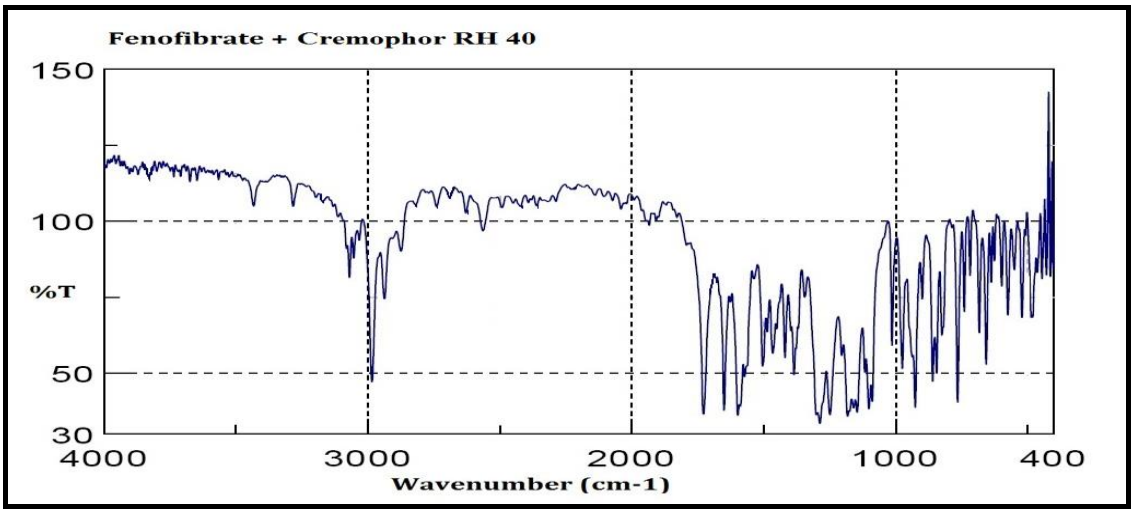

Figure 6.14: IR Spectrum of Fenofibrate + Cremophor RH 40

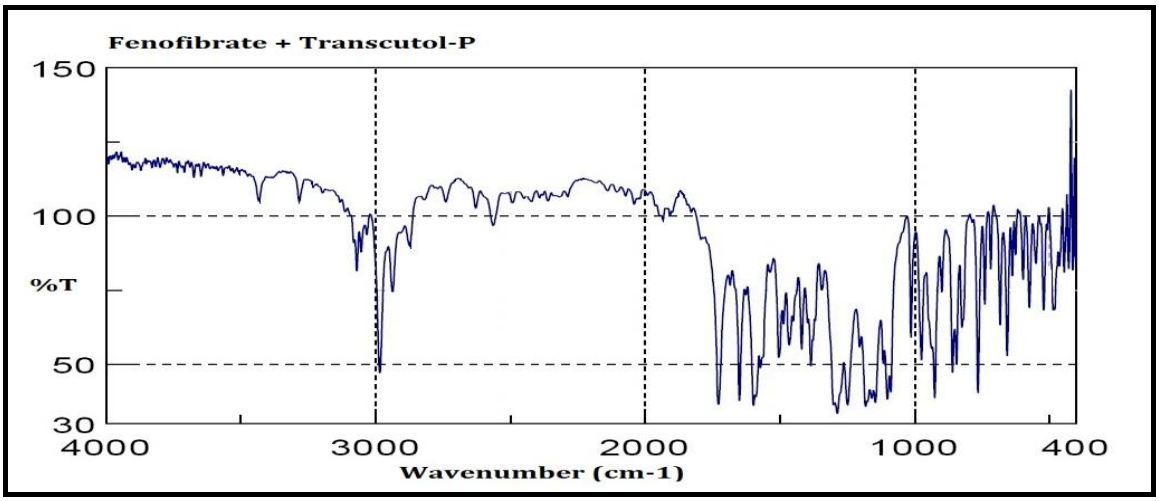

Figure 6.15: IR Spectrum of Fenofibrate + Transcutol-P

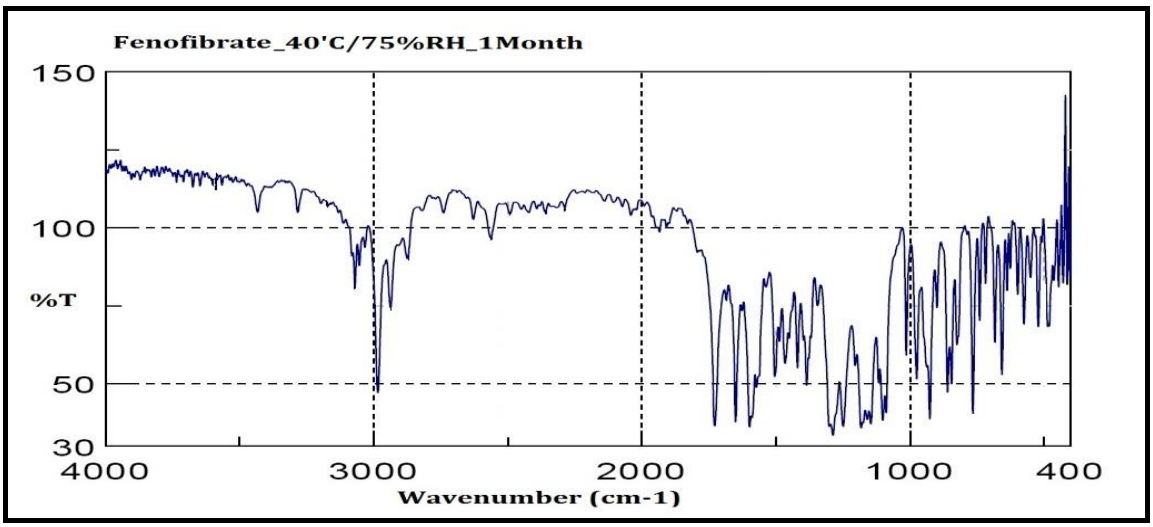

Figure 6.16: IR Spectrum of Fenofibrate (40°C/75%RH for 1 month)

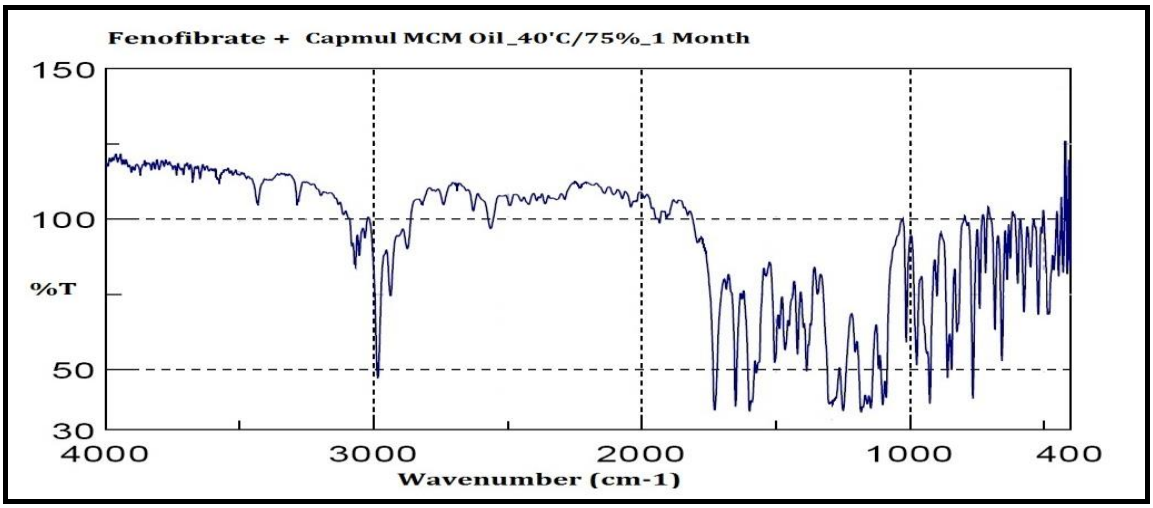

Figure 6.17: IR Spectrum of Fenofibrate + Capmul MCM oil (40°C/75%RH for 1 month)

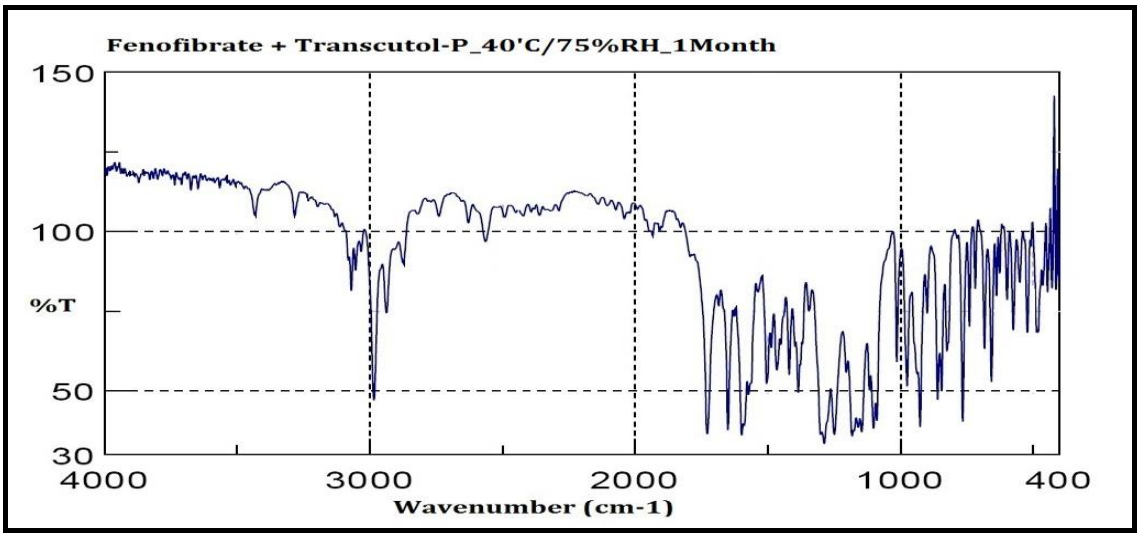

Figure 6.18: IR Spectrum of Fenofibrate + Transcutol-P (40°C/75%RH for 1 month)

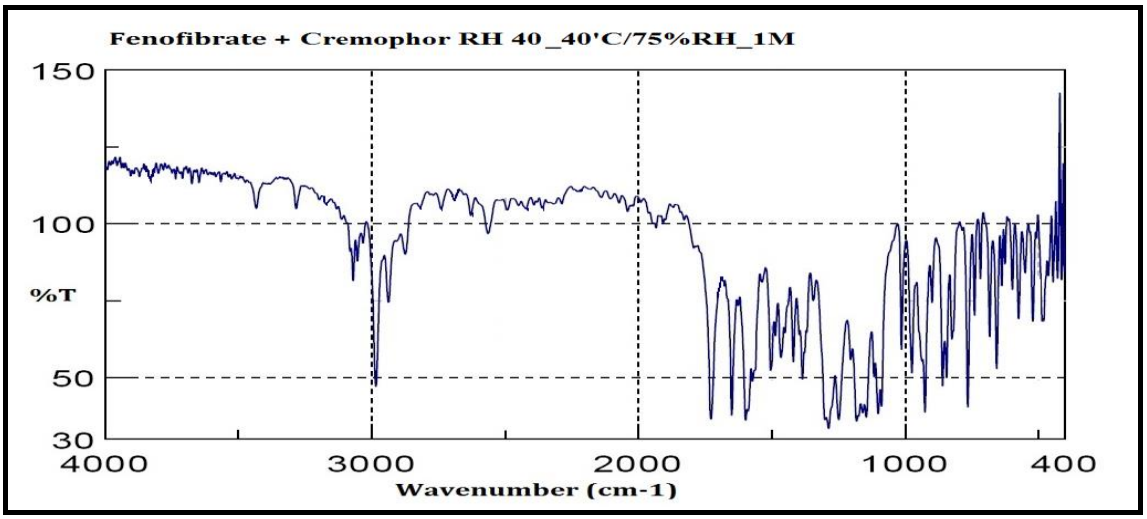

Figure 6.19: IR Spectrum of Fenofibrate + Cremophor RH 40 (40°C/75%RH for 1 month)

Table 6.5: Drug-Excipient compatibility for Fenofibrate		
Drug + Excipients	**R^2 Value ***	
	Initial	**40ºC/75% RH_1 Month**
Fenofibrate + Transcutol-P	0.9992	0.9846
Fenofibrate + Labrasol	0.9839	0.9872
Fenofibrate + Capmul MCM oil	0.9889	0.9739
Fenofibrate + Cremophor RH 40	0.9811	0.9782
*R^2 value should be not less than 0.9500 (more than 0.9500 R^2 value shows very less or absence of drug-excipients interaction		

The results of Drug-Excipient compatibility study represented that there was no interaction between active drugs and excipients.

6.3.3 Selection of Concentration of Oil, Surfactant and Cosurfactant

The selection of variable was based on the results of solubility data for Atorvastatin Calcium and Fenofibrate in oils and surfactants/co-surfactants, emulsifying ability of surfactant/co-surfactant, predicting drug solubility factors such as solubility parameter (δ), Required HLB value, Molecular weight, required chemical type of emulsifiers, solubilization capacity and Pseudo ternary phase diagram, dielectric constant (ε), dipole moment (μ), excipient fatty acid chain length, surface tension, viscosity etc. Capmul MCM was found satisfactory as oil, Cremophor RH 40 and Transcutol-P were found best as surfactant and cosurfactant on basis of solubility data. Selection of oil, surfactant and cosurfactant described in Section 5.3.2 of Chapter 5 in details. Ratio of Cremophor RH 40: Transcutol-P was selected as 3:1 and described in Section 5.3.3 of Chapter 5 in details.

Capmul MCM oil and Cremophor RH 40: Transcutol-P mixture (3:1) was used to found their suitable concentration in formulation development of SNEDDS of Fenofibrate and Atorvastatin Calcium. Preliminary batches were formulated and their results of dispersion status and dispersion time were presented in Table 6.6.

Table 6.6: Preliminary trials for development of Fenofibrate and Atorvastatin Calcium SNEDDS

Preliminary Batches	Oil (ml)	S:Co-S (3:1) (ml)	Dispersion	Dispersion time (Seconds)
P1	0.5	0.5	Turbid	68
P2	0.5	1.0	Clear & Translucent	60
P3	0.5	1.5	Clear & Transparent	44
P4	0.5	2.0	Clear & Transparent	41
P5	0.4	0.5	Clear & Translucent	65
P6	0.4	1.0	Clear & Transparent	64
P7	0.4	1.5	Clear & Translucent	60
P8	0.4	2.0	Clear & Transparent	58
P9	0.6	0.5	Clear & Translucent	71
P10	0.6	1.0	Clear & Translucent	63
P11	0.6	1.5	Clear & Transparent	46
P12	0.6	2.0	Clear & Transparent	45

The preliminary trials were carried out using different concentration of Capmul MCM oil (0.4mL – 0.6mL), and Cremophor RH 40 and Transcutol-P (3:1) (0.5mL – 2.0mL). The result of preliminary trial with 0.5mL of Capmul MCM oil (batch P3) was found satisfactory compared to other concentration (Table 6.6). Apart from Capmul MCM oil concentration, Concentration of Cremophor RH 40: Transcutol-P mixture (3:1) was also important in formulation development of SNEDDS and 1.5mL of Cremophor RH 40: Transcutol-P mixture (3:1) was found appropriate in preliminary study.

6.3.4 Optimization of SNEDDS of Fenofibrate and Atorvastatin Calcium using factorial design

The concentration of Capmul MCM oil and concentration of surfactant/Cosurfactant play important role in stable formulation of Self Nanoemulsifying drug delivery system (SNEDDS); hence concentration of Capmul MCM oil (0.5mL) and concentration of Cremophor RH 40:Transcutol-P (3:1) (1.5mL) were selected as independent variables in factorial design on the basis of the results of preliminary trials (Table 6.6). The 3^2 factorial design was employed using concentration of Capmul MCM oil and concentration of surfactant/Cosurfactant as independent variable X_1 and X_2 respectively. The Globule size (GS) (Y_1), Polydispersity index (PDI) (Y_2), Zeta potential (Y_3), drug release at 15 minutes of Fenofibrate (Y_4) and drug release at 15 minutes of Atorvastatin Calcium (Y_5) were selected as dependent variables. The coded and actual value of independent variable were shown in Table 6.7. The runs and responses for factorial batches were presented in Table 6.8.

Table 6.7: Factors and levels of independent variables in 3^2 factorial design for formulation of Fenofibrate and Atorvastatin Calcium SNEDDS

Independent variables	Level		
	Low (-1)	Medium (0)	High (+1)
Capmul MCM oil concentration (X_1), (mL)	0.4	0.5	0.6
Cremophor RH 40: Transcutol-P (3:1) concentration (X_2), (mL)	1.2	1.5	1.8

Table 6.8: Experimental runs and measured responses of 3^2 factorial design for SNEDDS of Fenofibrate and Atorvastatin Calcium

Batch	X_1	X_2	Globule size (nm) (Y_1)	PDI (Y_2)	Zeta potential (mV) (Y_3)	Drug release at 15 min for Fenofibrate (Y_4)	Drug release at 15 min for Atorvastatin Calcium (Y_5)
T1	-1	-1	357.0	0.428	-15.12	91.8	92.4
T2	0	-1	64.1	0.283	-16.40	93.9	93.6
T3	1	-1	55.8	0.221	-17.12	93.1	91.6
T4	-1	0	332.0	0.427	-15.68	93.8	92.9
T5	0	0	20.7	0.189	-27.96	96.7	97.4
T6	1	0	44.0	0.233	-17.60	94.1	93.4
T7	-1	1	307.0	0.426	-15.96	93.5	92.3
T8	0	1	26.6	0.195	-24.28	94.5	95.8
T9	1	1	29.2	0.191	-21.12	95.0	96.0

Multiple regression analysis was carried out for the responses using MS Excel. The reduced model was obtained by using significant terms ($p > 0.05$ was considered non-significant and such termed were neglected) for all the responses. The contour and response surface plot were constructed using Design Expert version 11 (Demo version).

(a) Globule size (Y_1)

A full model equation of globule size (Y_{FGS}) was written as Equation 6.5 [8, 9].

For globule size (GS),

$$Y_{FGS} = 31.9889 - 144.5000X_1 - 19.0167X_2 + 150.3667X_1^2 + 7.7167X_2^2 + 5.8500X_1X_2$$ …… (Equation 6.5)

The results of coefficients estimated by multiple regression for globule size (GS) was presented in Table 6.9.

Table 6.9: Coefficients estimated by multiple linear regression for globule size (Y_1)			
Factors	**Coefficients**	**Calculated t values**	**p-values**
Intercept	31.9889	4.3825	0.02199*
X_1	-144.5000	-36.1437	0.00004**
X_2	-19.0167	-4.7566	0.00176**
X_1^2	150.3667	21.7148	0.00021**
X_2^2	7.7167	1.1143	0.34635
X_1X_2	5.8500	1.1947	0.31803

**very significant ($p<0.01$), *significant ($p<0.05$)

The globule size for batch T1 to T9 ranges from 20.7 to 357.0nm. The coefficient of X_1 was -144.5000 and X_2 was -19.0167, which indicated that large negative value of X_1 was predominantly reducing the globule size of SNEDDS. The regression coefficient of X_1^2 was 150.3667, X_2^2 was 7.7167 and X_1X_2 was 5.8500, which indicated their positive influence on globule size. When the coefficients of the two independent variables in Equation 6.5 were compared, the value for the variable X_1(b1= -144.5000) was found to be maximum and hence the variable X_1 was considered to be a major contributing variable for GS.

The reduced model for globule size (Y_{RGS}) was presented as Equation 6.6 [8, 9].

For Globule size (GS),

$Y_{RGS} = 37.1333 – 144.5000X_1 – 19.0167X_2 + 150.3667X_1^2$ ……. (Equation 6.6)

The summary of full and reduced model for globule size was presented in Table 6.10

Table 6.10: Summary of full and reduced model for globule size (GS) (Y1)		
Response	(Y_{FGS})	(Y_{RGS})
Model	Full	Reduced
b0	31.9889	37.1333
b1	-144.5000	-144.5000
b2	-19.0167	-19.0167
b11	150.3667	150.3667

b22	7.7167	-
b12	5.8500	-
R^2	0.9983	0.9968

The results of ANOVA were present in Table 6.11.

Table 6.11: ANOVA of full model and reduced model for Globule Size (GS) (Y_1)

		DF	SS	MS	F	R	R^2	Ad. R^2
Regression	**FM**	5	172927.6	34585.51	360.64	0.9991	0.9983	0.9955
	RM	3	172671.6	57557.19	529.32	0.9984	0.9968	0.9949
Error	**FM**	3	287.701	95.900				
	RM	5	543.685	108.737				

FM = full model, RM = Reduced model

SSE2-SSE1 = 543.685-287.701 = 255.984

No. of parameters omitted = 2

MS of Error (full model) = 95.900

F calculated = (255.984/2)/95.900 = 1.334

F tabulated = (5-3)/2 = 9.28

The F–statistic of the results of ANOVA confirmed the omission of non-significant terms of Equation 6.5 The calculated F value for globule size was 1.334 which was less than the tabulated F value 9.28 (α=0.05, V1= 3 and V2= 3), so it was concluded that the neglected terms did not significantly contributed in the prediction of GS. The goodness of fit of the model was checked by the determination coefficient (R^2). In the case of GS, the values of the determination coefficients (R^2= 0.9983 for full model and 0.9968 for reduced model) indicated that over 90% of the total variations are explained by the model. The values of adjusted determination coefficients (adj R^2= 0.9955 for full model and 0.9949 for reduced model) for GS. The values of correlation coefficients (R=0.9991 for full model and 0.9984 for reduced model) for GS.

The predicted value along with their actual value for globule size were shown in Table 6.12 for globule size which showed about the present error which should be minimal.

Table 6.12: Observed and predicted responses for globule size (GS) (Y_1)				
Batch No	**Observed GS**	**Predicted GS**	**Residual Values**	**% Error**
T1	357.0	351.0167	5.9833	1.6760
T2	64.1	56.1500	7.9500	12.4025
T3	55.8	62.0167	-6.2167	-11.1410
T4	332.0	332.0000	0.0000	0.0000
T5	20.7	37.1333	-16.4333	-79.3879
T6	44.0	43.0000	1.0000	2.2727
T7	307.0	312.9533	-5.9833	-1.9490
T8	26.6	18.1167	8.4833	31.8922
T9	29.2	23.9833	5.2167	17.8653

(b) Polydispersity index (PDI) (Y_2)

A full model equation of polydispersity index (Y_{PDI}) was written as Equation 6.7 [8, 9].

For polydispersity index (PDI),

$Y_{FPDI} = 0.2172 - 0.1060X_1 - 0.0200X_2 + 0.0987X_1^2 + 0.0077X_2^2 - 0.007X_1X_2$ …… (Equation 6.7)

The results of coefficients estimated by multiple regression for polydispersity index (PDI) was presented in Table 6.13.

Table 6.13: Coefficients estimated by multiple linear regression for polydispersity index (PDI) (Y_2)			
Factors	**Coefficients**	**Calculated t values**	**p-values**
Intercept	0.2172	8.2286	0.00375**
X_1	-0.1060	-7.3310	0.00524**
X_2	-0.0200	-1.3832	0.26056
X_1^2	0.0987	3.9397	0.02914*
X_2^2	0.0077	0.3061	0.77951
X_1X_2	-0.0070	-0.3952	0.71905

**very significant ($p<0.01$), *significant ($p<0.05$)

The polydispersity index for batch T1 to T9 ranges from 0.189 to 0.428. The coefficient of X_1 was -0.1060 and X_2 was -0.0200, which indicated that large negative value of X_1 was predominantly reducing the polydispersity index of SNEDDS. The regression coefficient of X_1^2 was 0.0987 and X_2^2 was 0.0077, which indicated their positive influence on polydispersity index. When the coefficients of the two independent variables in Equation 6.7 were compared, the value for the variable X_1(b1= -0.1060) was found to be maximum and hence the variable X_1 was considered to be a major contributing variable for PDI.

The reduced model for polydispersity index (Y_{RPDI}) was presented as Equation 6.8 [8, 9].
For polydispersity index (PDI),

$Y_{RPDI} = 0.2223 – 0.1060X_1 – 0.0200X_2 + 0.0987X_1^2$ .....…….. (Equation 6.8)

The summary of full and reduced model for polydispersity index was presented in Table 6.14.

Table 6.14: Summary of full and reduced model for polydispersity index (PDI) (Y_2)

Response	(Y_{FPDI})	(Y_{RPDI})
Model	Full	Reduced
b0	0.2172	0.2223
b1	-0.1060	-0.1060
b2	-0.0200	-0.0200
b11	0.0987	0.0987
b22	0.0077	-
b12	-0.0070	-
R^2	0.9597	0.9563

The results of ANOVA were present in Table 6.15.

Table 6.15: ANOVA of full model and reduced model for Polydispersity index (PDI) (Y_2)

		DF	SS	MS	F	R	R^2	Ad. R^2
Regression	**FM**	5	0.0896	0.01792	14.286	0.9796	0.9597	0.8925
	RM	3	0.0892	0.0297	36.502	0.9779	0.9563	0.9301
Error	**FM**	3	0.0003	0.0012				
	RM	5	0.0040	0.0008				

FM = full model, RM = Reduced model

SSE2-SSE1 = 0.0040-0.003 = 0.0037

No. of parameters omitted = 2

MS of Error (full model) = 0.0012

F calculated = (0.0037/2)/0.0012 = 1.541

F tabulated = (5-3)/2 = 9.28

The F–statistic of the results of ANOVA confirmed the omission of non-significant terms of Equation 6.7. The calculated F value for polydispersity index was 1.541 which was less than the tabulated F value 9.28 (α=0.05, V_1= 3 and V_2= 3), so it was concluded that the neglected terms did not significantly contributed in the prediction of PDI. The goodness of fit of the model was checked by the determination coefficient (R^2). In the case of PDI, the values of the determination coefficients (R^2= 0.9597 for full model and 0.9563 for reduced model) indicated that over 90% of the total variations are explained by the model. The values of adjusted determination coefficients (adj R^2= 0.8925 for full model and 0.9301 for reduced model) for PDI. The values of correlation coefficients (R=0.9796 for full model and 0.9779 for reduced model) for PDI.

The predicted value along with their actual value for PDI were shown in Table 6.16 for polydispersity index which showed about the present error which should be minimal.

Table 6.16: Observed and predicted response for polydispersity index (PDI) (Y_2)				
Batch No	**Observed PDI**	**Predicted PDI**	**Residual Values**	**% Error**
T1	0.428	0.4470	-0.0190	-4.4393
T2	0.283	0.2423	0.0407	14.3816
T3	0.221	0.2350	-0.0140	-6.3348
T4	0.427	0.4270	0.0000	0.0000
T5	0.189	0.2233	-0.0333	-17.6190
T6	0.233	0.2150	0.0180	7.7253
T7	0.426	0.4070	0.0190	4.4601
T8	0.195	0.2023	-0.0073	-3.7436
T9	0.191	0.1950	-0.0040	-2.0942

(c) Zeta potential (ZP) (Y_3)

A full model equation of zeta potential (Y_{FZP}) was written as Equation 6.9 [8, 9].

For zeta potential,

$$Y_{FZP} = -24.2667 - 1.5133X_1 - 2.1200X_2 + 5.7800X_1^2 + 2.0800X_2^2 - 0.7900X_1X_2$$ …… (Equation 6.9)

The results of coefficients estimated by multiple regression for zeta potential (ZP) was present in Table 6.17.

Table 6.17: Coefficients estimated by multiple linear regression for zeta potential (ZP) (Y_3)			
Factors	**Coefficients**	**Calculated t values**	**p-values**
Intercept	-24.2667	-8.7485	0.00314**
X_1	-1.5133	-0.9960	0.39262
X_2	-2.1200	-1.3954	0.25724
X_1^2	5.7800	2.1965	0.11554
X_2^2	2.0800	0.7904	0.48699
X_1X_2	-0.7900	-0.4245	0.69976

**very significant ($p<0.01$), *significant ($p<0.05$)

The zeta potential for batch T1 to T9 ranges from -27.96 to -15.12. The coefficient of X_1 was -1.5133 and X_2 was -2.1200, which indicated that large negative value of X_2 was predominantly reducing the zeta potential of SNEDDS. The regression coefficient of X_1^2 was 5.7800 and X_2^2 was 2.0800, which indicated their positive influence on zeta potential. When the coefficients of the two independent variables in Equation 6.9 were compared, the value for the variable X_2(b2= -2.1200) was found to be maximum and hence the variable X_2 was considered to be a major contributing variable for zeta potential.

The reduced model for zeta potential (Y_{RZP}) was presented as Equation 6.10 [8, 9].

For Zeta potential (ZP),

$Y_{RZP} = -19.0267 - 1.5133X_1 - 2.1200X_2$ ..…….....……. (Equation 6.10)

The summary of full and reduced model for zeta potential was presented in Table 6.18

Table 6.18: Summary of full and reduced model for zeta potential (ZP) (Y_3)

Response	(Y_{FZP})	(Y_{RZP})
Model	Full	Reduced
b0	-24.2667	-19.0267
b1	-1.5133	-1.5133
b2	-2.1200	-2.1200
b11	5.7800	-
b22	2.0800	-
b12	-0.7900	-
R^2	0.7407	0.2541

The results of ANOVA were present in Table 6.19.

Table 6.19: ANOVA of full model and reduced model for Zeta Potential (ZP) (Y_3)

		DF	SS	MS	F	R	R^2	Ad. R^2
Regression	**FM**	5	118.673	23.734	1.713	0.8606	0.7407	0.3084
	RM	2	40.707	20.353	1.021	0.5040	0.2541	0.0054
Error	**FM**	3	41.547	13.849				
	RM	6	119.513	19.918				

FM = full model, RM = Reduced model

SSE2-SSE1 = 119.513-41.547 = 77.966

No. of parameters omitted = 3

MS of Error (full model) = 13.849

F calculated = (77.966 /3)/13.849 = 1.876

F tabulated = (5-2)/3 = 9.28

The F–statistic of the results of ANOVA confirmed the omission of non-significant terms of Equation 6.9. The calculated F value for zeta potential was 1.876 which was less than the tabulated F value 9.28 (α=0.05, V_1= 3 and V_2= 3), so it was concluded that the neglected terms did not significantly contributed in the prediction of zeta potential. The goodness of fit of the model was checked by the determination coefficient (R^2). In the case of ZP, the values of the determination coefficients (R^2= 0.7407 for full model and 0.2541 for reduced model) indicated that over 90% of the total variations are explained by the model. The values of adjusted determination coefficients (adj R^2= 0.3084 for full model and 0.0054 for reduced model) for ZP. The values of correlation coefficients (R=0.8606 for full model and 0.5040 for reduced model) for ZP.

The predicted value along with their actual values were shown in Table 6.20 for zeta potential which showed about the present error which should be minimal.

Table 6.20: Observed and predicted responses for zeta potential (ZP) (Y_3)

Batch No	Observed ZP	Predicted ZP	Residual Values	% Error
T1	-15.12	-15.3933	0.2733	1.8075
T2	-16.40	-16.9067	0.5067	3.0896
T3	-17.12	-18.4200	1.3000	7.0575
T4	-15.68	-17.5133	1.8333	11.6920
T5	-27.96	-19.0267	-8.9333	-31.9503
T6	-17.60	-20.5400	2.9400	16.7045
T7	-15.96	-19.6333	3.6733	23.0157
T8	-24.28	-21.1467	-3.1333	-12.9049
T9	-21.12	-22.6600	1.5400	7.2917

(d) Drug release at 15 minutes for Fenofibrate (DRF) (Y_4)

A full model equation of drug release at 15 minutes for Fenofibrate (Y_{FDRF}) was written as Equation 6.11 [8, 9].

For drug release at 15 minutes for Fenofibrate (DRF),

$Y_{FDRF} = 95.8556 + 0.5167X_1 + 0.7000X_2 – 1.4833X_1^2 – 1.2333X_2^2 + 0.0500X_1X_2$

…… (Equation 6.11)

The results of coefficients estimated by multiple regression for drug release at 15 minutes for Fenofibrate (DRF) was present in Table 6.21.

Table 6.21: Coefficients estimated by multiple linear regression for drug release at 15 minutes for Fenofibrate (DRF) (Y_4)

Factors	Coefficients	Calculated t values	p-values
Intercept	95.8556	141.2240	0.00000**
X_1	0.5167	1.3898	0.25878
X_2	0.7000	1.8829	0.15625
X_1^2	-1.4833	-2.3036	0.10464
X_2^2	-1.2333	-1.9153	0.15133
X_1X_2	0.0500	0.1098	0.91949

**very significant ($p<0.01$), *significant ($p<0.05$)

The drug release at 15 minutes for Fenofibrate (DRF) for batch T1 to T9 ranges from 91.8 to 96.7. The coefficient of X_1 was 0.5167 and X_2 was 0.7000, which indicated that large positive value of X_2 was predominantly increasing the drug release at 15 minutes for Fenofibrate (DRF) of SNEDDS. The regression coefficient of X_1^2 was -1.4833 and X_2^2 was -1.2333, which indicated their positive influence on drug release at 15 minutes for Fenofibrate (DRF). When the coefficients of the two independent variables in Equation 6.11 were compared, the value for the variable X_2 (b2= 0.7000) was found to be maximum and hence the variable X_2 was considered to be a major contributing variable for drug release at 15 minutes for Fenofibrate (DRF).

The reduced model for drug release at 15 minutes for Fenofibrate (Y_{RDRF}) was presented as Equation 6.12 [8, 9].

For drug release at 15 minutes for Fenofibrate (DRF),

$Y_{RDRF} = 94.0444 + 0.5167X_1 + 0.7000X_2$ ……..…… (Equation 6.12)

The summary of full and reduced model for drug release at 15 minutes for Fenofibrate (DRF) was presented in Table 6.22

Table 6.22: Summary of full and reduced model for drug release at 15 minutes for Fenofibrate (DRF) (Y_4)

Response	(Y_{FDRF})	(Y_{RDRF})
Model	Full	Reduced
b0	95.8556	94.0444
b1	0.5167	0.5167
b2	0.7000	0.7000
b11	-1.4833	-
b22	-1.2333	-
b12	0.0500	-
R^2	0.8282	0.3136

The results of ANOVA were present in Table 6.23.

Table 6.23: ANOVA of full model and reduced model for Drug release at 15 minutes for Fenofibrate (DRF) (Y_4)

		DF	SS	MS	F	R	R^2	Ad. R^2
Regression	**FM**	5	11.994	2.398	2.892	0.9100	0.8282	0.5419
	RM	2	4.541	2.270	1.370	0.5600	0.3136	0.0848
Error	**FM**	3	2.487	0.829				
	RM	6	9.940	1.656				

FM = full model, RM = Reduced model

SSE2-SSE1 = 9.940 – 2.487 = 7.453

No. of parameters omitted = 3

MS of Error (full model) = 0.829

F calculated = (7.453 /3)/0.829 = 2.996

F tabulated = (5-2)/3 = 9.28

The F–statistic of the results of ANOVA confirmed the omission of non-significant terms of Equation 6.11. The calculated F value for drug release at 15 minutes for Fenofibrate was 2.996 which was less than the tabulated F value 9.28 (α=0.05, V_1= 3 and V_2= 3), so it was concluded that the neglected terms did not significantly contributed in the prediction of drug release at 15 minutes for Fenofibrate. The goodness of fit of the model was checked by the determination coefficient (R^2). In the case of DRF, the values of the determination coefficients (R^2= 0.8282 for full model and 0.3136 for reduced model) indicated that over 90% of the total variations are explained by the model. The values of adjusted determination coefficients (adj R^2= 0.5419 for full model and 0.0848 for reduced model) for DRF. The values of correlation coefficients (R=0.9100 for full model and 0.5600 for reduced model) for DRF.

The predicted value along with their actual values were shown in Table 6.24 for drug release at 15 minutes for Fenofibrate which showed about the present error which should be minimal.

Table 6.24: Observed and predicted responses for drug release at 15 minutes for Fenofibrate (DRF) (Y_4)

Batch No	Observed DRF	Predicted DRF	Residual Values	% Error
T1	91.8	92.8278	-1.0278	-1.1196
T2	93.9	93.3444	0.5556	0.5916
T3	93.1	93.8611	-0.7611	-0.8175
T4	93.8	93.5278	0.2772	0.2955
T5	96.7	94.0444	2.6556	2.7462
T6	94.1	94.5611	-0.4611	-0.4900
T7	93.5	94.2278	-0.7278	-0.7783
T8	94.5	94.7444	-0.2444	-0.2586
T9	95.0	95.2611	-0.2611	-0.2748

(e) Drug release at 15 minutes for Atorvastatin Calcium (DRA) (Y_5)

A full model equation of drug release at 15 minutes for Atorvastatin Calcium (Y_{FDRA}) was written as Equation 6.13 [8, 9].

For drug release at 15 minutes for Atorvastatin Calcium (DRA),

$Y_{FDRA} = 96.2333 + 0.5667X_1 + 1.0833X_2 - 2.5000X_1^2 - 0.9500X_2^2 + 1.1250X_1X_2$ …..... (Equation 6.13)

The results of coefficients estimated by multiple regression for drug release at 15 minutes for Atorvastatin calcium (DRA) was present in Table 6.25.

Table 6.25: Coefficients estimated by multiple linear regression for drug release at 15 minutes for Atorvastatin Calcium (DRA) (Y_5)

Factors	Coefficients	Calculated t values	p-values
Intercept	96.2333	121.9224	0.00000**
X_1	0.5667	1.3107	0.28124
X_2	1.0833	2.5058	0.08725
X_1^2	-2.5000	-3.3386	0.04443*
X_2^2	-0.9500	-1.2687	0.29404
X_1X_2	1.1250	2.1247	0.12362

**very significant ($p<0.01$), *significant ($p<0.05$)

The drug release at 15 minutes for Atorvastatin Calcium for batch T1 to T9 ranges from 91.6 to 97.4. The coefficient of X_1 was 0.5667 and X_2 was 1.0833, which indicated that large positive value of X_2 was predominantly increasing the drug release at 15 minutes for Atorvastatin Calcium (DRA) of SNEDDS. The regression coefficient of X_1^2 was -2.5000 and X_2^2 was -0.9500, which indicated their positive influence on drug release at 15 minutes for Atorvastatin Calcium (DRA). When the coefficients of the two independent variables in Equation 6.13 were compared, the value for the variable X_2(b2= 1.0833) was found to be maximum and hence the variable X2 was considered to be a major contributing variable for drug release at 15 minutes for Atorvastatin Calcium (DRA).

The reduced model for drug release at 15 minutes for Atorvastatin Calcium (Y_{RDRA}) was presented as Equation 6.14 [8, 9].

For drug release at 15 minutes for Atorvastatin Calcium (DRA),

$Y_{RDRA} = 95.6000 + 0.5667X_1 + 1.0833X_2 - 2.5000X_1^2$ ……..... (Equation 6.14)

The summary of full and reduced model for drug release at 15 minutes for Atorvastatin Calcium (DRA) was presented in Table 6.26.

Table 6.26: Summary of full and reduced model for drug release at 15 minutes for Atorvastatin Calcium (DRA) (Y_5)

Response	(Y_{FDRA})	(Y_{RDRA})
Model	Full	Reduced
b0	96.2333	95.6000
b1	0.5667	0.5667
b2	1.0833	1.0833
b11	-2.5000	-2.500
b22	-0.9500	-
b12	1.1250	-
R^2	0.8938	0.6772

The results of ANOVA were present in Table 6.27.

Table 6.27: ANOVA of full model and reduced model for Drug Release at 15 minutes for Atorvastatin Calcium (DRA)(Y_5)

		DF	SS	MS	F	R	R^2	Ad. R^2
Regression	**FM**	5	28.335	5.667	5.053	0.9454	0.8938	0.7169
	RM	3	21.468	7.156	3.4970	0.8229	0.6772	0.4835
Error	**FM**	3	3.364	1.121				
	RM	5	10.231	2.046				

FM = full model, RM = Reduced model

SSE2-SSE1 = 10.231 – 3.364 = 6.867

No. of parameters omitted = 2

MS of Error (full model) = 1.121

F calculated = (6.867/2)/1.121 = 3.062

F tabulated = (5-3)/2 = 9.28

The F–statistic of the results of ANOVA confirmed the omission of non-significant terms of Equation 6.13. The calculated F value for drug release at 15 minutes for Atorvastatin

Calcium (DRA) was 3.062 which was less than the tabulated F value 9.28 (α=0.05, V_1= 3 and V_2= 3), so it was concluded that the neglected terms did not significantly contributed in the prediction of drug release at 15 minutes for Atorvastatin Calcium. The goodness of fit of the model was checked by the determination coefficient (R^2). In the case of DRA, the values of the determination coefficients (R^2= 0.8938 for full model and 0.6772 for reduced model) indicated that over 90% of the total variations are explained by the model. The values of adjusted determination coefficients (adj R^2= 0.7169 for full model and 0.4835 for reduced model) for DRA. The values of correlation coefficients (R=0.9454 for full model and 0.8229 for reduced model) for DRA.

The predicted value along with their actual values were shown in Table 6.28 for drug release at 15 min of Atorvastatin Calcium which showed about the present error which should be minimal.

Table 6.28: Observed and predicted responses for Drug Release at 15 minutes for Atorvastatin Calcium (DRA)(Y_5)

Batch No	Observed DRA	Predicted DRA	Residual Values	% Error
T1	92.4	91.4500	0.9500	1.0281
T2	93.6	94.5167	-0.9167	-0.9794
T3	91.6	92.5833	-0.9833	-1.0735
T4	92.9	92.5333	0.3667	0.3947
T5	97.4	95.6000	1.8000	1.8480
T6	93.4	93.6667	-0.2667	-0.2855
T7	92.3	93.6167	-1.3167	-1.4265
T8	95.8	96.6833	-0.8833	-0.9220
T9	96.0	94.7500	1.2500	1.3021

Contour Plots and Response Surface Plots

Two dimensional contour plots were constructed for all dependent variables i.e. globule size (GS), polydispersity index (PDI), zeta potential (ZP) and drug release at 15 minutes for Fenofibrate (DRF) and Atorvastatin Calcium (DRA) and shown in Figure 6.20, 6.22, 6.24, 6.26 and 6.28. Response surface plots are very helpful in learning about both the main and interaction effects of the independent variables [10, 18].

(a) Globule size (GS)

Figure 6.20 showed contour plot for globule size (GS) at prefixed values of 50, 100, 150 200, 250, and 300nm. The contour plot was found to be linear, thus the relationship between independent variables for GS could be linear.

Figure 6.21 showed the response surface plot obtained as a function of concentration of Capmul MCM oil and concentration of Cremophor RH 40: Transcutol-P (3:1) for globule size (GS). A decrease in globule Size (GS) with increase in the concentration of Capmul MCM oil and concentration of Cremophor RH 40: Transcutol-P (3:1) was observed.

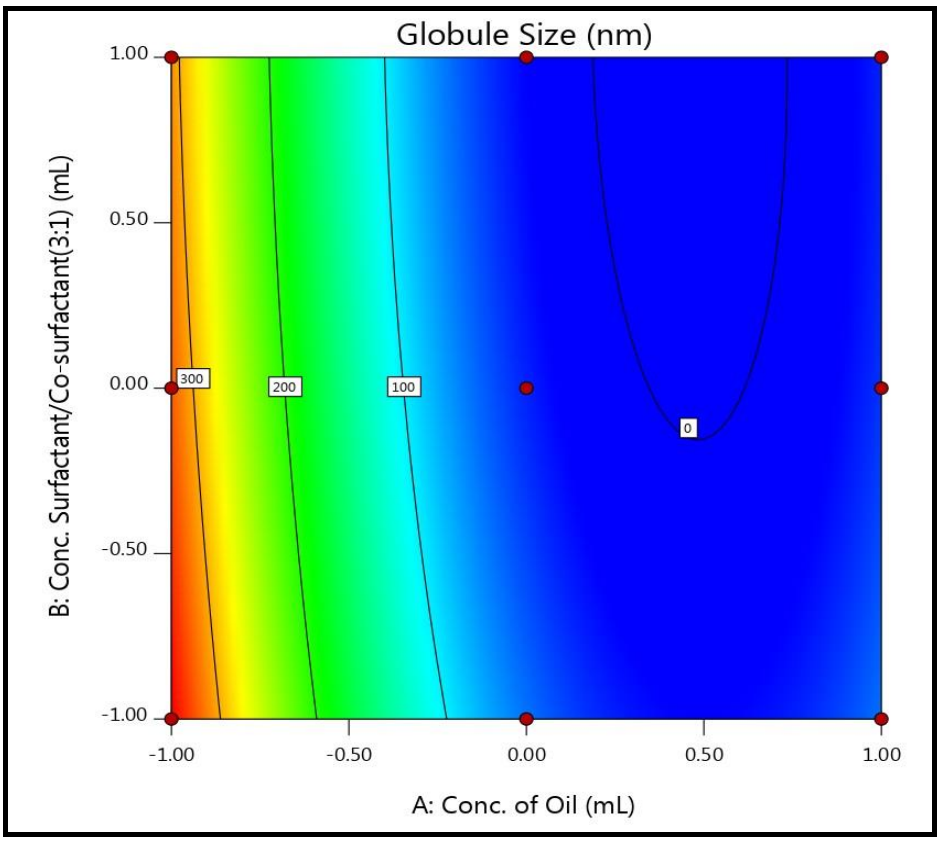

Figure 6.20: Contour plot for the effect on globule size

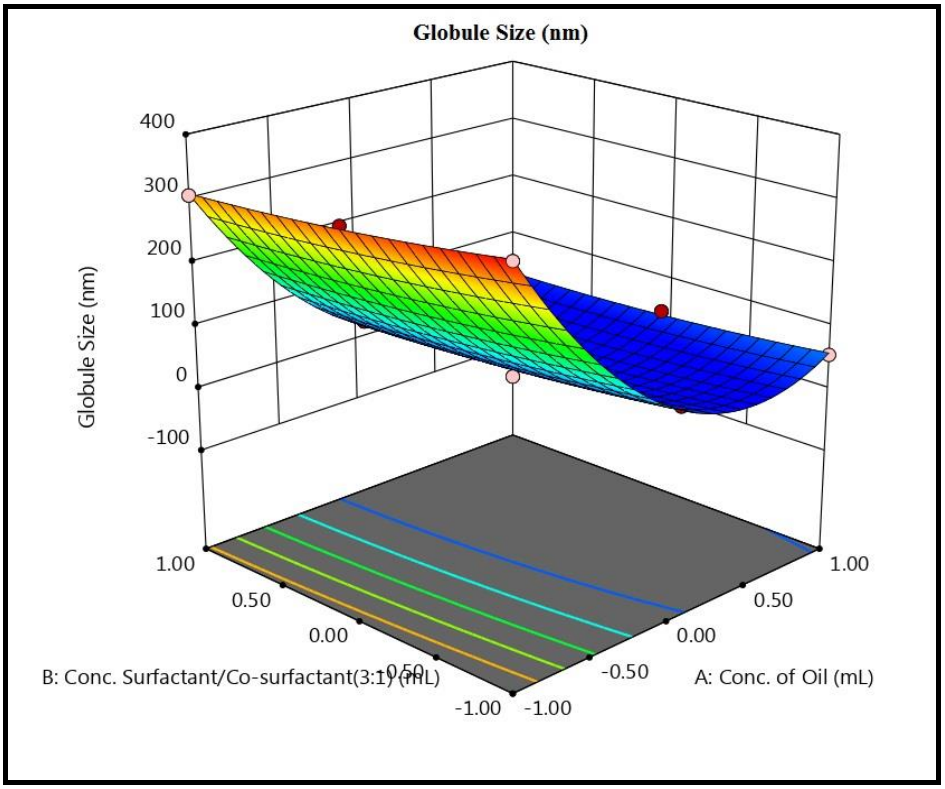

Figure 6.21: 3D surface plot for the effect on Globule size (GS)

(b) Polydispersity index (PDI)

Figure 6.22 showed contour plot for polydispersity index (PDI) at prefixed values of 0.2260, 0.2500, 0.3000, 0.3500, and 0.4000. The contour plot was found to be linear. Hence, the relationship between independent variables for polydispersity index (PDI) could be linear.

Figure 6.23 showed the response surface plot obtained as a function of concentration of Capmul MCM oil and concentration of Cremophor RH 40: Transcutol-P (3:1) for polydispersity index (PDI). A decrease in polydispersity index (PDI) with increase in the concentration of Capmul MCM oil and concentration of Cremophor RH 40: Transcutol-P (3:1) was observed.

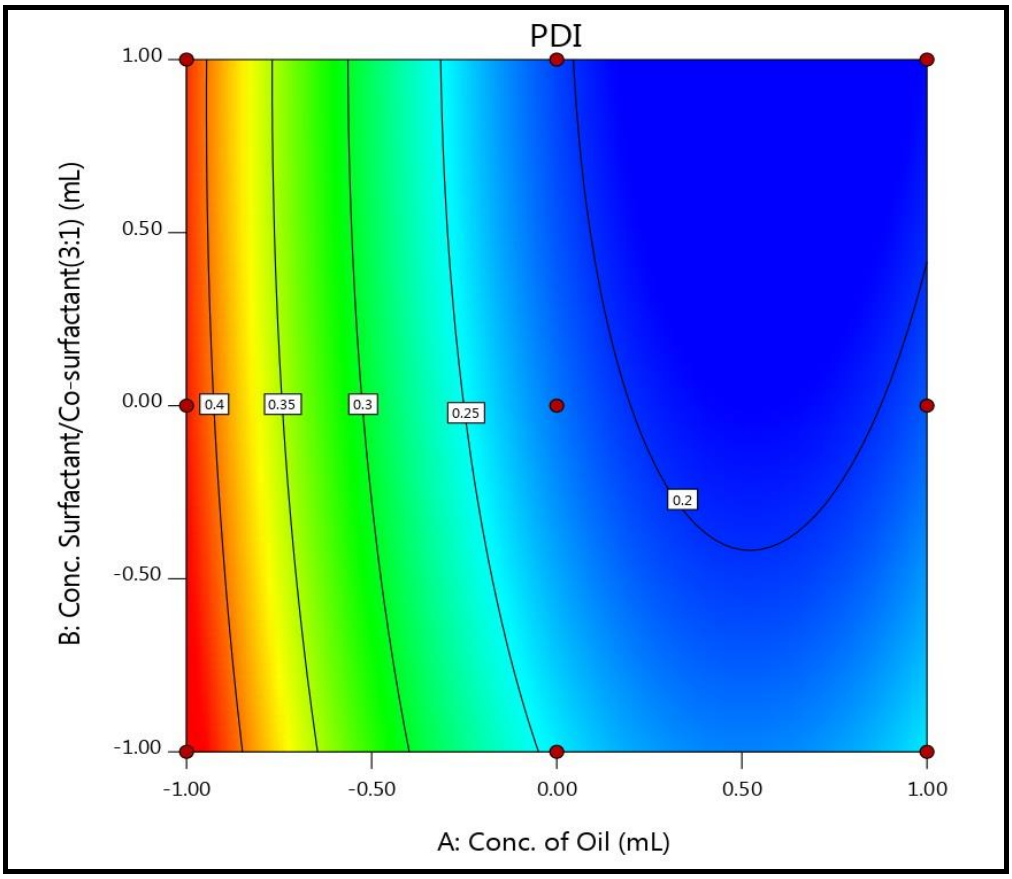

Figure 6.22: Contour plot for the effect on PDI

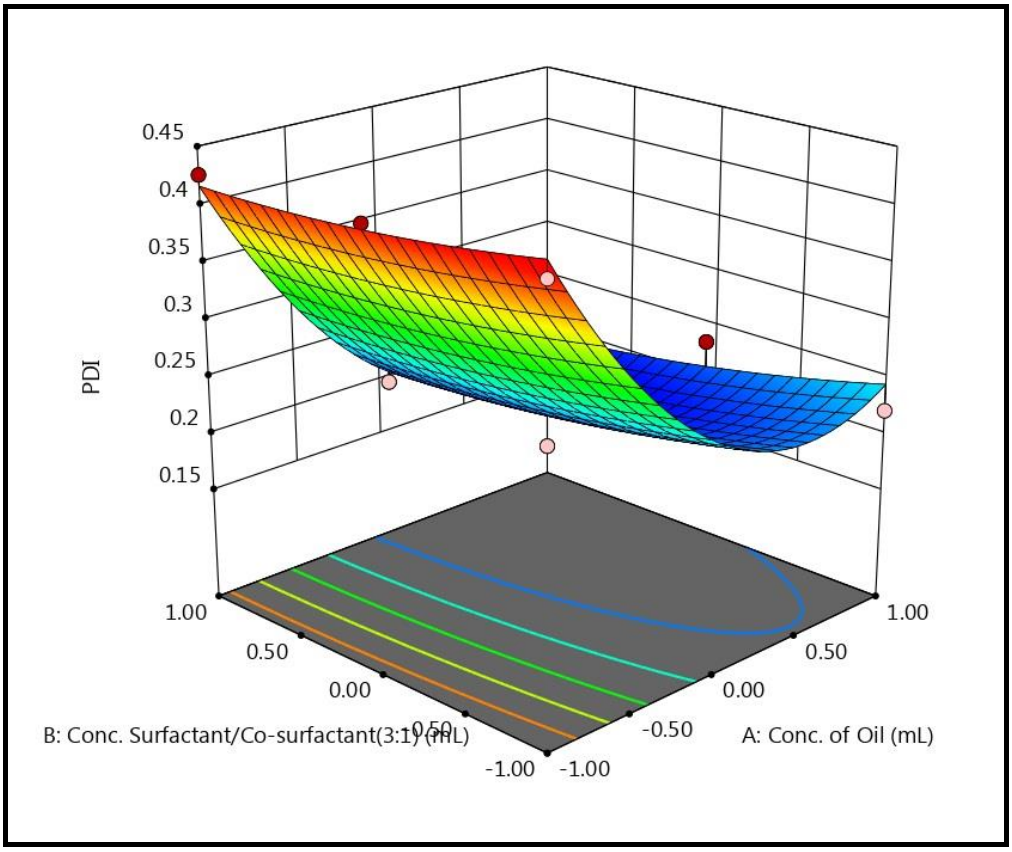

Figure 6.23: 3D surface plot for the effect on Polydispersity Index (PDI)

(c) Zeta Potential (ZP)

Figure 6.24 showed contour plot for zeta potential (ZP) at prefixed values of -15.5, -18.5, -20.5, and -22.5. The contour plot was found to be non-linear. Hence, the relationship between independent variables for zeta potential (ZP) could be non-linear because zeta potential may not be directly proportional to variable X_1 & X_2.

Figure 6.25 showed the response surface plot obtained as a function of concentration of Capmul MCM oil and concentration of Cremophor RH 40: Transcutol-P (3:1) for zeta potential (ZP). A decrease in zeta potential (ZP) with increase in the concentration of Capmul MCM oil and concentration of Cremophor RH 40: Transcutol-P (3:1) was observed up to some concentration.

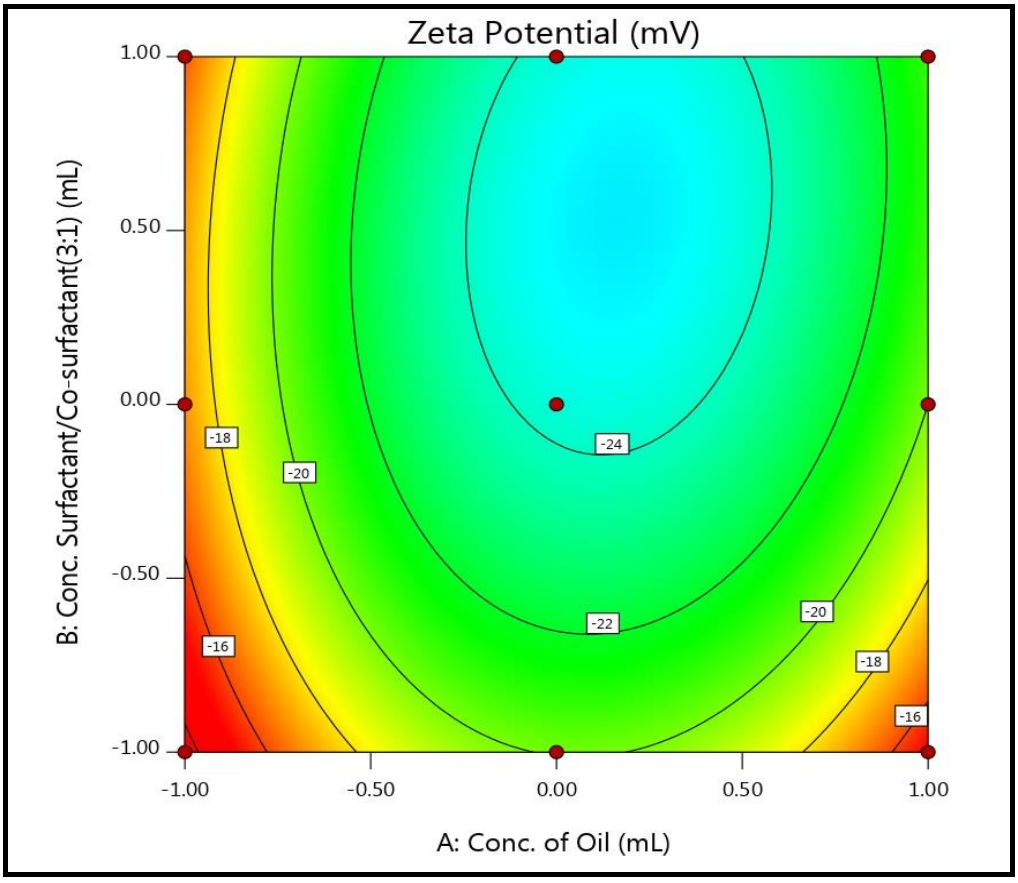

Figure 6.24: Contour plot for the effect on Zeta Potential

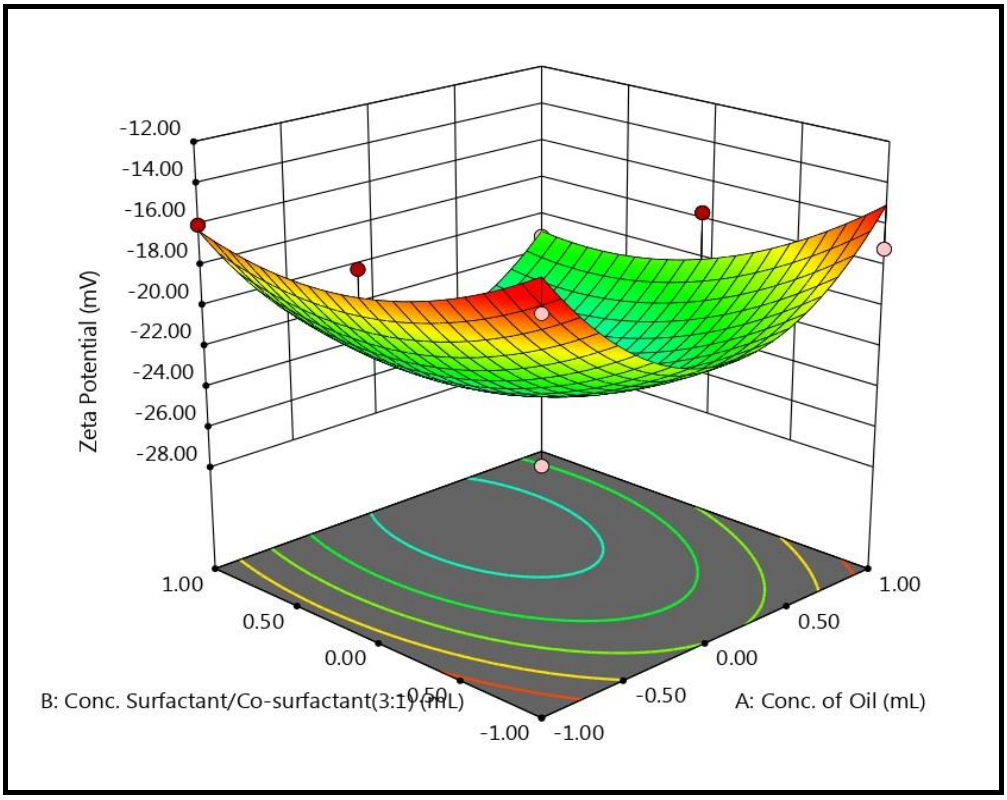

Figure 6.25: 3D surface plot for the effect on Zeta potential (ZP)

(d) Drug release at 15 minutes for Fenofibrate (DRF)

Figure 6.26 showed contour plot for drug release at 15 minutes for Fenofibrate (DRF) at prefixed values of 92.55, 93.55, 94.55, and 95.55. The contour plot was found to be non-linear. Hence, the relationship between independent variables for drug release at 15 minutes for Fenofibrate (DRF) could be non-linear because drug release at 15 minutes for Fenofibrate (DRF) may not be directly proportional to variable X_1 & X_2.

Figure 6.27 showed the response surface plot obtained as a function of concentration of Capmul MCM oil and concentration of Cremophor RH 40: Transcutol-P (3:1) for drug release at 15 minutes for Fenofibrate (DRF). An increase in drug release with increase in the concentration of Capmul MCM oil and concentration of Cremophor RH 40: Transcutol-P (3:1) was observed.

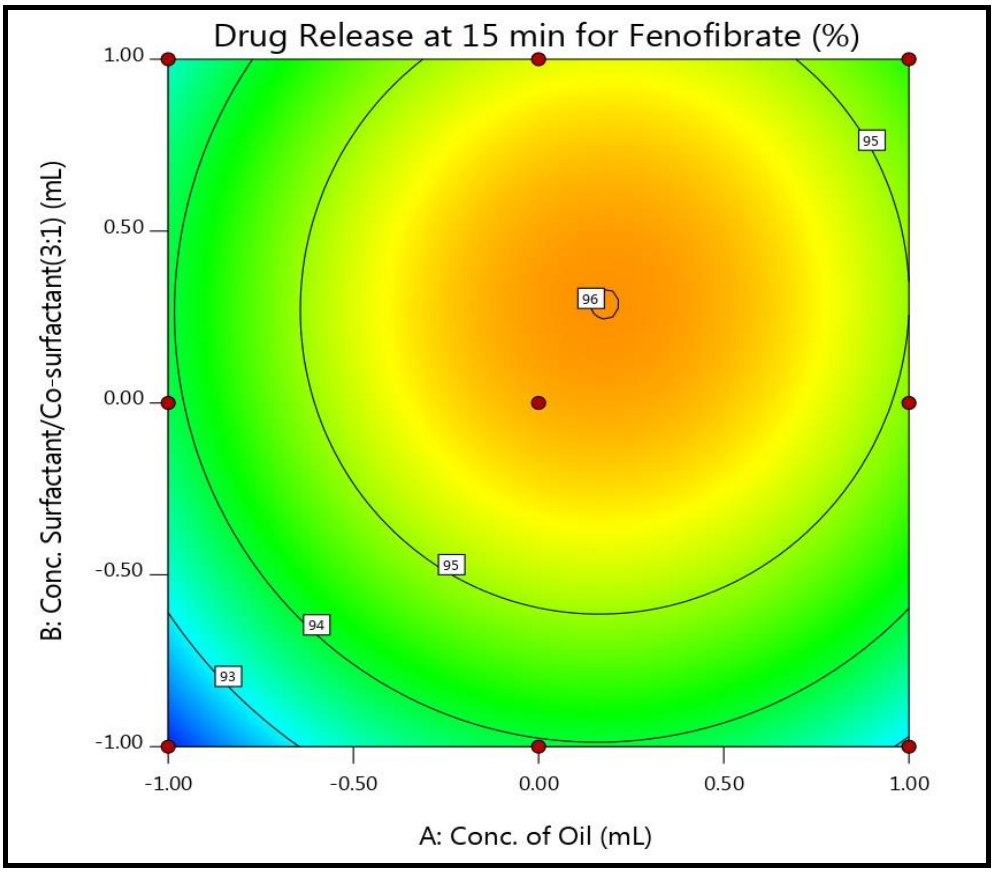

Figure 6.26: Contour plot for the effect on Drug Release at 15 min for Fenofibrate

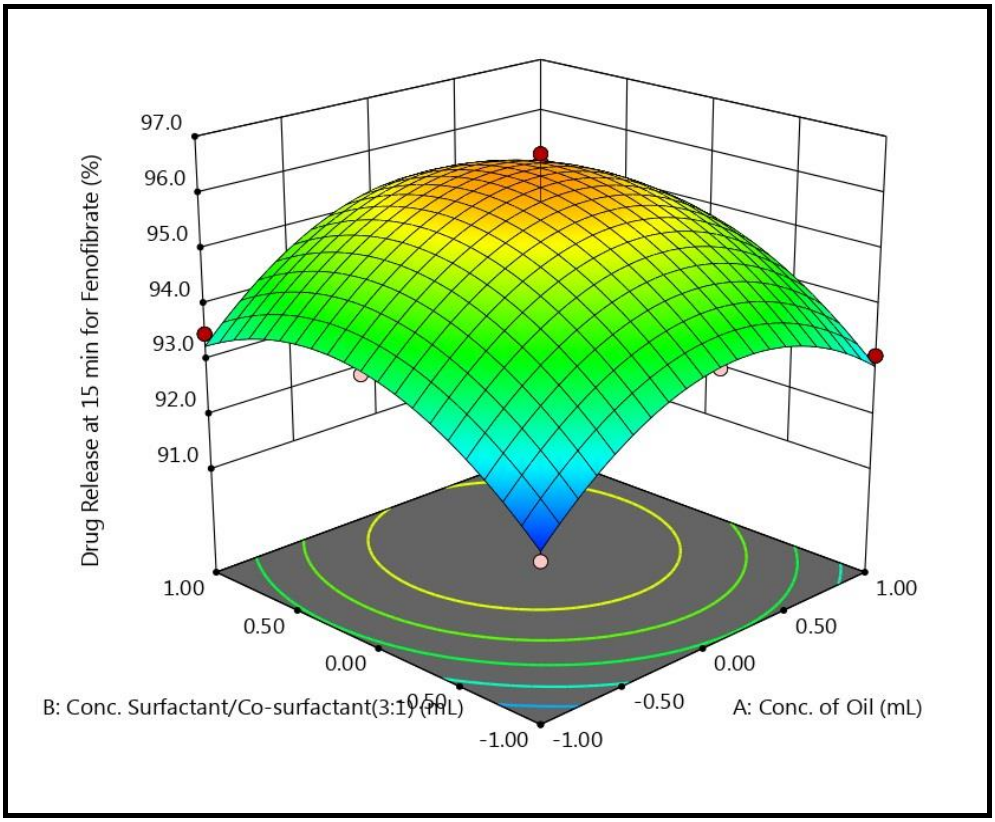

Figure 6.27: 3D surface plot for the effect on drug release (Fenofibrate)

(e) Drug release at 15 minutes for Atorvastatin Calcium (DRA)

Figure 6.28 showed contour plot for drug release at 15 minutes for Atorvastatin Calcium (DRA) at prefixed values of 92.75, 93.75, 94.75, and 95.5. The contour plot was found to be non-linear. Hence, the relationship between independent variables for drug release at 15 minutes for Atorvastatin Calcium (DRA) could be non-linear because drug release at 15 minutes for Atorvastatin Calcium (DRA) may not be directly proportional to variable X_1 & X_2.

Figure 6.29 showed the response surface plot obtained as a function of concentration of Capmul MCM oil and concentration of Cremophor RH 40: Transcutol-P (3:1) for drug release at 15 minutes for Atorvastatin Calcium (DRA). An increase in drug release with increase in the concentration of Capmul MCM oil and concentration of Cremophor RH 40: Transcutol-P (3:1) was observed.

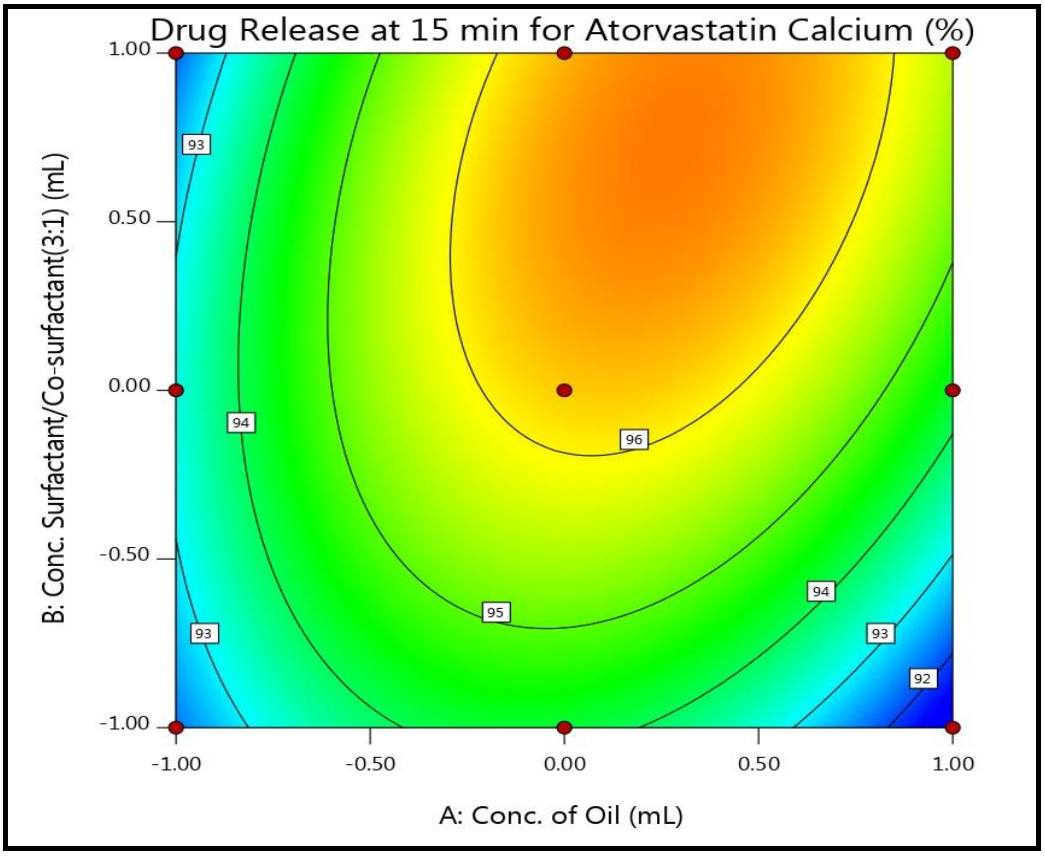

Figure 6.28: Contour plot for the effect on Drug Release at 15 min for Atorvastatin Calcium

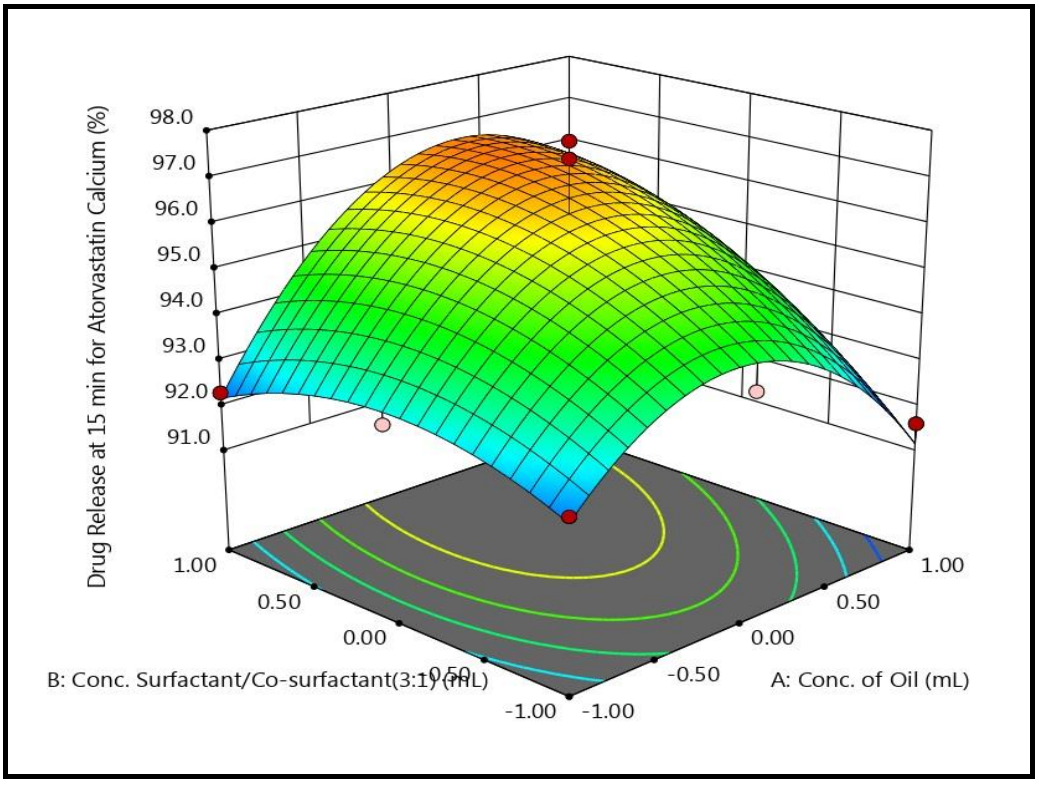

Figure 6.29: 3D surface plot for the effect on drug release (Atorvastatin Calcium)

Optimization of SNEDDS Formulation

The desirability function approach is a technique for the simultaneous determination of optimum settings of input variables that can determine optimum performance levels for one or more responses [10, 18]. The desirability procedure involves two steps:

(1) Finding the levels of the independent variables that simultaneously produce the most desirable predicted responses on the dependent variables

(2) Maximize the overall desirability with respect to the controllable factors.

Desirabilities range from zero to one for any given response. The program combines individual desirabilities into a single number and then searches for the greatest overall desirability nearer to 1. A value of one represents the case where all goals are met perfectly.

By using numerical optimization, a desirable value for each input factor and response can be selected. Therein, the possible input optimizations that can be selected include: the range, maximum, minimum, target, none (for responses) and set so as to establish an optimized output value for a given set of conditions.

Optimized formulation was selected by arbitrarily fixing the criteria of 20.7 – 357nm of the Globule size (GS), 0.189 – 0.428 Polydispersity index (PDI), -30mV to -21mV Zeta potential (ZP), more than 95% drug released at 15 minutes for Fenofibrate, and more than 95% drug released at 15 minutes for Atorvastatin Calcium. These constrains were shown in Table 6.29 for the SNEDDS formulation. The recommended concentrations of the independent variables were calculated by the Design Expert software using overlay plot with desirability approach (Figure 6.30 – 6.33). The results gave one optimized solution with theoretical target profile characteristics which were shown in Table 6.30.

Table 6.29: Constrains for SNEDDS Formulation

Name	Goal	Lower limit	Upper limit	Importance
Conc. of Capmul MCM oil	is in range	-1	1	+++
Conc. of Cremophor RH 40:Transcutol-P (3:1)	is in range	-1	1	+++
Globule Size (nm)	minimize	20.7	357	+++
Polydispersity index	maximize	0.189	0.428	+++
Zeta potential (mV)	is target = -27	-30	-21	+++
Drug Release at 15 minutes for Fenofibrate	is in range	95	96.7	+++
Drug Release at 15 minutes for Atorvastatin calcium	is in range	95	97.4	+++

Table 6.30: Solution proposed by Design Expert

Sol. Run	Conc. of oil (mL)	Conc. of S:Co-s (3:1) (mL)	GS (nm)	PDI	ZP (mV)	Drug Release at 15 min for Fenofibrate	Drug Release at 15 min for Atorvastatin Calcium
1	0.471	1.608	79.85	0.250	-23.75	95.67	96.01

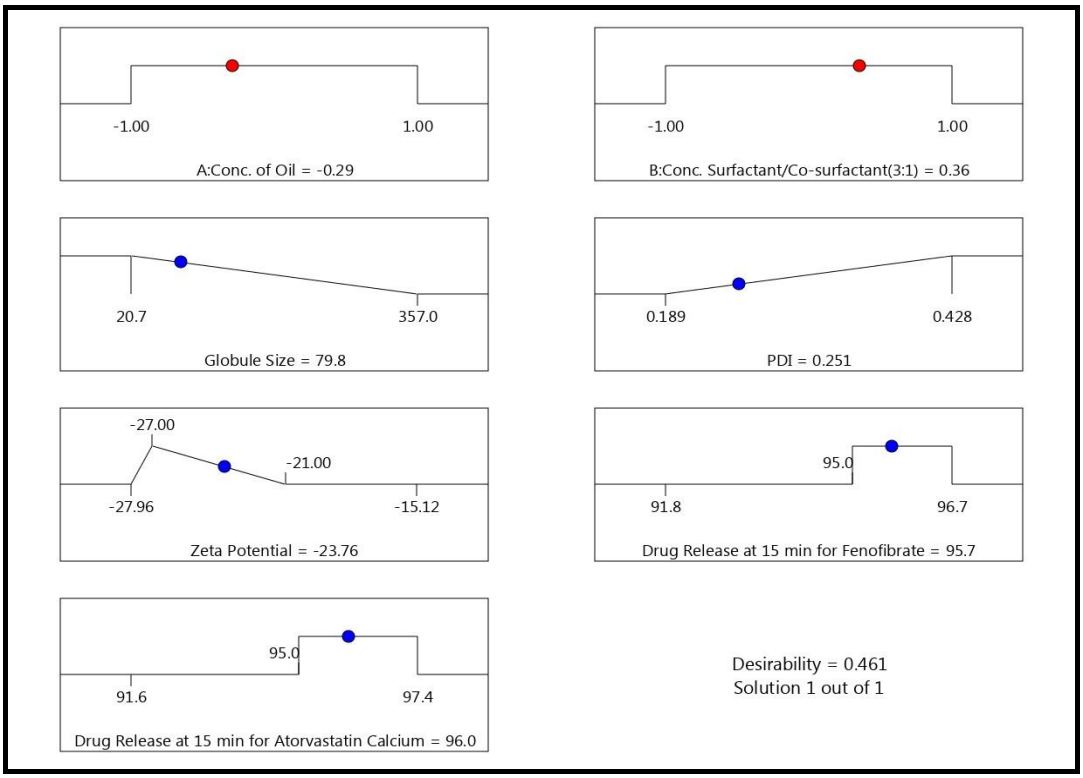

Figure 6.30: Desirability ramp for optimization of SNEDDS formulation

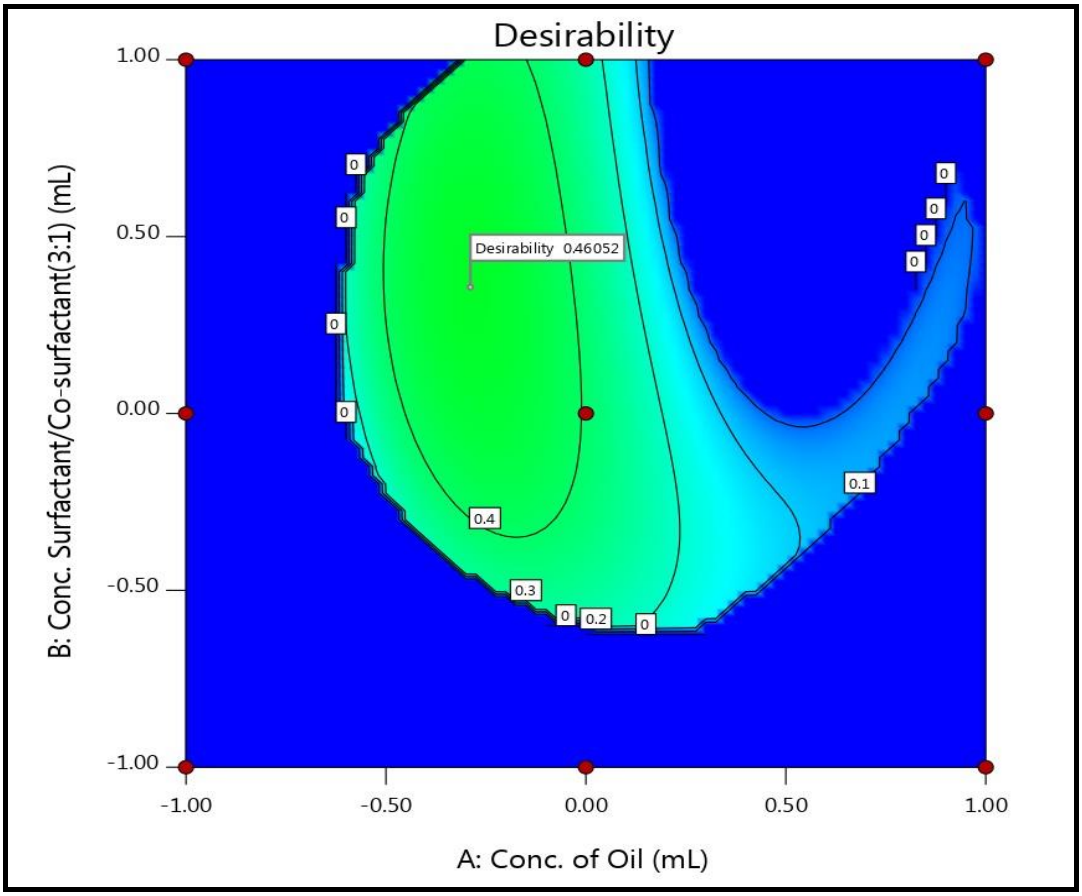

Figure 6.31: Desirability contour plot for optimization of SNEDDS formulation

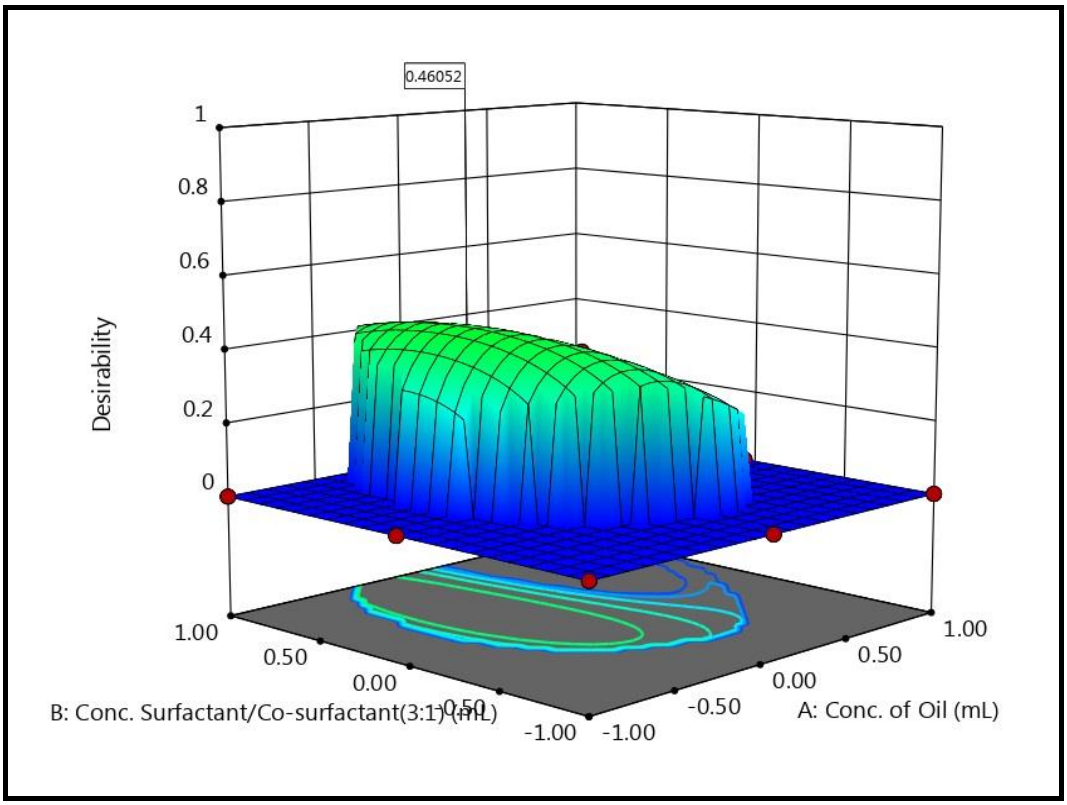

Figure 6.32: Desirability 3D surface plot for optimization of SNEDDS formulation

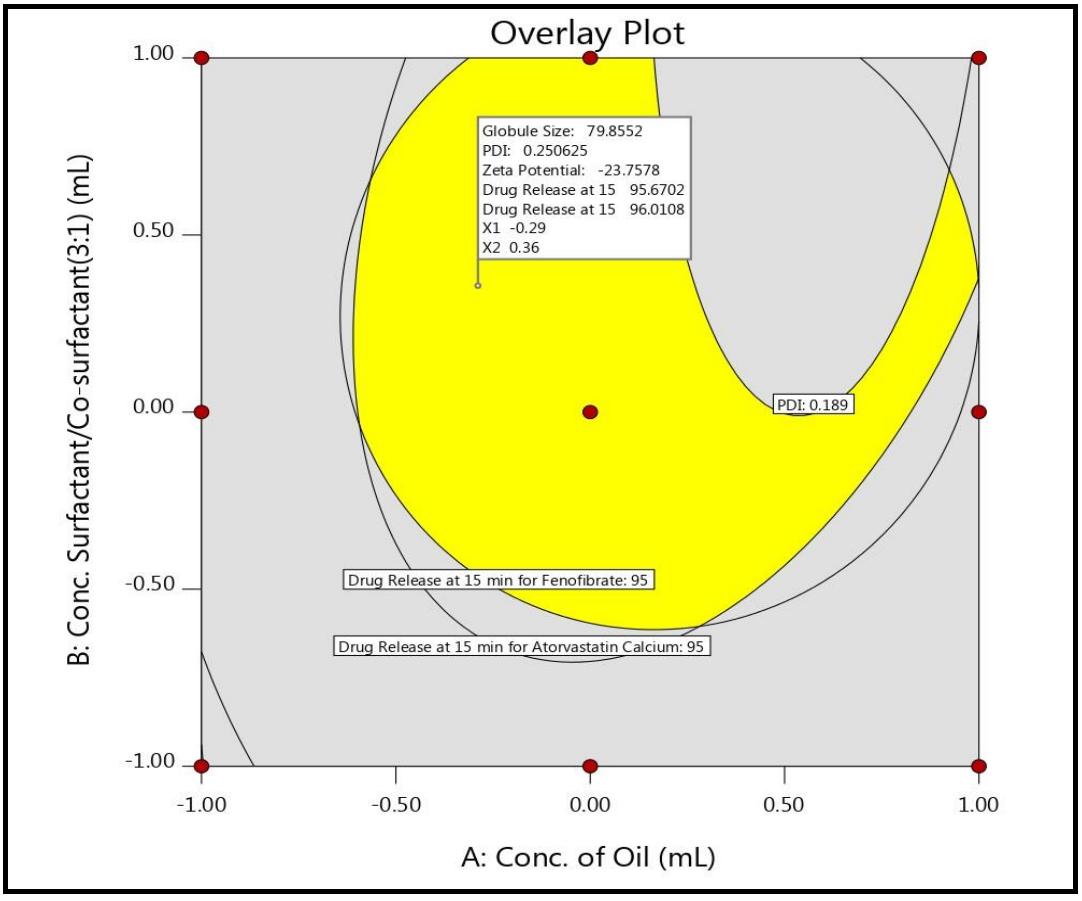

Figure 6.33: Overlay plot for optimization of SNEDDS formulation

Figure 6.30 showed desirability ramp for optimization of SNEDDS formulation. The ramp display combined the individual graphs for easier interpretation. The dot on each ramp reflected the factor setting or response prediction for that solution. The height of the dot showed how desirable it is.

Figure 6.31 showed desirability contour plot for optimization of SNEDDS formulation. The contour plot with graduated colours – cool blue for lower desirability and warm yellow for higher. Design-Expert software sets a flag at the optimal point.

Figure 6.33 showed the overlay plot obtained from Design Expert. Grayed area (Shaded areas) on the graphical optimization plot did not meet the selection criteria. The plot, yellow area indicated the area in which the optimized formulation can be formulated. In this yellow portion, the values of all variables i.e. Globule size, Polydispersity index, Zeta potential, drug release at 15 minutes for Fenofibrate, and drug release at 15 minutes for Atorvastatin calcium were selected. The point indicating toggle flag showed the value of X_1 and X_2 for optimized formulation.

Table 6.31: Optimized formulation of Fenofibrate and Atorvastatin Calcium SNEDDS (Batch OP1)

Material used	Quantity per Unit (mL)	Quantity per Unit (%)
Capmul MCM oil	0.471	23.62%
Cremophor RH 40	1.206	57.27%
Transcutol-P	0.402	19.11%

Check point batch analysis

Two different check point batches (C1 and C2) of Fenofibrate and Atorvastatin Calcium SNEDDS were prepared according to the levels of factors as shown in Table 6.32. The check points was evaluated for globule size, polydispersity index, zeta potential, drug release at 15 minutes for Fenofibrate, and drug release at 15 minutes for Atorvastatin Calcium. The experimentally and theoretically computed values of GS, PDI, ZP, drug release at 15 minute for fenofibrate and Atorvastatin Calcium were presented in Table 6.32. The results were compared using student 't' test, the difference was found to be non-significant ($p<0.05$) in both cases. Ratio confirmed the utility of established contour plots

and reduced polynomial equation for both GS, PDI, ZP, and drug release at 15 minute for Fenofibrate and Atorvastatin Calcium in the preparation of SNEDDS containing Fenofibrate and Atorvastatin Calcium.

Table 6.32: Composition and results of Check point batches

Batches	**C1**		**C2**	
X_1	0.5		-0.5	
X_2	-0.5		0.5	
Response	**Predicted**	**Experimental**	**Predicted**	**Experimental**
Globule size (nm) [Y_1]	132.39	127.3	7.42	11.56
Polydispersity index [Y_2]	0.288	0.291	0.203	0.194
Zeta potential (mV) [Y_3]	-22.42	-22.15	-21.76	-20.33
Drug release at 15 minutes for Fenofibrate [Y_4]	95.26	95.6	95.06	93.9
Drug release at 15 minutes for Atorvastatin Calcium [Y_5]	95.35	95.8	94.81	93.6

6.3.5 Evaluation parameters of Fenofibrate and Atorvastatin Calcium SNEDDS of factorial design batches

The refractive index, %transmittance, drug content, effect of dilution, viscosity, pH, self-emulsification and precipitation, centrifugation and freeze-thaw cycle, in vitro drug release and in vitro drug diffusion studies were done for factorial design batches.

(a) Refractive Index and Turbidimetric Evaluation

The results of refractive index and % transmittance of batches T1 to T9 were shown in Table 6.33. The refractive index and percent transmittance data proved that transparency of system [6, 19].

Table 6.33: Refractive Index and %Transmittance of various SNEDDS formulations

Batches	Refractive Index	% Transmittance
	Water (250 ml)	Water (250 ml)
T1	1.373	91.36
T2	1.359	97.43
T3	1.352	97.86
T4	1.369	92.75
T5	1.338	100.0
T6	1.347	98.14
T7	1.362	93.52
T8	1.339	99.31
T9	1.342	98.92

(b) Measurement of Globule Size, Polydispersity Index, and Zeta Potential

Globule size distribution following self nanoemulsification is a critical factor to evaluate self-nanoemulsion system. The smaller droplets have larger interfacial surface area will be provided for drug [20, 21]. Globule size analysis, Polydispersity Index and Zeta Potential data were shown in Table 6.34.

Table 6.34: Droplet size analysis, Polydispersity Index, and Zeta Potential data of SNEDDS formulation

Batches	Globule Size (nm)	Polydispersity Index	Zeta Potential (mV)
T1	357.0	0.428	-15.12
T2	64.1	0.283	-16.40
T3	55.8	0.221	-17.12
T4	332.0	0.427	-15.68
T5	20.7	0.189	-27.96
T6	44.0	0.233	-17.60
T7	307.0	0.426	-15.96
T8	26.6	0.195	-24.28
T9	29.2	0.191	-21.12

Generally, an increase of electrostatic repulsive forces between microemulsion/nanoemulsion droplets prevents the coalescence of microemulsion/nanoemulsion droplets. On the contrary, a decrease of electrostatic repulsive forces will cause phase separation [20, 21]. Fenofibrate and Atorvastatin Calcium SNEDDS (T5) was diluted with distil water, and resulted zeta potential was -27.96 mV. Several studies have reported that the zeta potential played an important role in stability of microemulsion/nanoemulsion.

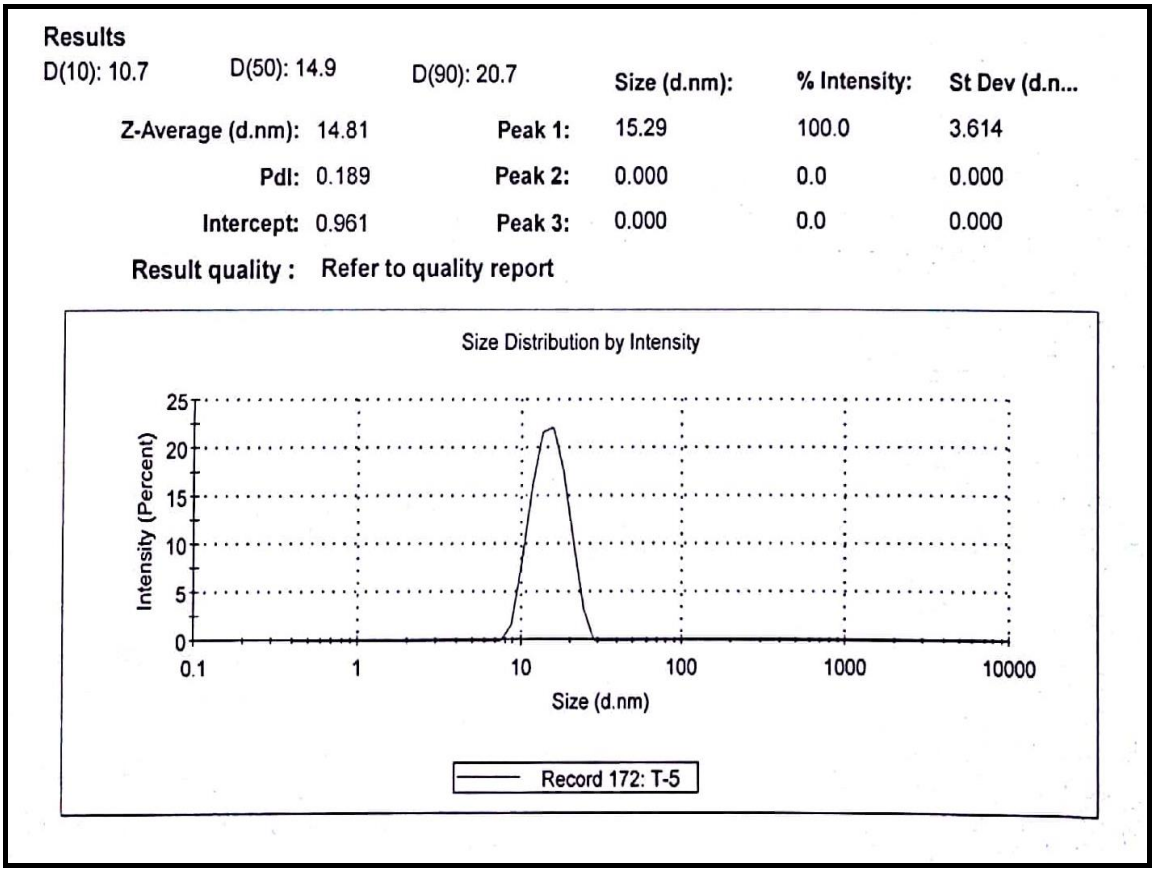

Figure 6.34: Droplet size and Polydispersity index of SNEDDS formulation T5

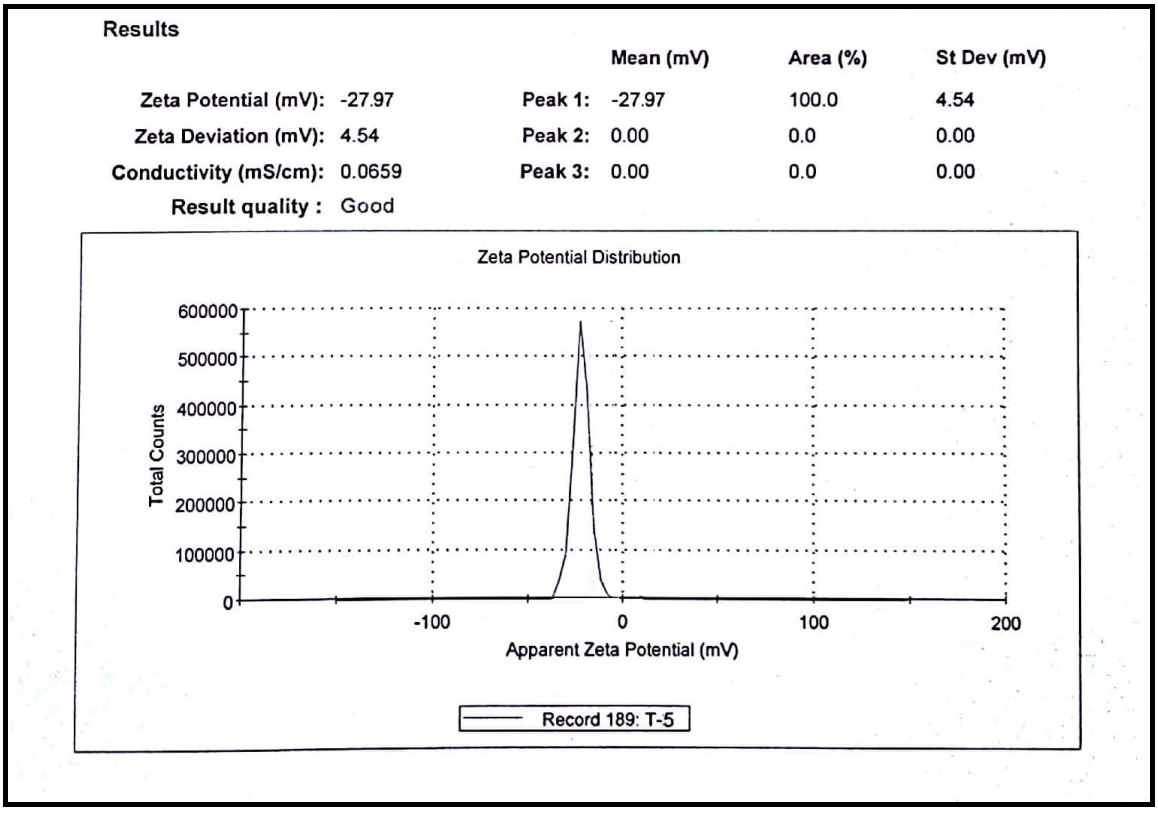

Figure 6.35: Zeta potential of SNEDDS formulation T5

(c) Drug Content

Drug content of SNEDDS formulation can be found by methanolic extract of SNEDDS was analyzed by HPLC at 248nm for Fenofibrate and Atorvastatin Calcium respectively. Drug content of various formulation shown in Table 6.35 (n=3) and Table 6.36 (n=3).

Table 6.35: Drug content in various SNEDDS formulations (Fenofibrate)

Batches	% Drug Content			Average	Standard Deviation
	I	II	III		
T1	99.1	98.3	99.3	98.9	0.53
T2	98.3	98.6	98.9	98.6	0.30
T3	99.4	100.2	99.1	99.6	0.57
T4	99.8	99.1	100.4	99.8	0.65
T5	100.2	101.1	100.5	100.6	0.46
T6	99.2	100.4	99.5	99.7	0.62
T7	101.4	99.8	100.6	100.6	0.80
T8	99.6	100.7	99.9	100.1	0.57
T9	100.3	99.1	100.9	100.1	0.92

Table 6.36: Drug content in various SNEDDS formulations (Atorvastatin Calcium)

Batches	% Drug Content			Average	Standard Deviation
	I	II	III		
T1	99.6	98.5	99.8	99.3	0.70
T2	98.9	98.7	98.5	98.7	0.20
T3	99.2	100.5	99.7	99.8	0.66
T4	98.4	98.8	99.6	98.9	0.61
T5	100.1	99.8	100.3	100.1	0.25
T6	99.9	98.6	99.2	99.2	0.65
T7	100.5	99.4	100.1	100.0	0.56
T8	100.3	99.1	98.9	99.4	0.76
T9	99.7	99.3	100.2	99.7	0.45

(d) Effect of Dilution and Aqueous Phase Composition on SNEDDS

Effect of dilution and aqueous phase composition on SNEDDS were shown in Table 6.37. Data was shown for various SNEDDS formulation at 25 ± 2°C for 24 hour.

Table 6.37: Effect of dilution and aqueous phase composition on SNEDDS formulation

Batches	Medium	Drug Precipitation	Phase Separation
T1	Distil water	Not found	Not found
T2	Distil water	Not found	Not found
T3	Distil water	Not found	Not found
T4	Distil water	Not found	Not found
T5	Distil water	Not found	Not found
T6	Distil water	Not found	Not found
T7	Distil water	Not found	Not found
T8	Distil water	Not found	Not found
T9	Distil water	Not found	Not found

Ability of nanoemulsion to be diluted without any phase separation and drug precipitation is essential for its use as a drug delivery system. The results indicated that SNEDDS can be diluted up to 1,000-fold without any phase separation or drug precipitation and remained stable over a period of 24 hr. Aqueous phase composition also did not affect physical stability of resulting nanoemulsion. These results were in contrast with phospholipids based microemulsion systems described in literature that became turbid leading to phase separation after dilution [22, 23]. This suggested that Cremophor RH 40 and Transcutol-P system could reside at interface for sufficiently longer period despite larger dilutions, producing stable microemulsion/nanoemulsion. In addition, drug was not precipitated even after large dilution up to 24 hours, thus confirming solvent capacity of nanoemulsion.

(e) Measurement of Viscosity and pH of SNEDDS

Viscosity of SNEDDS was measured by using Brookfield viscometer at 25°C temperature. Spindle S-61 was selected for measurement of viscosity of various SNEDDS formulations. Viscosity measurement was done at 30 rpm before and after dilution with water. pH of SNEDDS formulations were measured by using pH meter at room temperature. pH of SNEDDS formulations were also measured before and after dilution with distil water [19, 20]. Viscosity and pH data of SNEDDS formulation was shown in Table 6.38.

Table 6.38: Viscosity and pH of various SNEDDS formulations				
Batches	**Viscosity (CP)**		**pH**	
	Dilution		**Dilution**	
	Before	**After**	**Before**	**After**
T1	97.8	1.04	7.731	6.423
T2	114.9	1.01	7.681	6.466
T3	105.6	1.08	7.659	6.531
T4	106.0	1.04	7.186	6.505
T5	109.4	1.02	7.710	6.496
T6	107.3	1.03	7.522	6.481
T7	104.5	1.05	7.539	6.493
T8	117.0	1.02	7.485	6.512
T9	115.0	1.05	7.565	6.501

Viscosity data has shown that viscosity of formulation before dilution was greater than after dilution of formulation. Data has shown that viscosity of formulation after dilution was near to viscosity of water.

(f) Self-Emulsification and Precipitation Assessment

The results of self nanoemulsification and precipitation studies were shown in Table 6.39.

Table 6.39: Self-emulsification and Precipitation of various SNEDDS formulations			
Batches	**Dispersion Time (second)**	**Clarity**	**Precipitation**
T1	70	Translucent to clear	Stable
T2	55	clear	Stable
T3	55	clear	Stable
T4	58	Translucent to clear	Stable
T5	40	clear	Stable
T6	50	clear	Stable
T7	62	Translucent to clear	Stable
T8	43	clear	Stable
T9	42	clear	Stable

Formulation T5 and T9 showed less dispersion time, clear and stable nanoemulsion.

(g) Centrifugation and Freeze– Thaw Cycle

The effect of centrifugation and freeze–thaw cycling on phase separation of nanoemulsion and precipitation of drug is shown in Table 6.40. Both accelerated tests are carried out to ascertain stability of nanoemulsion under stress conditions.

Table 6.40: Centrifugation and Freeze-Thaw Cycle data of various SNEDDS formulations

Batches	Centrifugation		Freeze-thaw cycle	
	Phase separation	Drug precipitation	Phase separation	Drug precipitation
T1	No	Slight	No	Slight
T2	No	No	No	No
T3	No	No	No	No
T4	No	No	No	No
T5	No	No	No	No
T6	No	No	No	No
T7	No	No	No	No
T8	No	No	No	No
T9	No	No	No	No

Batches which did not show any drug precipitation, phase separation after centrifugation confirming its stable nature. Similarly, batches which survived freeze–thaw cycling as it was found to be reconstituted without any phase separation, drug precipitation after exposure to freeze–thaw cycling [22, 24].

(h) In Vitro drug release Study

Drug release from the SNEDDS batch T5 was found to be significantly higher as compared with that of plain fenofibrate and Atorvastatin calcium drug powder and marketed drug formulation (Figure 6.36 and 6.37). It could be suggested that the SNEDDS formulation resulted in spontaneous formation of a nanoemulsion with a small droplet size, which permitted a faster rate of drug release into the aqueous phase, much faster than that of plain fenofibrate and Atorvastatin calcium drug powder and marketed drug

formulation. Thus, this greater availability of dissolved fenofibrate and Atorvastatin calcium from the SNEDDS formulation could lead to higher absorption and higher oral bioavailability.

Table 6.41: Comparison of drug release profile of various SNEDDS formulation with pure drug and marketed formulation (Fenofibrate)

Batches	% Drug Release (Fenofibrate) (Mean ± SD)						
	Time (Minutes)						
	0	10	15	20	30	45	60
T1	0±0	88.6±1.9	91.8±2.1	96.0±1.1	98.4±0.4	99.4±0.2	99.5±0.2
T2	0±0	90.7±2.1	93.9±1.8	96.7±0.5	98.7±0.3	99.2±0.4	99.7±0.2
T3	0±0	90.3±1.1	93.1±1.5	95.8±1.1	98.4±0.4	99.4±0.2	99.6±0.2
T4	0±0	90.6±1.8	93.8±1.3	97.1±1.5	98.7±0.9	99.3±1.1	99.5±0.5
T5	0±0	92.6±1.5	96.7±1.2	99.5±1.1	100.1±0.2	99.8±0.1	99.6±0.3
T6	0±0	91.2±2.0	94.1±2.0	96.9±1.4	98.9±0.6	99.5±0.5	99.7±0.3
T7	0±0	90.1±2.0	93.5±1.8	96.7±0.6	98.3±0.7	98.9±0.6	99.2±0.4
T8	0±0	91.2±2.0	94.5±1.1	96.9±1.4	98.9±0.6	99.5±0.5	99.7±0.3
T9	0±0	91.2±1.7	95.0±1.2	97.5±1.2	98.9±0.4	99.2±0.2	99.5±0.3
Pure drug	0±0	7.3± 0.7	17.6±0.8	19.1±0.5	27.4±1.1	38.7±0.4	48.2±0.5
Fenostat	0±0	14.1±0.2	22.8±0.7	23.2±0.8	32.4±0.5	47.5±1.4	59.3±1.4

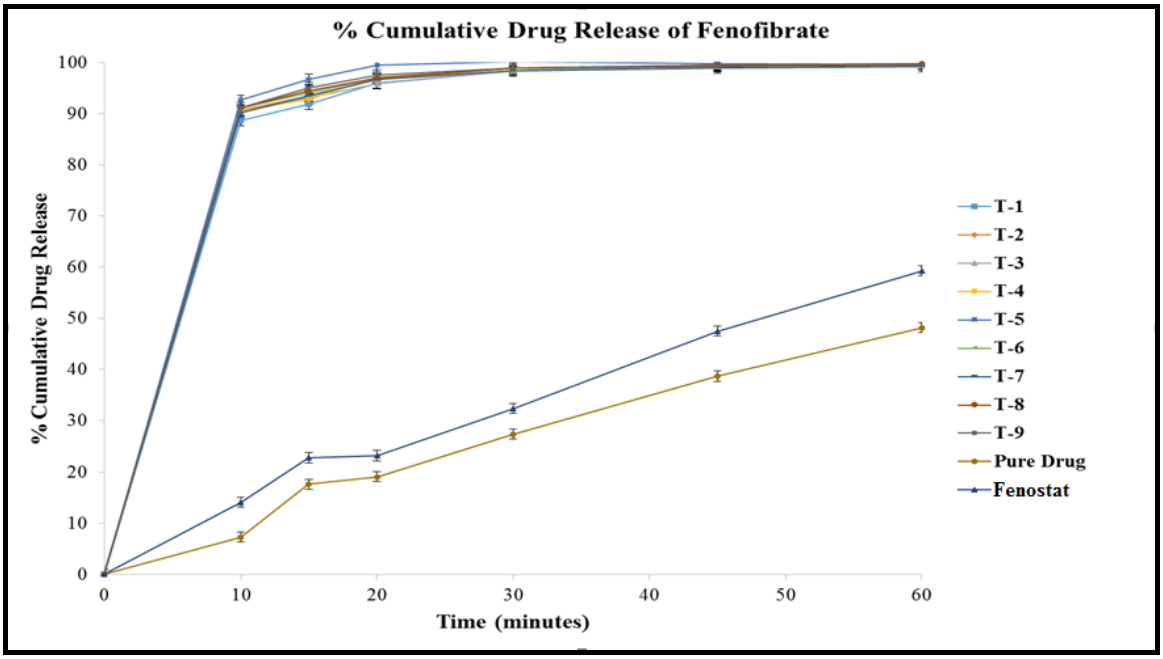

Figure 6.36: Comparison of drug release profile of various SNEDDS formulation with pure drug and marketed formulation (Fenofibrate)

Table 6.42: Comparison of drug release profile of various SNEDDS formulation with pure drug and marketed formulation (Atorvastatin Calcium)

Batches	% Drug Release (Atorvastatin Calcium) (Mean ± SD)						
	Time (Minutes)						
	0	10	15	20	30	45	60
T1	0±0	87.6±1.9	92.4±2.0	94.9±2.2	99.1±0.9	99.4±0.7	99.5±0.7
T2	0±0	89.6±1.5	93.6±0.7	96.2±1.6	99.4±0.5	99.7±0.4	100.0±0.1
T3	0±0	88.7±1.8	91.6±4.3	96.0±1.6	99.1±0.8	99.8±0.3	100.1±0.3
T4	0±0	89.8±1.5	92.9±0.7	95.6±1.6	98.8±0.4	99.6±0.5	99.7±0.6
T5	0±0	92.6±1.5	97.4±1.2	99.5±1.1	100.1±0.2	99.8±0.1	99.6±0.3
T6	0±0	88.8±2.4	93.4±0.8	95.9±1.4	98.8±1.0	99.5±0.4	100.1±0.4
T7	0±0	89.3±1.8	92.3±1.5	95.9±1.6	99.5±0.3	99.6±0.4	99.9±0.3
T8	0±0	93.3±0.7	95.8±0.4	99.0±0.8	99.4±0.3	99.6±0.3	99.8±0.2
T9	0±0	93.1±1.0	96.0±0.6	99.0±0.4	99.3±0.6	99.4±0.4	99.4±0.6
Pure drug	0±0	6.3± 1.7	13.3±0.9	18.7±0.8	26.6±1.3	36.7±0.7	46.5±0.5
Fenostat	0±0	13.6±1.2	18.9±0.8	24.7±0.7	31.6±0.5	46.5±1.1	57.6±1.0

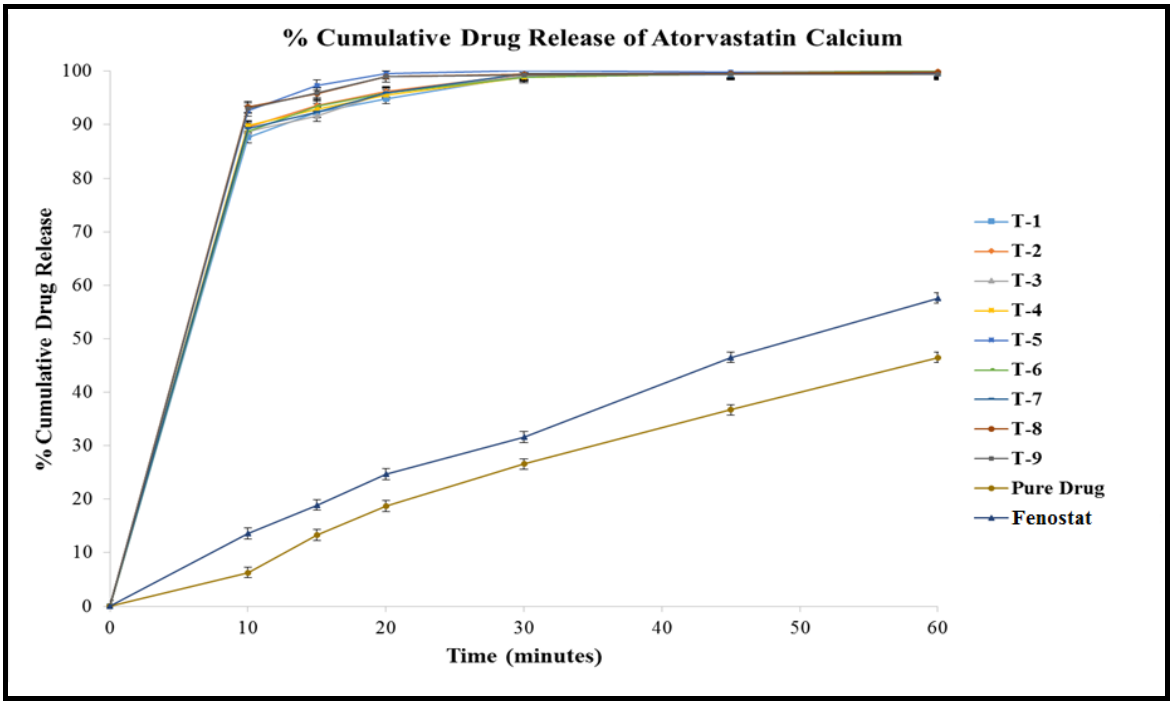

Figure 6.37: Comparison of drug release profile of various SNEDDS formulation with pure drug and marketed formulation (Atorvastatin Calcium)

(i) In Vitro Diffusion Study

To understand characteristics of drug release from SNEDDS, an in vitro release study was carried out. When SNEDDS encountered aqueous media, drug existed in system in different forms including a free molecular form, or mixed in micelles or in nanoemulsion droplets. Under this circumstance, it is necessary to separate isolated drug molecules from those trapped by micelles or nanoemulsion for a real in vitro release test. Therefore, it is not rational to use routine release approach in this case.

Comparison of diffusion profile of various SNEDDS formulation with pure drug and marketed formulation were shown in Table 6.43 and 6.44, Figure 6.38 and 6.39.

Table 6.43: Comparison of diffusion profile of various SNEDDS formulation with pure drug and marketed formulation (Fenofibrate)

Batches	% Drug Diffusion (Fenofibrate) (Mean ± SD)						
	Time (Minutes)						
	0	30	60	120	180	240	360
T1	0±0	31.6±2.6	53.4±1.7	66.1±1.2	78.4±0.9	82.3±0.7	86.2±0.6
T2	0±0	41.4±3.4	66.3±2.5	76.3±2.3	84.6±1.4	86.8±1.2	89.7±0.7
T3	0±0	42.3±3.1	65.3±1.9	78.6±1.4	85.7±0.9	87.4±0.9	90.6±0.8
T4	0±0	38.3±2.8	57.5±1.8	68.4±1.7	79.8±0.9	84.3±1.2	87.2±0.8
T5	0±0	50.2±2.5	79.5±1.7	93.6±1.2	95.3±1.1	96.8±0.8	97.5±0.8
T6	0±0	46.8±2.2	75.5±1.9	88.2±1.6	91.3±1.6	92.7±1.3	93.1±0.9
T7	0±0	40.2±3.5	56.7±2.6	68.2±1.9	78.3±1.6	86.2±1.4	89.2±1.4
T8	0±0	45.3±2.3	77.2±2.3	88.4±1.7	90.2±1.5	93.4±1.3	95.6±1.5
T9	0±0	47.2±2.7	76.2±2.2	87.3±1.4	90.1±1.6	92.2±1.5	93.7±1.3
Pure drug	0±0	7.1± 3.7	18.2±2.5	23.8±2.3	33.4±1.4	36.2±1.5	38.4±1.3
Fenostat	0±0	15.7±3.2	25.3±2.8	34.7±2.5	46.4±1.9	58.8±1.7	61.5±1.6

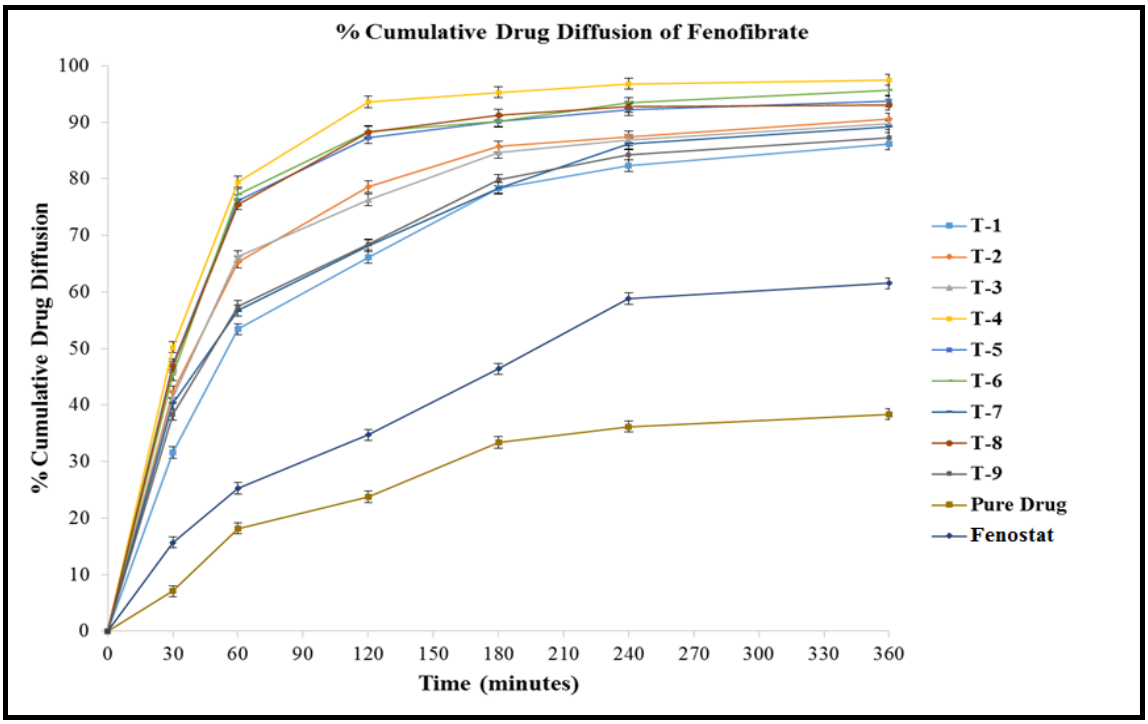

Figure 6.38: Comparison of diffusion profile of various SNEDDS formulation with pure drug and marketed formulation (Fenofibrate)

Table 6.44: Comparison of diffusion profile of various SNEDDS formulation with pure drug and marketed formulation (Atorvastatin Calcium)

Batches	% Drug Diffusion (Atorvastatin Calcium) (Mean ± SD)						
	Time (Minutes)						
	0	30	60	120	180	240	360
T1	0±0	33.3±2.2	51.5±1.9	67.3±1.7	77.3±1.8	83.2±1.3	85.8±1.4
T2	0±0	40.1±2.7	65.7±2.8	78.7±2.0	83.7±1.5	86.2±1.6	88.3±1.1
T3	0±0	41.8±2.1	67.7±2.2	76.3±2.4	84.3±1.7	86.7±1.9	89.2±0.9
T4	0±0	36.4±3.7	55.8±2.5	66.7±2.2	80.1±1.7	83.7±1.8	86.9±1.4
T5	0±0	52.4±2.2	78.4±1.9	92.5±1.4	96.2±1.2	97.4±0.9	98.2±0.6
T6	0±0	43.8±3.3	76.7±2.2	87.4±1.9	89.8±1.8	94.1±1.6	95.2±1.5
T7	0±0	41.7±3.0	54.9±2.8	67.8±2.9	77.8±1.8	87.1±1.2	89.9±1.5
T8	0±0	43.8±3.3	76.7±2.2	87.4±1.9	89.8±1.8	94.1±1.6	95.2±1.5
T9	0±0	46.6±3.1	75.7±2.6	85.7±2.4	91.4±1.8	92.8±1.7	94.2±1.1
Pure drug	0±0	8.5± 3.9	17.6±2.8	25.2±2.6	35.3±2.3	38.5±2.5	40.2±1.7
Fenostat	0±0	16.3±3.7	26.7±3.8	36.2±3.1	48.7±2.6	59.2±1.8	64.2±1.9

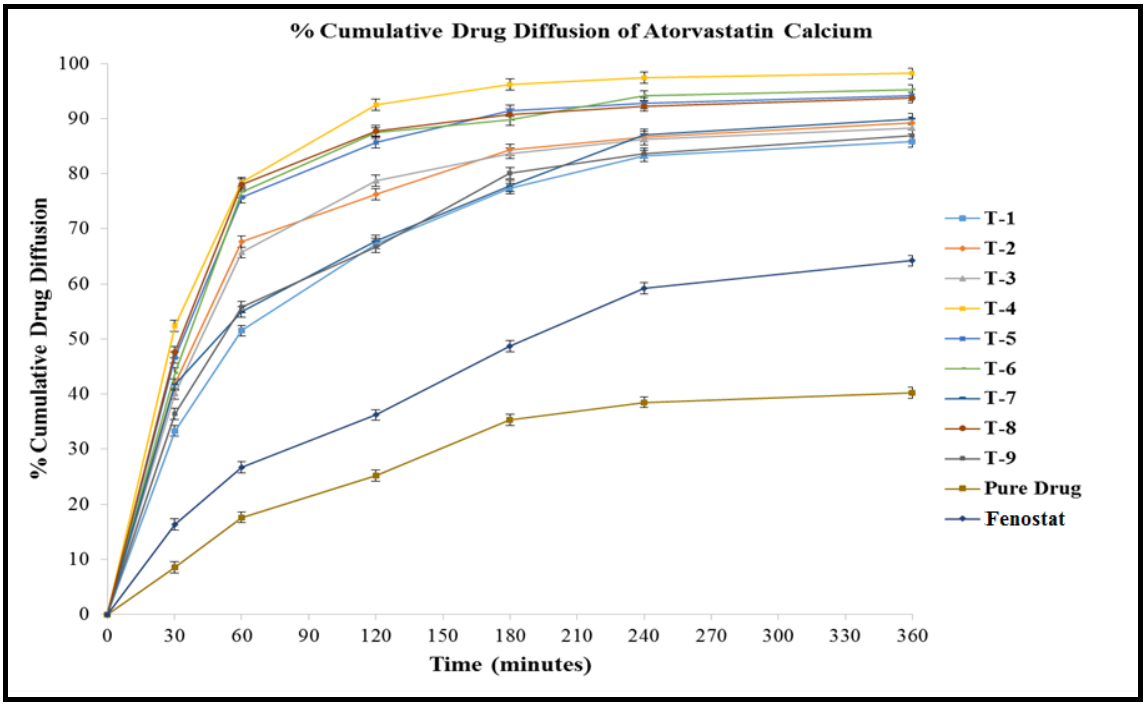

Figure 6.39: Comparison of diffusion profile of various SNEDDS formulation with pure drug and marketed formulation (Atorvastatin Calcium)

In case of SNEDDS (T5), more than 95% of Fenofibrate and Atorvastatin Calcium were diffused in 180 minutes. The diffusion percentage of Fenofibrate and Atorvastatin Calcium from SNEDDS form was significantly higher than that of Fenofibrate and Atorvastatin Calcium from pure drug suspension and Fenofibrate and Atorvastatin Calcium capsule (Marketed formulation as conventional capsule). Order of drug diffusion through dialysis membrane was T5 > T9 > T8 > T6> T2 > T3 > T4 > T7 > T1 > Marketed formulation > Pure drug.

It could suggest that Fenofibrate and Atorvastatin Calcium dissolved perfectly in SNEDDS form could be diffused due to small droplet size, which permits a faster rate of drug diffusion into aqueous phase, faster than pure drug suspension and marketed formulation capsule and it could affect bioavailability. The diffusion rate of Fenofibrate and Atorvastatin Calcium from SNEDDS batch T5 was faster than SNEDDS than other formulation. So, increasing droplet size of nanoemulsion could decrease diffusion rate of drug and it might suggest that diffusion rate of drug could be controlled by regulating mean globule size.

6.3.6 Stability study of Fenofibrate and Atorvastatin Calcium SNEDDS optimized batch (OP1)

Stability study of optimized batch (OP1) was conducted up to 6 months at two different storage conditions:

1. Room temperature
2. Accelerated condition (40°C & 75% RH)

Stability chamber was used for accelerated condition. The change in globule size, zeta potential, drug content and drug release at 15 minutes for Fenofibrate and Atorvastatin calcium were carried out periodically to determine the stability of drug in the formulation at various storage conditions.

6.3.6.1 Results of Globule size and Zeta potential at storage conditions

Globule size and Zeta potential of optimized batch (OP1) were measured by Zetasizer at periodic intervals.

Globule size and Zeta potential were measured after 1, 3 and 6 months. The results were recorded in Table 6.45 and Table 6.46.

Table 6.45: Globule size of optimized batch at storage conditions

Storage Conditions	Average of Globule Size (nm)			
	Initial	1 Month	3 Month	6 Month
Room Temperature	78.3	79.2	82.1	82.5
Accelerated Conditions	78.3	79.8	83.3	83.9

Table 6.46: Zeta Potential of optimized batch at storage conditions

Storage Conditions	Zeta Potential (mV)			
	Initial	1 Month	3 Month	6 Month
Room Temperature	-23.13	-22.38	-22.24	-21.79
Accelerated Conditions	-23.13	-22.45	-22.92	-21.47

6.3.6.2 Drug content determination at storage conditions

Drug content was measured by HPLC method as described in Chapter 4. Table 6.47 and 6.48 showed results of chemical drug stability during the storage conditions. It was concluded that there was no significant change in drug amount during 6 months. The optimized batch (OP1) was found stable chemically.

Table 6.47: Drug content of optimized batch at storage conditions				
Storage Conditions	**% Assay (±) SD (For Fenofibrate)**			
	Initial	**1 Month**	**3 Month**	**6 Month**
Room Temperature	100.2 ± 0.46	100.1 ± 0.27	99.7 ± 0.52	99.4 ± 0.38
Accelerated Conditions	100.2 ± 0.46	100.0 ± 0.58	99.4 ± 0.65	99.1 ± 0.62

Table 6.48: Drug content of optimized batch at storage conditions				
Storage Conditions	**% Assay (±) SD (For Atorvastatin Calcium)**			
	Initial	**1 Month**	**3 Month**	**6 Month**
Room Temperature	100.4 ± 0.25	99.6 ± 0.46	99.3 ± 0.46	98.7 ± 0.58
Accelerated Conditions	100.4 ± 0.25	99.4 ± 0.63	99.1 ± 0.82	98.3 ± 0.28

6.3.6.3 Drug release at 15 minutes for Fenofibrate and Atorvastatin Calcium determination at storage conditions

Drug release at 15 minutes for Fenofibrate and Atorvastatin Calcium was performed as method described in Section 4.10 of Chapter 4. Table 6.49 and 6.50 showed results of chemical drug stability during the storage conditions. It was concluded that there was more than 90% drug dissolution was achieved in 15 minutes during 6 months. The optimized batch (OP1) was found stable chemically.

Table 6.49: Drug release at 15 minutes for Fenofibrate for optimized batch at storage conditions				
Storage Condition	**% Drug release at 15 minute for Fenofibrate (±) SD**			
	Initial	**1 Month**	**3 Month**	**6 Month**
Room Temperature	96.2 ± 1.2	97.4 ± 1.7	97.7 ± 2.4	96.4 ± 1.9
Accelerated Condition	96.2 ± 1.2	97.1 ± 1.6	96.9 ± 2.1	96.1 ± 2.5

Table 6.50: Drug release at 15 minutes for Atorvastatin Calcium for optimized batch at storage conditions				
Storage Condition	**% Drug release at 15 minute for Atorvastatin Calcium (±) SD**			
	Initial	**1 Month**	**3 Month**	**6 Month**
Room Temperature	97.1 ± 1.2	97.4 ± 1.7	97.7 ± 2.4	96.4 ± 1.9
Accelerated Condition	97.1 ± 1.2	97.1 ± 1.6	96.9 ± 2.1	96.1 ± 2.5

6.3.7 Comparison of in vitro drug release between optimized batch (OP1), pure drug powder, and marketed product

The Fenofibrate and Atorvastatin Calcium release of optimized batch (OP1) was compared with pure drug powder and marketed capsule product. The marketed product was FENOSTAT of Ordain Health Care Global Pvt Ltd. which is a conventional capsule formulation.

Table 6.51: Comparison of Fenofibrate release profile of batch OP1 with pure drug and marketed formulation

Batches	% Drug Release (Mean ± SD)						
	Time (Minutes)						
	0	10	15	20	30	45	60
OP1	0±0	92.1±1.5	96.2±1.2	99.7±1.1	100.1±0.2	99.6±0.1	99.7±0.3
Pure drug	0±0	7.3± 0.7	17.6±0.8	19.1±0.5	27.4±1.1	38.7±0.4	48.2±0.5
Fenostat	0±0	14.1±0.2	22.8±0.7	23.2±0.8	32.4±0.5	47.5±1.4	59.3±1.4

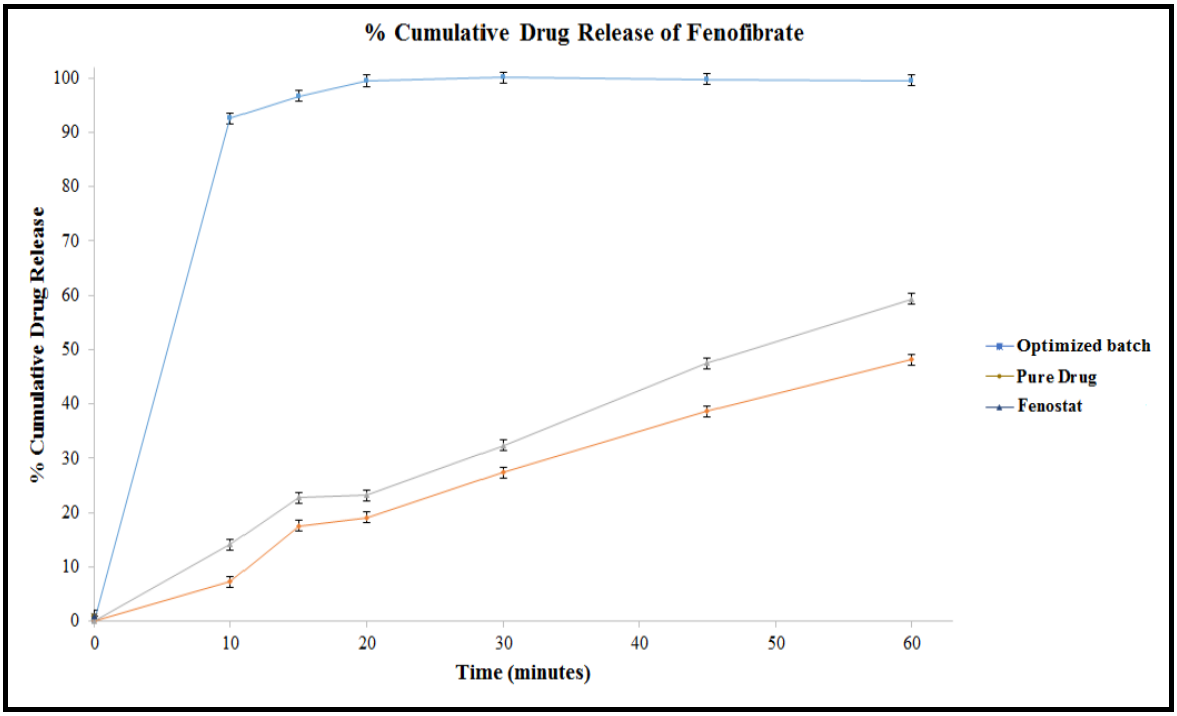

Figure 6.40: Comparison of drug release profile of optimized SNEDDS batch with pure drug and marketed formulation (Fenofibrate)

Table 6.52: Comparison of Atorvastatin Calcium release profile of batch OP1 with pure drug and marketed formulation

Batches	% Drug Release (Mean ± SD)						
	Time (Minutes)						
	0	10	15	20	30	45	60
OP1	0±0	92.4±1.5	97.1±1.2	99.3±1.1	100.0±0.2	99.8±0.1	99.7±0.3
Pure drug	0±0	6.3± 1.7	13.3±0.9	18.7±0.8	26.6±1.3	36.7±0.7	46.5±0.5
Fenostat	0±0	13.6±1.2	18.9±0.8	24.7±0.7	31.6±0.5	46.5±1.1	57.6±1.0

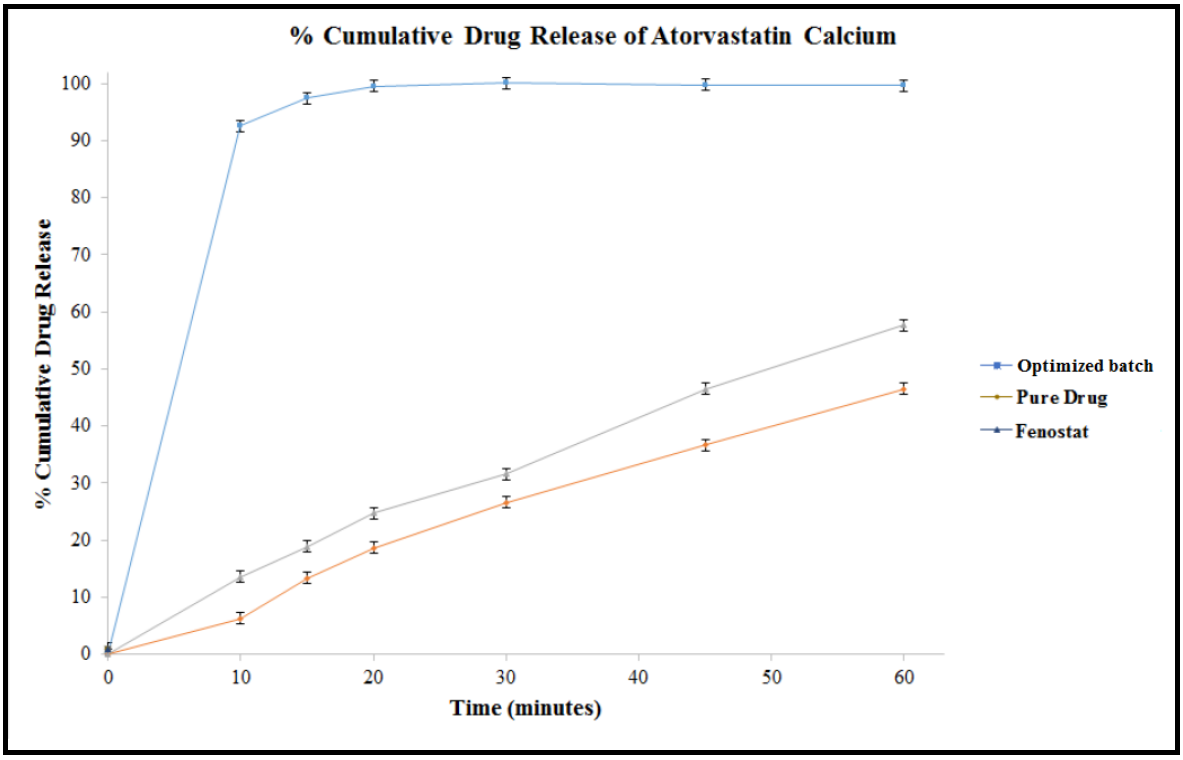

Figure 6.41: Comparison of drug release profile of various optimized SNEDDS batch with pure drug and marketed formulation (Atorvastatin Calcium)

Table 6.53: Similarity factor (f2) for optimized batch (OP1) and marketed formulation (Fenostat)

Time points (min)	% Drug Release (Mean)							
	Fenofibrate				Atorvastatin Calcium			
	OP1	Fenostat	Diff.	$(Diff.)^2$	OP1	Fenostat	Diff.	$(Diff.)^2$
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
10	92.1	14.1	78.0	6084.0	92.4	13.6	78.8	6209.4
15	96.2	22.8	73.4	5387.6	97.1	18.9	78.2	6115.2
20	99.7	23.2	76.5	5852.3	99.3	24.7	74.6	5565.2
30	100.1	32.4	67.7	4583.3	100.0	31.6	68.4	4678.6
45	99.6	47.5	52.1	2714.4	99.8	46.5	53.3	2840.9
60	99.7	59.3	40.4	1632.2	99.7	57.6	42.1	1772.4
(Sum of Diff.)^2			26253.67		**(Sum of Diff.)^2**		27181.70	
/ n			3750.52		**/ n**		3883.10	
+ 1			3751.52		**+ 1**		3884.10	
- 0.5			0.02		**- 0.5**		0.02	
x 100			1.63		**x 100**		1.60	
Log			0.21		**Log**		0.21	
x 50			10.64		**x 50**		10.27	
F2 value			10.64		**F2 value**		10.27	

Table 6.54: Dissolution Efficiency (DE) for optimized batch (OP1) and marketed formulation (Fenostat)

Time points (min)	% Drug Release (Mean)							
	Fenofibrate				Atorvastatin Calcium			
	OP1	Area	Fenostat	Area	OP1	Area	Fenostat	Area
0	0.0	460.5	0.0	70.5	0.0	462.0	0.0	68.0
10	92.1	470.8	14.1	92.3	92.4	473.8	13.6	81.3
15	96.2	489.8	22.8	115.0	97.1	491.0	18.9	109.0
20	99.7	999.0	23.2	278.0	99.3	996.5	24.7	281.5
30	100.1	1497.8	32.4	599.3	100.0	1498.5	31.6	585.8
45	99.6	1494.8	47.5	801.0	99.8	1496.3	46.5	780.8
60	99.7	-	59.3	-	99.7	-	57.6	-
	DE	**90.21**	**DE**	**32.60**	**DE**	**90.30**	**DE**	**31.77**

Drug release from the optimized batch (OP1) was found to be better as compared with that of pure drug powder and marketed drug formulation (Figure 6.40 and 6.41). It could be suggested that the optimized SNEDDS batch resulted in spontaneous formation of a nanoemulsion with a small globule size, which permitted a faster drug release into the aqueous phase than that of pure drug powder and marketed formulation. The similarity factor (F2) and dissolution efficiency of the optimized batch (OP1) and marketed formulation (Fenostat) were calculated (Table 6.53 and 6.54) [25 – 27]. The F2 value of optimized formulation was 10.64 for Fenofibrate and 10.27 for Atorvastatin Calcium. The result of F2 value indicated the dissimilarity between optimized formulation and marketed formulation [25]. The dissolution efficiency of marketed formulation was 32.60 for Fenofibrate and 31.77 for Atorvastatin Calcium. The dissolution efficiency of optimized formulation was 90.21 for Fenofibrate and 90.30 for Atorvastatin Calcium. So, it can be concluded that the optimized formulation was superior than marketed formulation. Thus, the greater availability of dissolved Fenofibrate and Atorvastatin Calcium from the SNEDDS formulation could lead to higher absorption and higher oral bioavailability.

6.4 Conclusion

SNEDDS are isotropic mixtures made up of oil, surfactant and sometimes cosurfactant or cosolvent. In an aqueous environment a homogeneous, transparent (or at least translucent), isotropic and thermodynamically stable dispersion will result up on mild agitation. SNEDDS is best suited for dosage for development of poorly soluble drugs. Fenofibrate and Atorvastatin Calcium are BCS class II drugs having low solubility and high permeability. The present study was aimed to explore stable SNEDDS formulation development using 3^2 factorial design for dissolution improvement compared to marketed formulation of Fenofibrate and Atorvastatin Calcium. On the basis of results of preliminary trials, Capmul MCM as oil, Cremophor RH 40 as surfactant and Transcutol-P as cosurfactant were used for formulation development of Fenofibrate and Atorvastatin Calcium SNEDDS. The 3^2 factorial design was employed using concentration of Capmul MCM oil and Cremophor RH 40: Transcutol-P (3:1) as independent variables. The Globule size (GS), Zeta potential (ZP), Polydispersity index (PDI) and drug release at 15 minute for Fenofibrate and Atorvastatin Calcium were selected as dependent variables. Multiple regression analysis, contour plot and response surface plot were used to study the main and interaction effects of the variables on the responses. Simple linear equation, or interactive equation or quadratic model was fitted by carrying out multiple regression analysis and F-statistic to identify statistically significant term. The optimized batch was selected on the basis arbitrary criteria using Design Expert employing overlay plot with desirability approach. The batch containing 0.471ml of Capmul MCM oil, 1.608ml of Cremophor RH 40: Transcutol-P (3:1) was selected as optimized SNEDDS formulation. The check point batches were prepared to validate the evolved equations. The optimized formulation was subjected to in vitro dissolution to evaluate drug release as compared to marketed product. The stability study for optimized batch was conducted at room temperature and 40°C & 75% RH. The optimized formulation was found stable and more than 90% drug dissolution was achieved within 15 minutes. The desirable goals can be achieved by systematic formulation approach in shortest possible time with reduced number of experiments for formulation development of Fenofibrate and Atorvastatin Calcium SNEDDS formulation using factorial design.

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CHAPTER – 7

Summary

7. Summary

SNEDDS are be used to enhance solubility of drugs that are poorly water soluble. These particular advantage make it a unique dosage form. It is also simple strategy that has various advantages over other techniques. Fenofibrate and Atorvastatin Calcium are BCS class II drugs having low solubility and high permeability. There is a need exists for developing a formulation of Fenofibrate and Atorvastatin Calcium, which provides improved bioavailability with minimizing variation. The aim of present work was to prepare a stable formulation of Fenofibrate and Atorvastatin calcium which improves dissolution profile of drug compare to currently marketed formulation, which ultimately enhances oral bioavailability, reduces patient to patient absorption variability and to reduce the food effect on absorption of drug compare to marketed formulation.

Outline of Chapter 1 Aim and Objectives:

The Chapter 1 summarized the aim and objectives. There is a need exists for developing a formulation of Fenofibrate and Atorvastatin Calcium, which provides improved bioavailability with minimizing variation. The aim of present work was to prepare a stable formulation of Fenofibrate and Atorvastatin Calcium which improves dissolution profile of drug compared to currently marketed formulation, which ultimately enhances oral bioavailability compared to marketed formulation.

The objectives of the present research work were:

(i) To develop a stable formulation for self-Nanoemulsifying drug delivery system (SNEDDS) in order to enhance solubility and release rate of poorly soluble Antilipidemic drugs (Fenofibrate and Atorvastatin Calcium).

(ii) In silico prediction of drugs solubility in lipid vehicle using several factors like solubility parameter (δ), HLB value, partition coefficient, relative molecular mass (MW), fatty acid chain length, solubilization capacity.

(iii) To select most suitable vehicle and perceive role of lipid vehicle in pseudo ternary phase diagram behavior to find nanoemulsion area in SNEDDS containing Fenofibrate and Atorvastatin Calcium.

(iv) To study physicochemical properties of Self Nano Emulsifying Drug Delivery System (SNEDDS).

(v) To study effect of dilution of SNEDDS formulation that formation of spontaneous curvature of surfactant layer changes.

(vi) To study in-vitro drug release and drug diffusion profile by using a suitable in-vitro dissolution model.

Outline of Chapter 2 Introduction:

This chapter described the introduction to use atorvastatin/fenofibrate fixed-combination preparation when treatment with both atorvastatin (for prevention of cardiovascular events) and fenofibrate (decrease elevated serum total and LDL-cholesterol, triglyceride, and apolipoprotein B (apo B) concentrations, and to increase HDL-cholesterol concentrations in the management of primary hypercholesterolemia and mixed dyslipidemia, including heterozygous familial hypercholesterolemia and other causes of hypercholesterolemia) is appropriate. Introduction to SNEDDS which included need for formulating SNEDDS, available methods for preparation of SNEDDS, formulation considerations of SNEDDS, characterization of SNEDDS with details of each characterization parameters and applications of SNEDDS technology in pharmaceutical industry.

Outline of Chapter 3 Review of Literature:

In review of literature, SNEDDS as drug delivery system with specific advantages and limitations, Fenofibrate and Atorvastatin Calcium as model drugs with their chemical, pharmacological and physico-chemical properties were summarized from the available

library, website sources and prior arts. The oral bioavailability problem of Fenofibrate and Atorvastatin Calcium in currently marketed dosage form of Fenofibrate and Atorvastatin Calcium was mentioned in details.

Outline of Chapter 4 Materials and Methods:

It mentioned the list of various materials and instruments used in this research work with their source. It also described all the methods in details such as analytical method, formulation method, characterization method and stability study which were explored in the present investigation.

Outline of Chapter 5 Role of lipid vehicle in Pseudo Ternary Phase Diagram in formulation development of SNEDDS containing poorly water soluble drugs:

The present study showed the importance of selecting a surfactant with the proper HLB for specific oils, as well as the type of surfactant/co-surfactant. Solubility parameter (δ), Required HLB (RHLB), required chemical type of emulsifiers and solubilization capacity appears to be useful as a criterion for selection of surfactant/co-surfactant. The solubility parameter (δ) of Fenofibrate and Atorvastatin Calcium are closest solubility parameter (δ) of Capmul MCM. Blend of better surfactant/co-surfactant was obtained when surfactant and co-surfactant at higher and lower HLB level respectively were blended. The greater the difference between the hydrophilic and lipophilic surfactants, the better the coverage by blends at the interface. The study also showed the importance of the structural similarities between the lipophilic tails of the surfactant blends.

The pseudo ternary phase diagram of the system containing the surfactant co-surfactant and the oily phase was constructed. The Area enclosed within the solid line represented the region of self-emulsification. Within this area a ternary mixture formed a fine oil in water emulsion with only gentle agitation. This was possible as surfactants strongly localized to the surface of the emulsion droplet reduces interfacial free energy and provide a mechanical barrier to coalescence resulting in a thermodynamically spontaneous dispersion. The co-surfactants increased interfacial fluidity by penetrating into the surfactant film creating void space among surfactant molecules. Selected lipid vehicle were Capmul MCM as oil, Cremophor RH 40 as surfactant and Transcutol-P as cosurfactant.

The present study was to select appropriate lipid vehicle and to understand role of lipid vehicle in pseudo ternary phase diagram behaviour to find nanoemulsion area in formulation development of SNEDDS containing Fenofibrate and Atorvastatin Calcium. On the basis of solubility study, Solubility parameter (δ), Required HLB (RHLB), required chemical type of emulsifiers and solubilization capacity, selection appropriate lipid vehicle were Capmul MCM as oil, Cremophor RH 40 as surfactant and Transcutol-P as cosurfactant.

Outline of Chapter 6 Formulation and Development of Fenofibrate and Atorvastatin Calcium SNEDDS Using 3^2 Factorial Design:

SNEDDS are isotropic mixtures made up of oil, surfactant and sometimes cosurfactant or cosolvent. In an aqueous environment a homogeneous, transparent (or at least translucent), isotropic and thermodynamically stable dispersion will result up on mild agitation. SNEDDS is best suited for dosage for development of poorly soluble drugs. Fenofibrate and Atorvastatin Calcium are BCS class II drugs having low solubility and high permeability. The present study was aimed to explore stable SNEDDS formulation development using 3^2 factorial design for dissolution improvement compared to current marketed formulation of Fenofibrate and Atorvastatin Calcium.

Concentration of Capmul MCM, Cremophor RH 40: Transcutol-P (3:1) play important role in stable formulation of SNEDDS; hence both were selected as independent variables in factorial design.

The Globule size, Polydispersity index, Zeta potential and drug release at 15 minute for Fenofibrate and Atorvastatin calcium were selected as dependent variables. Multiple regression analysis, contour plot and 3D response surface plot were used to study the main and interaction effects of the variables on the responses. Simple linear equation, or interactive equation or quadratic model was fitted by carrying out multiple regression analysis and F-statistics to identify statistically significant term.

The optimized batch was selected on the basis arbitrary criteria using Design Expert employing overlay plot with desirability approach. The batch containing 0.471ml of Capmul MCM oil, 1.608ml of Cremophor RH 40: Transcutol-P (3:1) was selected as optimized SNEDDS formulation.

The check point batches were prepared to validate the evolved equations. The optimized formulation was subjected to in vitro dissolution to evaluate drug release as compared to marketed product.

The stability study for optimized batch was conducted at room temperature and 40°C & 75% RH. The optimized formulation was found stable and more than 90% drug dissolution was achieved within 15 minutes.

The desirable goals can be achieved by systematic formulation approach in shortest possible time with reduced number of experiments for formulation development of Fenofibrate and Atorvastatin Calcium SNEDDS formulation using factorial design.

Future aspects of SNEDDS technology

SNEDDS technologies have provided the pharmaceutical industry with new strategies for resolving issues associated with poorly soluble molecules. Production techniques have been successfully employed for large-scale production of SNEDDS. For marketed products requiring life-cycle management opportunities, SNEDDS formulation strategies provide a means to incorporate an old drug into a new drug-delivery platform, thus opening new avenues for addressing unmet medical needs. Screening efforts to improve the water solubility of a compound will be a thing of the past, and more emphasis will be placed on efficacy and safety that will shorten development times and bring new therapies and diagnostic agents for challenging diseases that have yet to be controlled or eradicated. The applications of SNEDDS in parenteral and oral routes have been very well investigated and applications in pulmonary and ocular delivery have been realized.

However, their applications in buccal, nasal and topical delivery are still awaiting exploration. The era of nanotechnology in the pharmaceutical industry has begun. During the next decade, it will be interesting to see if all the promises envisioned become a reality.

CHAPTER – 8

Patent filing, Paper Publication and Poster Presentation

8.1 Patent Filing

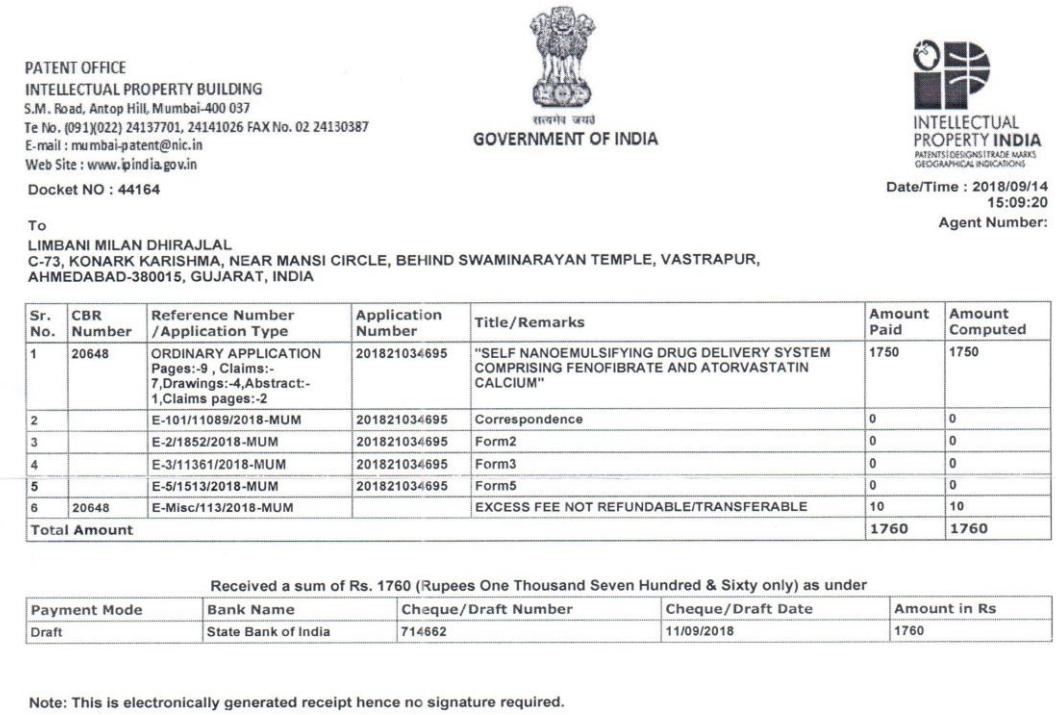
Patent Office: Mumbai Intellectual Property and Trademark office

Date of Filing: 14th September 2018

Patent Application No.: 201821034695

Title of Invention: "Self nanoemulsifying drug delivery system comprising fenofibrate and atorvastatin calcium"

Name of inventors: Milan Dhirajlal Limbani and Dr. Lakshamanbhai D. Patel



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LIMBANI MILAN DHIRAJLAL
C-73, KONARK KARISHMA, NEAR MANSI CIRCLE, BEHIND SWAMINARAYAN TEMPLE, VASTRAPUR,
AHMEDABAD-380015, GUJARAT, INDIA

Sr. No.	CBR Number	Reference Number /Application Type	Application Number	Title/Remarks	Amount Paid	Amount Computed
1	20648	ORDINARY APPLICATION Pages:-9 , Claims:-7,Drawings:-4,Abstract:-1,Claims pages:-2	201821034695	"SELF NANOEMULSIFYING DRUG DELIVERY SYSTEM COMPRISING FENOFIBRATE AND ATORVASTATIN CALCIUM"	1750	1750
2		E-101/11089/2018-MUM	201821034695	Correspondence	0	0
3		E-2/1852/2018-MUM	201821034695	Form2	0	0
4		E-3/11361/2018-MUM	201821034695	Form3	0	0
5		E-5/1513/2018-MUM	201821034695	Form5	0	0
6	20648	E-Misc/113/2018-MUM		EXCESS FEE NOT REFUNDABLE/TRANSFERABLE	10	10
Total Amount					1760	1760

Received a sum of Rs. 1760 (Rupees One Thousand Seven Hundred & Sixty only) as under

Payment Mode	Bank Name	Cheque/Draft Number	Cheque/Draft Date	Amount in Rs
Draft	State Bank of India	714662	11/09/2018	1760

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CBR Number : 20648 **CBR date: 14-09-2018**

Application Type: ORDINARY APPLICATION
Priority Number:
Priority Date:
Priority Country: Not Selected

To,

LIMBANI MILAN DHIRAJLAL

LIMBANI MILAN DHIRAJLAL, C-73, KONARK KARISHMA, NEAR MANSI CIRCLE, BEHIND SWAMINARAYAN TEMPLE, VASTRAPUR, AHMEDABAD-380015, GUJARAT, INDIA

Received documents purporting be to an application for patent numbered 201821034695 dated 14-09-2018 by LIMBANI MILAN DHIRAJLAL of C-73, KONARK KARISHMA, NEAR MANSI CIRCLE, BEHIND SWAMINARAYAN TEMPLE, VASTRAPUR, AHMEDABAD-380015, GUJARAT, INDIA relating to "SELF NANOEMULSIFYING DRUG DELIVERY SYSTEM COMPRISING FENOFIBRATE AND ATORVASTATIN CALCIUM" together with the Complete and fee(s) of ₹1750 (One Thousand Seven Hundred & Fifty only).

Note:

1. In case of Patent Application accompanied by a Provisional Specification, a complete Specification should be filed within 12 months from the date of filing of the Provisional Specification, failing which the application will be deemed to be abandoned under Section 9(1) of the Patent Act, 1970.
2. You may withdraw the application at any time before the grant of patent, if you with so. If, in addition to withdrawal, you also wish to pravent the publication of application in the Patent Office Journal, the application should be withdrawn within fifteen months from the date of priority of date of filing, whichever earlier.
3. If not withdrawn, your application will be published in the Patent Office Journal after eighteen months from the date of priority of date of filing, whichever is earlier.
4. If you with to get your application examined, you should file a request for examination in Form-18 within 48 months from the date of priority or date of filing, whichever is earlier, failing which the application will be treated as withdrawn by the applicant under Section 11(B)(4) of the Patent Act, 1970.

(For Controller of Patents)

8.2 Poster Presentation

National conference: 7th National Seminar "Pharma vision - 2016"

Name and Place: Babaria Institute of Pharmacy, Vadodara, Gujarat, India

Date of Conference: 17 - 18 September 2016

Title of Poster: "Studies on drug solubilization and Role of lipid vehicle in Pseudo Ternary Phase diagram in formulation development of SNEDDS containing poorly water soluble drug"

Name of Authors: Milan D. Limbani and Dr. L.D. Patel

ABSTRACT

Purpose: The purpose of this study was to select appropriate lipid vehicle and understand role of lipid vehicle in pseudo ternary phase diagram behavior to find nanoemulsion area in formulation development of self-nano emulsifying drug delivery system (SNEDDS) containing Fenofibrate and Atorvastatin Calcium.

Method: In silico prediction of drug solubility in a lipid vehicle remains challenging task. Solubility, Solubility Parameter (δ), Required HLB (RHLB), required chemical type of emulsifiers and Solubilization capacity appeared to be useful as a criterion for the selection of surfactant/co-surfactant. The present study showed the importance of selecting a surfactant with the proper HLB for specific oils, as well as the type of surfactant/co-surfactant. Selection of lipid vehicle based on solubility study, calculation of solubility parameter (δ), Determination of Required HLB (RHLB) of Capmul MCM Oil, Determination of required chemical type of Emulsifiers, Measurement of solubilization capacity, Construction of Pseudo-Ternary Phase Diagrams.

Results: Fenofibrate and Atorvastatin Calcium had highest solubility in Capmul MCM Oil, Cremophor RH 40 and Transcutol-P as compare to other surfactant and co-surfactant. Capmul MCM Oil as oil, Cremophor RH 40 as surfactant and Transcutol-P as co-surfactant were selected for optimal SNEDDS formulation resulting in improved drug loading capability. The solubility parameter calculated for Atorvastatin calcium is $\delta_{T\ (ATR)}$ = 15.27 $(cal/cm^3)^{½}$. Capmul MCM that has the closest solubility parameter (16.86 $(cal/cm^3)^{1/2}$) to that of Atorvastatin calcium and hence it provided the highest solubility among all lipids used. The same correlation could be observed with Fenofibrate. The calculated solubility parameter for Fenofibrate is $\delta_{T\ (FENO)}$ = 16.46 $(cal/cm^3)^{½}$ and the lipid that has closest solubility parameter is Capmul MCM (Capmul MCM = 16.86 $(cal/cm^3)^{1/2}$. These preliminary tests showed that the approximate RHLB for Capmul MCM is 12.84.

The results for the solubilization capacity of blends of surfactants/co-surfactant showed that Cremophor RH 40/Transcutol-P (3:1) at HLB 12.3 has the highest solubilization capacity compared with the Labrasol/Transcutol-P (3:1) at HLB 10.1. The larger area of oil-in-water microemulsion/nanoemulsion formed by Cremophor RH 40/Transcutol-P (3:1) is due to the large molecular packing ratio of Cremophor RH 40/Transcutol-P, which is classified as a strong solubilizer.

Conclusion: Solubility Parameter (δ), Required HLB (RHLB), required chemical type of emulsifiers and Solubilization capacity appeared to be useful as a criterion for the selection of surfactant/co-surfactant along with pseudo ternary phase diagrams in formulation development of SNEDDS.


Knowledge Character Unity

AKHIL BHARATIYA VIDYARTHI PARISHAD

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8.3 Paper Publication

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   Title of Manuscript: "Studies on Drug Solubilization and Role of Lipid Vehicle in Pseudo Ternary Phase Diagram in Formulation Development of SNEDDS Containing Poorly Water Soluble Drugs"
   Name of Authors: Milan D. Limbani, Dr. L. D. Patel
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Research Article

Studies on Drug Solubilization and Role of Lipid Vehicle in Pseudo Ternary Phase Diagram in Formulation Development of SNEDDS Containing Poorly Water Soluble Drug

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ABSTRACT

The purpose of this study was to select appropriate lipid vehicle and understand role of lipid vehicle in pseudo ternary phase diagram behaviour to find nanoemulsion area in formulation development of self nano emulsifying drug delivery system (SNEDDS) containing Fenofibrate and Atorvastatin Calcium. *In silico* prediction of drug solubility in a lipid vehicle remains challenging task. However, it has identified several factors that could be useful in predicting drug solubility in a particular excipient. These factors include the solubility parameter (δ), HLB value, partition coefficient, Molecular weight (MW), Dielectric constant (ε), dipole moment (μ) excipient fatty acid chain length, saponification value and viscosity. Non-ionic surfactant blends of Labrasol/Transcutol-P and Cremophor RH 40/Transcutol-P in different ratio were screened based on their solubilization capacity with water for Capmul MCM oil. High solubilization capacity was obtained by Cremophor RH 40/Transcutol-P (3:1) compared with other surfactant/co-surfactant ratio. High HLB blends of Cremophor RH 40/Transcutol-P (3:1) at HLB 12.3 has better solubilization capacity compared with the lower HLB values of Cremophor RH 40/Transcutol-P(2:1, 1:1) and Labrasol/Transcutol-P (3:1, 2:1, 1:1). All the selected blends of surfactants/co-surfactant were formed as oil-in-water microemulsions/nanoemulsion, and other dispersion systems varied in size and geometrical layout in the triangles. The high solubilization capacity and larger areas of the oil-in-water microemulsions/nanoemulsion systems were due to the structural similarity between the lipophilic tail of Cremophor RH 40 and the glycerides group of the Capmul MCM oil. This study also suggested that the pseudo ternary phase diagram behaviour of Capmul MCM oil, water, and non-ionic surfactant/co-surfactant is not affected by the HLB value.

Keywords: Solubility Parameter (δ), Pseudo ternary phase diagram, Capmul MCM oil, non-ionic surfactant/co-surfactant, SNEDDS, Microemulsion, Nanoemulsion.

INTRODUCTION

An increasing number of recently discovered drug substances exhibit poor water solubility and hence low absorption after oral administration. An example of such a compound suffering from lower solubility and poor bioavailability are Fenofibrate, Atorvastatin, Pitavastatin, Simvastatin, etc.

Several strategies to improve the solubility and dissolution of such poorly water soluble drugs have been developed and described in literature like use of surfactants, lipids, permeation enhancer, micronization, salt formation, cyclodextrin complexation, nanoparticles, etc. Among these lipid base system the self nano emulsifying drug delivery system (SNEDDS) is promising technology to improve the dissolution rate and rate and extent absorption of poorly water soluble drugs.[1,2]

Nanoemulsion is a clear, isotropic, thermodynamically stable colloidal system which may be formed spontaneously by the chemical energy of surfactants, combinations of surfactants, and co-surfactants upon mixing a suitable oil phase and water without any mechanical energy input.[3,4] It has many advantages compared with conventional emulsions, including increased drug-loading and enhanced transdermal delivery.[4,5]

In silico prediction of drug solubility in a lipid vehicle remains challenging task. However, it has identified several factors that could be useful in predicting drug solubility in a particular excipient. These factors include the solubility parameter (δ), HLB value, partition coefficient, Molecular weight (MW), Dielectric constant (ε), dipole moment (μ) excipient fatty acid chain length, saponification value, surface tension and viscosity.

To the best of our knowledge, no information is available in the literature on the usefulness of solubility parameter, required HLB (RHLB), and required chemical type of emulsifiers or solubilization capacity for solubilising vehicles as criterion for the selection of surfactant/cosurfactant in the formulation development of SNEDDS using Fenofibrate or Atorvastatin Calcium.

The purpose of this study was to select appropriate lipid vehicle and to understand role of lipid vehicle in pseudo ternary phase diagram behaviour to find nanoemulsion area in formulation development of self nano emulsifying drug delivery system (SNEDDS) containing Fenofibrate or Atorvastatin Calcium.

Solubility Parameter (δ), Required HLB (RHLB), required chemical type of emulsifiers and Solubilization capacity appeared to be useful as a criterion for the selection of surfactant/co-surfactant.

The present study showed the importance of selecting a surfactant with the proper HLB for specific oils, as well as

the type of surfactant/co-surfactant. The solubility parameter (δ) of Fenofibrate and Atorvastatin Calcium are closest solubility parameter (δ) of Capmul MCM. Blend of better surfactant/co-surfactant was obtained when surfactant and co-surfactant at higher and lower HLB level respectively were blended. The greater the difference between the hydrophilic and lipophilic surfactants, the better the coverage by blends at the interface. The study also showed the importance of the structural similarities between the lipophilic tails of the surfactant blends. The pseudo ternary phase diagrams for mixtures of Capmul MCM oil with non-ionic surfactant/co-surfactant and water were constructed in this study.[6,7] The micelles discussed in this study have potential applications, advantages, and usefulness in the pharmaceutical industry as SNEDDS by various routes of administration, as well as in cosmetics and personal care products.[8,9]

MATERIALS AND METHODS

Materials

Labrasol and Transcutol-P were generous gift from Gattefose for research. Cremophor RH 40 was gifted from BASF. Capmul MCM oil was gifted from Abitech. Atorvastatin calcium was gifted from MSN. Fenofibrate was gifted from DIVI's Lab. All other chemicals used were of analytical grade.

Methods

Solubility Study

Screening of solubilizing excipient was done by determining the solubility of Fenofibrate and Atorvastatin Calcium in different solubilizing vehicle like oils, surfactants and co-surfactants (Table 1). An excess quantity of Fenofibrate or/and Atorvastatin Calcium were added to the 2 ml of the solubilizing vehicle. Both components were mixed in a vial for 5 min using cyclomixer (REMI, Mumbai, India). The mixture in vial was shaken at 25 ± 1.0°C for 48 hour using controlled temperature rotary shaker. The mixtures were centrifuged using R-4C DX Laboratory Centrifuge (REMI, Mumbai, India) at 5000 rpm for 15 minute. The supernatant was separated and Fenofibrate and Atorvastatin Calcium were extracted in methanol. The drug content was analysed using Shimadzu 1700 UV-Visible spectrophotometer at 287 and 246 nm for Fenofibrate or Atorvastatin Calcium, respectively.

Selection of Blend of Surfactant/Co-surfactant (Lipid Vehicle)

Selection of surfactant is critical step in formulating the desired nanoemulsion. Each surfactant or oil has a specific HLB. The corrected HLB of the selected surfactant or blend of surfactant and co-surfactant that match the HLB of the selected oil provides the lowest interface tension between the oil and water phases. The HLB of the selected surfactant and blend of surfactant and co-surfactant reflects the stability of the system at lower levels, and can be obtained when the HLBs of the surfactant or blend of surfactant: co-surfactant and oil are similar.[10]

Capmul MCM is a mono-diglyceride of medium chain fatty acids (mainly caprylic and capric). It is an excellent solvent for many organic compounds including steroids.

Polyoxyl 35 hydrogenated castor oil is a non-ionic solubiliser and emulsifier made by reacting hydrogenated castor oil with ethylene oxide in a molar ratio of 1: 40. It has many uses as a nonionic surfactant, emollient, and thickening agent in skin preparations.

Labrasol (Caprylocaproyl polyoxyl-8 glycerides) is a non-ionic solubiliser and emulsifier. It is mixture of monoesters, diesters and triesters of glycerol and monoesters and diesters of polyethylene glycol with a mean relative molecular weight between 200 and 400. They are produced by partial alcoholysis of medium chain triglycerides with polyethylene glycol, by esterification of glycerol and polyethylene glycol with caprylic acid and capric acid, or as a mixture of glycerol esters and ethylene oxide condensate with caprylic acid and capric acid.

Transcutol-P (Diethylene glycol monoethyl ether) is non-ionic solubiliser and emulsifier. Structurally it is an alcohol and ether. It is a colorless, slightly viscous liquid with a mild pleasant odor.

Capmul MCM oil is composed of mono-diglyceride of medium chain fatty acids (mainly caprylic and capric) in which the side chains match the tail of non-ionic surfactant.

Therefore, non-ionic surfactants were chosen to study the phase diagram behaviour of Capmul MCM oil. Non-ionic surfactants are also recognized as being safe and biocompatible, and are not affected by pH changes in media because they are uncharged.

The non-ionic surfactants were chosen for screening to select a suitable blend of surfactant/co-surfactant that would best match Capmul MCM oil.

A blend of hydrophilic and lipophilic surfactants is needed to obtain longer stability of the dispersion phase at the lowest concentration levels.[11,12] A blend of surfactant/co-surfactant with an HLB that matches that of the oil phase will provide better solubilization and stability of the dispersion system produced. Therefore, the selection of surfactant blends at lower and higher HLB matching the HLB of oil is important in the formulation of a colloidal system.

Calculation of Solubility Parameter

Polarity of a solvent plays an important role in the solubility. Polar solvents are capable of solvating molecules through dipole interaction forces, particularly via hydrogen-bond formation, which is a major mechanism in the solubility of a compound. Polarity of solvents can be defined by dielectric constant (E), which is an important property related to the solubility and

hydrophilic-lipophilic balance.[13-16] It has been shown that the solubility of a solute decreased as the dielectric constant of solvent decreased.[17,18] An understanding of cohesive energy between drug and lipid molecules may help to determine how a lipid will behave as a solvent.

Cohesion is result of the London forces, polar interactions and specific ones like hydrogen bonding.[19,20] The commonly used approach in quantifying the cohesion between a solvent and a solute is the solubility parameter (δ), which is defined as the square root of the cohesive energy density, expressed as the energy of vaporization.

$$\delta = (CED)^{1/2} = (\Delta Ev/Vm)^{1/2} \quad \text{(Equation-1)}$$

Where CED is cohesive energy density ΔEv is the energy of vaporization and Vm is the molar volume.

This parameter may be useful to predict the solvating ability of a lipid or lipid mixture. When solubility parameters of lipid and drug are similar, they are expected to become miscible.[21, 2]

According to this calculation, the solubility parameter:

$$\delta_F = [\Sigma\Delta e/ \Sigma \Delta v]^{1/2} \quad \text{(Equation-2)}$$

Where Δe = the additive atomic group contributions for the energy of vaporization

Δv = the additive atomic group contributions for the molar volume

In this study, the group contribution method was used to calculate the solubility parameter from knowledge of the structural formula of the selected lipids and drug compounds.

Solubility parameters (δ_F) of lipids and drugs were calculated using the group contribution method devised by Fedor's (Equation-2).

$$\delta_F = [\Sigma\Delta e/ \Sigma\Delta v]^{1/2} \quad \text{(Equation-2)}$$

In this mode the contribution of hydrogen bonding is not included. Therefore, hydrogen bonding contribution (δ_H) was calculated as:

$$\delta_H = (5000m/V)^{1/2} \quad \text{(Equation-3)}$$

Where, m is the number of hydrogen donor and acceptors, and Vis the molar volume (MW/density).

Total solubility parameter (δ_T) was calculated by adding hydrogen bonding contribution (δ_H) to the Fedor's solubility parameter (δ_F):

$$\delta_T = (\delta_F^2 + \delta_H^2)^{1/2} \quad \text{(Equation-4)}$$

Solubility parameters for Atorvastatin calcium and Fenofibrate were calculated by equation 4. Atorvastatin calcium has $\delta_{T\ (ATR)}$ of 15.27 $(cal/cm^3)^{1/2}$ and Fenofibrate has $\delta_{T\ (FENO)}$ of 16.46 $(cal/cm^3)^{1/2}$.

Determination of Required HLB (RHLB) of Capmul MCM Oil

To determine RHLB (o/w) for emulsification of Capmul MCM oil, a matched pair of surfactants belonging to same chemical class but having different hydrophilicity i.e. Cremophor RH 40 (non-ionic hydrophilic surfactant) and Transcutol-P (lipophilic surfactant) were selected. The batches of eleven surfactants blends, ranging in HLB from straight Cremophor RH 40 (HLB = 15) to Transcutol-P (HLB = 4.5) were shown in Table 2.

Eleven test formulation containing 25% Capmul MCM (oily phase), 75% water and one of the above surfactant/co-surfactant blend (10% of weight of Capmul MCM) were prepared in test tubes. Test tubes were closed using stopper. Test tubes were shaken once (up and down in a quick, hard motion) and observed for emulsification.

Similarly eleven test formulations were also prepared in beakers. Further, contents of each beaker were stirred for 1 minute using magnetic stirrer at 600 rpm, transferred in test tubes and observed for separation. The time taken by emulsion for separation of a particular volume of Capmul MCM was recorded. Trials were performed in triplicate. Required HLB for Capmul MCM was determined based on ease of preparation and time for separation. Number of times the test tubes shaken till a homogenous milky emulsion formed and time of separation for Capmul MCM emulsions prepared using emulsifiers of different HLB were shown in Table 3.

Determination of required chemical type of Emulsifiers

To find out appropriate surfactants, one more formulation was prepared using pair of Labrasol and Transcutol-P in such a ratio to give HLB value 12.84 (which is required for Capmul MCM). Ease of preparation and time for separation was determined and compared with the emulsion prepared using Cremophor RH 40 and Transcutol-P mixtures. Number of times the test tubes shaken till a homogenous milky emulsion formed and time of separation for Capmul MCM emulsion prepared using surfactant/co-surfactant blend of same HLB but different chemical type was shown in Table 4.

The individual non-ionic hydrophilic surfactant Labrasol and Cremophor RH 40 was blended with the lipophilic surfactant Transcutol-P in ratios of 1:1, 2:1, 3:1 w/w to produce blends of surfactant/co-surfactant with various HLBs in the range of 8.1–12.5.

Measurement of solubilization capacity

The water solubilization capacity, i.e, minimum content of non-ionic surfactant required to form a nanoemulsion system with Capmul MCM oil, was performed as a criterion for optimization using the water titration method.[23] The results of solubilization capacity were used to select the best emulsifier to study the phase diagram behaviour of Capmul MCM oil.

The blend of surfactant/co-surfactant forming a clear system at the minimum concentration (oil-in-water microemulsion/nanoemulsion) was selected as the blend that best matched the HLB of Capmul MCM oil.

Construction of Pseudo-Ternary Phase Diagrams

Pseudo ternary phase diagrams were constructed based on the types of mixtures or dispersion systems formed when Capmul MCM oil-surfactant/co-surfactant mixtures were serially titrated with water at ambient temperature. Various weight to weight blends of selected surfactant/co-surfactant in the ratios of 1:1, 2:1 and 3:1 were produced to form surfactant/co-surfactant mixtures with HLB values of 8.1, 9.4, 10.1, 9.6, 11.4 and 12.3, respectively.[22]

The Capmul MCM oil and the blend of surfactant/co-surfactant at each HLB value were weighed separately in glass beakers, and were mixed and vortexed thoroughly in specific oil to surfactant/co-surfactant mixture ratios in the range of 0.25:4.75 – 4.5:0.5. Each mixture was slowly titrated with distilled water drop wise using a pipette. After each addition of water, the systems were vortexed for 10–20 seconds, and the final mixtures were vortexed for 2–3 minutes at room temperature. Initial visual observations of the resulting mixtures were categorized according to their physical characteristics. Microscopic examination was made of the final mixtures to identify the type of emulsion obtained using water-soluble dyes, i.e. Congo red and methylene blue. Details of the visual observation and microscopic identification of the resulting mixtures were recorded. The mixtures were stored for 24 hours at room temperature to achieve equilibrium. After equilibrium was reached, the final visual observation was recorded. The oil vertex in the triangle phase diagram represents Capmul MCM oil, the S/Cos vertex represents the surfactant/co-surfactant, and the remaining vertex represents the water phase.

To determine effect of drug addition on nanoemulsion boundary, phase diagrams were also constructed in presence of drug using drug-enriched oil as hydrophobic component. Phase diagrams were constructed using Tri plot v1-4 software.

RESULTS AND DISCUSSION

Solubility Study

Vehicles should have good solubilizing capacity for the drug substance, which is essential for formulating SNEDDS. The results of solubility of Fenofibrate and Atorvastatin Calcium in various vehicles were shown in Table 1. Fenofibrate and Atorvastatin Calcium had highest solubility in Capmul MCM Oil (Glyceryl Caprylate/Caprate) with comparison to other lipid vehicles. Fenofibrate and Atorvastatin Calcium had highest solubility in Cremophor RH 40 (Polyoxyl 40 hydrogenated Castor oil) and Transcutol-P as compare to other surfactant and co-surfactant. Capmul MCM Oil (Glyceryl Caprylate/Caprate) as oil, Cremophor RH 40 (Polyoxyl 40 hydrogenated Castor oil) as surfactant and Transcutol-P as co-surfactant were selected for optimal SNEDDS formulation resulting in improved drug loading capability. Furthermore, with respect to its safety, Capmul MCM Oil (Glyceryl Caprylate/Caprate), Cremophor RH 40 (Polyoxyl 40 hydrogenated Castor oil) and Transcutol-P are included in the FDA Inactive Ingredients Guide.

Selection of Blend of Surfactant/Co-Surfactant

Solubility Parameter (δ)

Lipids used were better solvents for Atorvastatin calcium or Fenofibrate in increasing solubility because Atorvastatin calcium and Fenofibrate has higher lipophilicity with a log P of 5.7 and 5.3 respectively.

The solubility parameter calculated for Atorvastatin calcium is $\delta_{T\ (ATR)}$ = 15.27 $(cal/cm^3)^{½}$ (Table 5). Capmul MCM that has the closest solubility parameter (16.86 $(cal/cm^3)^{1/2}$) to that of Atorvastatin calcium and hence it provided the highest solubility among all lipids used. The same correlation could be observed with Fenofibrate. The calculated solubility parameter for Fenofibrate is $\delta_{T\ (FENO)}$ = 16.46 $(cal/cm^3)^{½}$ and the lipid that has closest solubility parameter is Capmul MCM (Capmul MCM = 16.86 $(cal/cm^3)^{1/2}$ (Table 5). Overall, calculated solubility parameter appeared to be a good predictor for the expected solvent effects of the lipids. The predictions are exclusively based on molecular structure of compounds, and no experimental data required.

Required HLB (RHLB) of Capmul MCM Oil

The data of Table-2 showed that among the surfactant/co-surfactant blends (Cremophor RH 40/Transcutol-P), the composition at 80:20 ratio having HLB 12.84 gave an emulsion that is easy to prepare and take longer time for separation of components then the other ten mixtures. These preliminary tests showed that the approximate RHLB for Capmul MCM is 12.84.

Under the HLB system, it was found that the oils, waxes, and other materials likely to be incorporated in to emulsion had an individual required HLB. This means that a surfactant or blend of surfactant/co-surfactant, having desired RHLB will make more stable emulsion than the emulsifier of any other HLB value.

Required Chemical Type of Emulsifiers

The mixture of Labrasol and Transcutol-P having HLB 12.84 gave similar results for ease of preparation and time for separation (no significant difference) as that of mixture of Cremophor RH 40 and Transcutol-P having similar HLB.

The 80:20 mixture of Cremophor RH 40 and Transcutol-P having HLB 12.84 was selected as surfactant/co-surfactant blend for further study.

Solubilization Capacity

Reverse micelle systems have been an interesting area of research in various fields of science and technology, due to their capability to solubilize water in organic solvent in the presence of surfactant.[25] It is known that ethoxylated non-ionic hydrophilic surfactants tend to form reverse micelles in organic media.[26] The results for the reverse micelle systems in this study formed by screening series

surfactants/co-surfactant were shown in Table 6. Cremophor RH 40/Transcutol-P (3:1) showed a high solubilization capacity compared with other S/CoS Ratio. Cremophor RH 40 (Polyoxyl 40 hydrogenated castor oil) is a non-ionic solubiliser and emulsifier made by reacting hydrogenated castor oil with ethylene oxide in a molar ratio of 1: 40.

Labrasol/Transcutol-P (1:1) showed the lowest solubilization capacity compared with Cremophor RH 40/Transcutol-P (3:1) (Table 6). This indicated a weak interaction between the oil and surfactant/co-surfactant from the same fatty acid derivative.

The results of this study were consistent with the study showing that the maximum solubilization capacity of water depends upon the oxyethylene chain and the configuration of the polar head group and hydrocarbon moiety of non-ionic surfactants and on type of oil.[23]

The results for the solubilization capacity of blends of surfactants/co-surfactant showed that Cremophor RH 40/Transcutol-P (3:1) at HLB 12.3 has the highest solubilization capacity compared with the Labrasol/Transcutol-P (3:1) at HLB 10.1. These results indicated the importance of the more lipophilic tail group that is structurally similar to the group on the Capmul MCM oil, which enables the co-surfactants to be well packed at the interface. Thus, these results reflected the effect of the type of co-surfactant blend on the solubilization capacity. The high solubilization capacity was obtained when surfactant/co-surfactant having the highest and lowest HLB value were mixed together, as shown by the solubilization capacity result for Cremophor RH 40/Transcutol-P (3:1) compared with the Labrasol/Transcutol-P (3:1) blend (Table 6).

The results of the study indicated the importance of selection of a better surfactant/co-surfactant blend showing strong solubilization capacity, which accordingly gives high stability.

Pseudo Ternary Phase Diagrams

Pseudo Ternary phase diagrams were constructed in presence of Fenofibrate or Atorvastatin Calcium to obtain optimum concentrations of oil, water, surfactant, and co-surfactant. SNEDDS formed fine oil–water emulsions with only gentle agitation, upon its introduction into aqueous media.

Phase behaviour investigations of this system demonstrated suitable approach to determining water phase, oil phase, surfactant concentration, and co-surfactant concentration with which transparent, one phase low-viscous nanoemulsion system was formed.[27]

Since free energy required to form an emulsion is very low, formation is thermodynamically spontaneous.[26] Surfactants form a layer around emulsion droplets and reduce interfacial energy as well as providing a mechanical barrier to coalescence. The visual test measured apparent spontaneity of emulsion formation.

Figure 1-2 presented the pseudo ternary phase diagram for mixtures of Capmul MCM oil, S/CoS (Labrasol/Transcutol-P and Cremophor RH 40/Transcutol-P) and water at various component compositions. All types of dispersions, including conventional water-in-oil and oil-in-water emulsions, water-in-oil and oil-in-water microemulsions, can be formed by S/CoS mixtures. A large area of clear isocratic solution (oil-in-water microemulsion/nanoemulsion) is formed at the oil-S/CoS axis in oil-rich regions. The minimum content of Cremophor RH 40/Transcutol-P (3:1) at an HLB of 12.3 formed in an isocratic system is 11.05% (fenofibrate) and 8.796% (Atorvastatin Calcium). This minimum content of surfactant/co-surfactant in a microemulsion or nanoemulsion system is known as the surfactant solubilization capacity.[23]

The smaller the percentage of S/CoS in a microemulsion/nanoemulsion system, the higher the solubilization capacity of the S/CoS, the better the match of the oil and S/CoS HLB, and hence the higher the stability of the product. Based on solubilization capacity, Cremophor RH 40/Transcutol-P (3:1) was selected as the best S/Cos.

The larger area of oil-in-water microemulsion/nanoemulsion formed by Cremophor RH 40/Transcutol-P (3:1) is due to the large molecular packing ratio of Cremophor RH 40/Transcutol-P, which is classified as a strong solubiliser.[29] Recent research has also suggested that the solubilization capacity and formation of oil-in-water microemulsion/nanoemulsion was caused by the extent of packing at the interface and not because of the HLB or the specific hydrophobicity of the surfactants.[26]

The main disadvantage of microemulsion/nanoemulsion systems is the lack of biocompatibility due to high surfactant(s) concentrations which might lead to toxicity or skin irritation.[30] Use of Capmul MCM oil that form a reverse micelle system in any formulation can overcome the lack of biocompatibility of such microemulsion/nanoemulsion systems because a low concentration of S/Cos is used.

Figures 1-2 showed the behaviours of surfactant/co-surfactant blends of Cremophor RH 40/Transcutol-P (with HLB values of 9.6, 11.3, and 12.3), Capmul MCM oil, and water at various concentration levels. The dispersion systems formed by these mixtures had reflected the nature and behaviour of their component compositions. The dispersion systems in these phase diagrams differ geometrically from Labrasol/Transcutol-P phase diagram. They showed much smaller areas of oil-in-water microemulsion/nanoemulsion compared with Cremophor RH 40/Transcutol-P (HLB 12.3). They also showed variation in area for the microemulsion system and other types of dispersion. Cremophor RH 40/Transcutol-P (3:1) at an HLB of 12.3 formed a large oil-in-water microemulsion/nanoemulsion area. The smaller area of oil-in-water microemulsion/nanoemulsion was due to a

lower HLB, which increases the lipophilic character of the surfactant blend.[31]

It was also clear from the solubilization capacity results that the Cremophor RH 40/Transcutol-P (3:1) with an HLB of 12.3 was a stronger solubiliser for water in Capmul MCM oil than other blends of Cremophor RH 40/Transcutol-P and Labrasol/Transcutol-P with HLB values in the range of 8.1–12.3. The weak interaction between the oil and S/CoS at lower HLB values for forming a reverse micelle system was due to the weaker solubilization of water at the interface in the presence of high percentages of lipophilic surfactant in the blends.

However, excessive amount of co-surfactant will cause system to become less stability for its intrinsic high aqueous solubility and lead to droplet size increasing as a result of expanding interfacial film.[32,33]

Table 1: Solubility of Fenofibrate and Atorvastatin Calcium in Various Oil, Surfactant and Co-Surfactant

Material	Solubility (mg/ml) ± SD	
	Fenofibrate	Atorvastatin Calcium
Castor Oil	72.18 ± 0.15	11.60 ± 0.06
Labrafac PG	58.85 ± 0.14	28.14 ± 0.04
Oleic Acid	21.43 ± 0.11	19.40 ± 0.10
Capmul MCM Oil	**178.93 ± 0.38**	**52.97 ± 0.07**
Light Liquid Paraffin	25.70 ± 0.12	10.69 ± 0.09
Tween-80	74.80 ± 0.20	40.13 ± 0.04
Span-20	47.22 ± 0.24	26.06 ± 0.07
Labrafac Lipophile WL 1349	63.89 ± 0.22	42.02 ± 0.03
Cremophor EL	61.48 ± 0.18	30.43 ± 0.05
Labrasol	**119.93 ± 0.46**	**74.48 ± 0.08**
Capmul GMO-50	36.29 ± 0.14	26.74 ± 0.08
Captex 355	25.19 ± 0.08	14.31 ± 0.08
PEG-400	36.39 ± 0.11	38.67 ± 0.07
Propylene Glycol	34.17 ± 0.11	10.74 ± 0.09
Transcutol-P	**177.11 ± 0.43**	**82.28 ± 0.08**
Cremophor RH 40	**112.85 ± 0.31**	**71.32 ± 0.28**

Table 2: Surfactant/Co-surfactant blends Cremophor RH 40 and Transcutol-P in different weight ratio and having different calculated HLB

S. No.	Surfactant/Co-surfactant Blends		Calculated HLB
	Cremophor RH 40	Transcutol-P	
1	100	0	15.00
2	90	10	13.92
3	**80**	**20**	**12.84**
4	70	30	11.76
5	60	40	10.68
6	50	50	9.60
7	40	60	8.52
8	30	70	7.44
9	20	80	6.36
10	10	90	5.28
11	0	100	4.20

Table 3: Number of times the test tubes shaken till a homogenous milky emulsion forms and time of separation for Capmul MCM emulsions prepared using emulsifiers of different HLB

S. No.	Calculated HLB of Surfactant/co-surfactant blend	Number of times Test tubes shaken till a homogenous milky emulsion forms		Time taken by emulsion for separation (min)	
		Mean	SD	Mean	SD
1	15.00	3.6	0.21	42.7	2.08
2	13.92	3.2	0.15	47.0	2.65
3	**12.84**	**3.0**	**0.06**	**58.0**	**2.00**
4	11.76	5.3	0.26	45.7	1.53
5	10.68	6.1	0.31	43.7	2.52
6	9.60	8.1	0.25	37.0	2.65
7	8.52	9.4	0.35	32.3	2.52
8	7.44	12.0	0.25	28.7	3.06
9	6.36	12.4	0.31	22.7	2.52
10	5.28	16.3	0.36	18.0	2.65
11	4.20	No emulsification		2.3	0.58

Table 4: Number of times the test tube shaken till a milky emulsion forms and time for separation for Capmul MCM emulsion prepared using surfactant/co-surfactant blend of same HLB but different chemical type

S. No.	Surfactant/Co-surfactant blend and HLB	Number of times the test tubes shaken for emulsification		Time taken by emulsion for separation (min)	
		Mean	SD	Mean	SD
1	Cremophor RH 40 and Transcutol-P, 12.84	3.0	0.06	58	2.00
2	Labrasol and Transcutol-P, 12.84	4.0	0.25	51.3	1.53

Table 5: The Solubility Parameter of Selected Lipid Vehicles

Materials	δH	Δv	δF	δT
Atorvastatin Calcium	**9.79**	**939.3**	**11.72**	**15.27**
Fenofibrate	**8.08**	**306.45**	**14.34**	**16.46**
Capmul MCM Oil	**11.74**	**217.81**	**12.10**	**16.86**
Light Liquid Paraffin	7.03	405.12	7.62	10.36
Castor oil	7.81	982.56	5.34	9.46
Oleic acid	6.87	317.87	10.64	12.67
Labrafac PG	6.95	724.97	9.61	11.86
Tween-80	10.77	560.01	8.73	13.87
Span-20	11.58	335.72	6.34	13.20
Labrafac Lipophile WL 1349	8.19	522.01	9.39	12.46
Cremophor EL (Polyoxyl 35 castor oil)	10.15	2233	9.77	14.09
Cremophor RH 40	**10.40**	**2403.8**	**12.20**	**16.03**
Labrasol	**13.69**	**1094.3**	**6.68**	**15.23**
Capmul GMO-50 (Glyceryl Monooleate)	8.88	380.19	12.10	15.01
Captex 355	7.75	499.98	11.51	13.88
PEG-400	12.99	355.56	6.14	14.37
Propylene Glycol	16.53	73.163	6.82	17.88
Transcutol-P	**12.15**	**135.5**	**9.24**	**15.26**

Table 6: The Solubilization Capacity of Selected Surfactants and Surfactant Blends

Drug	Surfactant/Co-surfactant	HLB	Solubilization Capacity
Fenofibrate	Labrasol/Transcutol-P (1: 1)	8.1	29.688
	Labrasol/Transcutol-P (2: 1)	9.4	23.750
	Labrasol/Transcutol-P (3: 1)	10.1	12.180
	Cremophor RH 40/Transcutol-P (1: 1)	9.6	27.457
	Cremophor RH 40/Transcutol-P (2: 1)	11.4	20.652
	Cremophor RH 40/Transcutol-P (3: 1)	12.3	11.050
Atorvastatin Calcium	Labrasol/Transcutol-P (1: 1)	8.1	12.838
	Labrasol/Transcutol-P (2: 1)	9.4	11.047
	Labrasol/Transcutol-P (3: 1)	10.1	9.694
	Cremophor RH 40/Transcutol-P (1: 1)	9.6	12.179
	Cremophor RH 40/Transcutol-P (2: 1)	11.4	10.106
	Cremophor RH 40/Transcutol-P (3: 1)	12.3	8.796

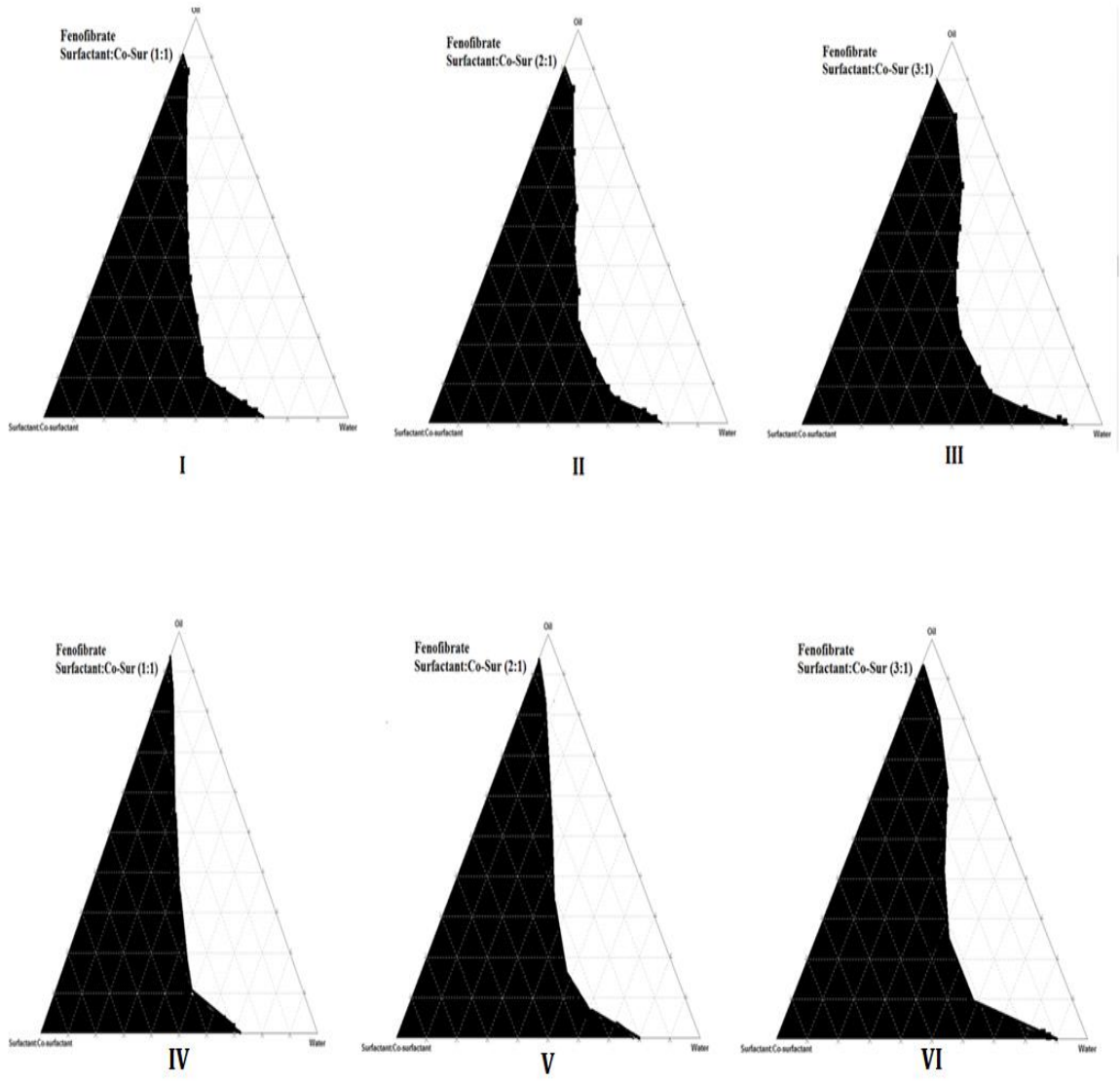

Figure 1: (I) S/Cos (Labrasol/Transcutol-P (1:1) at HLB - 8.1), **(II)** S/Cos (Labrasol/Transcutol-P (2:1) at HLB – 9.4), **(III)** S/Cos (Labrasol/Transcutol-P (3:1) at HLB – 10.1)**, (IV)** S/Cos (Cremophor/Transcutol-P (1:1) at HLB – 9.6), **(V)** S/Cos (Cremophor/Transcutol-P (2:1) at HLB – 11.3), **(VI)** S/Cos (Cremophor/Transcutol-P (3:1) at HLB – 12.3)

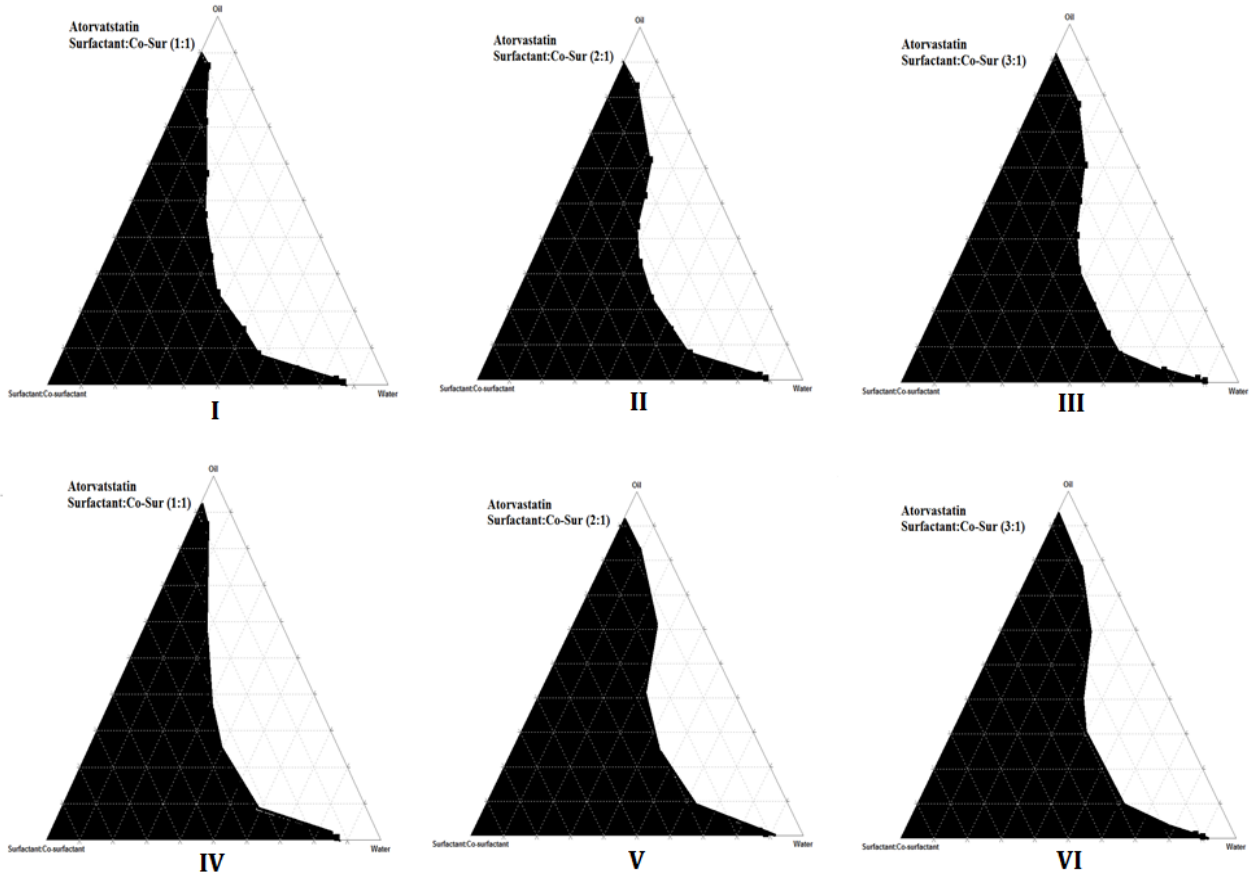

Figure 2: (I) S/Cos (Labrasol/Transcutol-P (1:1) at HLB - 8.1), **(II)** S/Cos (Labrasol/Transcutol-P (2:1) at HLB – 9.4), **(III)** S/Cos (Labrasol/Transcutol-P (3:1) at HLB – 10.1), **(IV)** S/Cos (Cremophor/Transcutol-P (1:1) at HLB – 9.6), **(V)** S/Cos (Cremophor/Transcutol-P (2:1) at HLB – 11.3), **(VI)** S/Cos (Cremophor/Transcutol-P (3:1) at HLB – 12.3)

CONCLUSION

The purpose of the present was to select appropriate lipid vehicle and to understand role of lipid vehicle in pseudo ternary phase diagram behaviour to find nanoemulsion area in formulation development of self-nanoemulsifying drug delivery system (SNEDDS) containing Fenofibrate and Atorvastatin Calcium. Solubility Parameter (δ), Required HLB (RHLB), required chemical type of emulsifiers and Solubilization capacity were determined for selection of blend of surfactant/co-surfactant.

The pseudo ternary phase diagrams for mixtures of Capmul MCM oil with non-ionic surfactant/co-surfactant and water were constructed in this study. The present study showed the importance of selecting a surfactant with the proper HLB for specific oils, as well as the type of surfactant/co-surfactant. The solubility parameter (δ) of Fenofibrate and Atorvastatin Calcium are closest solubility parameter (δ) of Capmul MCM. Blend of better surfactant/co-surfactant was obtained when surfactant and co-surfactant at higher and lower HLB level respectively were blended. The greater the difference between the hydrophilic and lipophilic surfactants, the better the coverage by blends at the interface. The study also showed the importance of the structural similarities between the lipophilic tails of the surfactant blends.

The SNEDDS have potential applications, advantages, and usefulness in the pharmaceutical industry as SNEDDS by various routes of administration, as well as in cosmetics and personal care products. Solubility Parameter (δ), Required HLB (RHLB), required chemical type of emulsifiers and Solubilization capacity appeared to be useful as a criterion for the selection of surfactant/co-surfactant along with pseudo ternary phase diagrams in formulation development of SNEDDS.

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FORMULATION AND EVALUATION OF SELF NANOEMULSIFYING DRUG DELIVERY SYSTEM CONTAINING POORLY WATER SOLUBLE ANTILIPIDEMIC DRUGS

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ABSTRACT

The present work was aimed for the formulation development of stable SNEDDS of Fenofibrate and Atorvastatin Calcium using concentration of oil and surfactant/cosurfactant on the basis of preliminary trials. The 3^2 factorial design was employed using concentration of Capmul MCM oil and Cremophor RH 40: Transcutol-P (3:1) as independent variables. The Globule size (GS), Polydispersity index (PDI), Zeta potential (ZP) and drug release at 15 minutes for Fenofibrate and Atorvastatin calcium were selected as dependent variables. The optimized batch was selected on the basis of arbitary criteria using Design Expert software employing overlay plot with desirability approach. The SNEDDS formulations were evaluated for their physico-chemical parameters such as globule size, zeta potential, polydispersity index, drug release profile and physico-chemical stability. The composition of optimized formulation consisted of Capmul MCM Oil as oil (0.471ml), Cremophor RH 40 as surfactant (1.206ml) and Transcutol-P as cosurfactant (0.402ml), containing 10mg of Atorvastatin and 45mg of Fenofibrate showing drug release for liquid SNEDDS formulation (>95%), droplet size (78.3nm), Zeta potential (-23.13), and infinite dilution capability. *In-vitro* drug release of the optimized batch was highly significant ($p<0.05$) as compared to marketed conventional capsule (Fenostat) of Fenofibrate and Atorvastatin Calcium.

KEYWORDS: Capmul MCM oil, non-ionic surfactant/co-surfactant, Nanoemulsion, SNEDDS.

INTRODUCTION

SNEDDS are isotropic mixtures made up of oil, surfactant and sometimes cosurfactant or cosolvent. In an aqueous environment a homogeneous, transparent (or at least translucent), isotropic and thermodynamically stable dispersion will result up on mild agitation.[1,2] SNEDDS is best suited for dosage for development of poorly soluble drugs. Lipophilic drug compounds which exhibit dissolution rate-limited absorption, these systems may offer an improvement in rate and extent of absorption.[1,2] SNEDDS formulations are known to reduce inter- and intra-individual variations in bioavailability, which is believed to be caused by a decreased sensitivity of formulation performance to pre-absorptive solubilisation and dietary status.

Atorvastatin Calcium inhibits HMG-CoA reductase and reduces serum concentrations of LDL-cholesterol, VLDL-cholesterol, Apo lipoprotein B and triglycerides.[3] Atorvastatin shows low aqueous solubility and rapidly absorbed after oral administration. Food decreases the rate and extent of drug absorption by approximately 25% and 9% respectively. Fenofibrate decrease LDL-cholesterol, triglyceride and Apo B concentrations and to increase concentrations of HDL-cholesterol in the management of mixed dyslipidemia and primary hypercholesterolemia, including heterozygous familial hypercholesterolemia.[4] Fenofibrate shows bioavailability problems due to poor water and physiological fluids solubility. Fenofibrate shows increase in absorption in fed condition of patient compare to fasting condition of patient. It exhibits additive antilipidemic effects when used concomitantly with other antilipidemic agents.[5]

The first step in oral absorption process is dissolution of the drug compound in gastrointestinal lumen contents, poor aqueous solubility is rapidly becoming the leading hurdle for formulation scientist working on oral delivery of such drug compounds. Hence, SNEDDS formulation were considered for enhance solubility, release rate and oral absorption of poorly soluble antilipidemic drugs.

To the best of our knowledge, no information is available in the literature on the improvement of Fenofibrate and Atorvastatin Calcium dissolution and bioavailability using mixture of Capmul MCM oil, Cremophor RH 40 and Transcutol-P by SNEDDS methodology. The present work described the formulation development of stable SNEDDS. The 3^2 factorial design was employed using concentration of Capmul MCM oil and Cremophor RH 40: Transcutol-P (3:1) as independent variables. The Globule size (GS),

Polydispersity index (PDI), Zeta potential (ZP) and drug release at 15 minutes for Fenofibrate and Atorvastatin calcium were selected as dependent variables. The optimized batch was selected on the basis of arbitary criteria using Design Expert software employing overlay plot with desirability approach.

Two check point batches was prepared and performed to validation the evolved polynomial equations in the formulation development of Fenofibrate and Atorvastatin Calcium SENDDS.

Stability study for optimized SNEDDS was performed as per ICH guidelines by keeping in stability chamber at room temperature (25°C) & 60% RH and accelerated condition (40°C) & 75% RH for 6 months. The optimized formulation was subjected to in vitro dissolution to evaluate improvement in drug release as compared to marketed product.

MATERIAL AND METHODS

Materials

Labrasol and Transcutol-P were generous gift from Gattefose for research. Cremophor RH 40 was gifted from BASF. Capmul MCM oil was gifted from Abitech. Atorvastatin calcium and Fenofibrate were gifted from Cadila Healthcare Limited. Water used in the preparation of formulations was distilled water, whereas ultra-pure water, used in analyses, was obtained with a Milli-Q apparatus. All other chemicals and reagents used were of pharmaceutical grade or HPLC grade.

METHODS

Solubility Study

Screening of solubilizing excipients was done by determining the solubility of Fenofibrate and Atorvastatin Calcium in different solubilizing vehicle like oils, surfactants and co-surfactants. An excess quantity of Fenofibrate or/and Atorvastatin Calcium were added to the 2 ml of the solubilizing vehicle. Both components were mixed in a vial for 5 minutes using cyclomixer. The mixtures in vial was shaken at room temperature for 48 hour using controlled temperature rotary shaker. The mixtures were centrifuged at 5000 RPM for 20 minutes. The drug content was analysed using UV-Visible spectrophotometer at 287 and 246 nm for Fenofibrate or Atorvastatin Calcium, respectively.[6,7]

Preliminary Trials

The oil and surfactant/co-surfactant mixture was used to found suitable concentration in formulation development of SNEDDS. The efficiency of self-emulsifying systems was measured from the rate of emulsification upon hydration with mild agitation. The time taken for the formation of fine emulsion was noted as dispersion time. Preliminary batches were formulated and their results of dispersion status as clear, turbid or clear & translucent in SNEDDS and dispersion time were recorded.

Preparation of SNEDDS[7]

Accurately weighed Fenofibrate and Atorvastatin Calcium were placed in a glass vial, and required quantity of oil, surfactant, and co-surfactant were added. The mixture was mixed by gentle stirring and vortex mixing at 40ºC on a magnetic stirrer, until drugs were dissolved.[7]

The mixture was stored at room temperature in closed container for further use.

3^2 factorial design for optimization of formulation parameters of Fenofibrate and Atorvastatin Calcium SNEDDS

The preliminary trials were carried out using different concentration of Capmul MCM oil, Cremophor RH 40 and Transcutol-P (3:1). On the basis of results of preliminary trials for selection of lipid vehicle, the concentration of Capmul MCM oil (X_1) and Concentration of Cremophor RH 40: Transcutol-P (3:1) (X_2) were taken as independent variables at three levels. The GS (Y_1), PDI (Y_2), ZP (Y_3), drug release at 15 minutes of Fenofibrate (Y_4) and drug release at 15 minutes of Atorvastatin Calcium (Y_5) were considered to play significant role in formulation performance of SNEDDS and all the five were taken as dependent parameters in present study. Multiple regression analysis, contour plot and 3D response surface plot were used to study the main and interaction effects of the variables. The responses were measured for each trials and then either simple linear equation, or interactive equation or quadratic equation model was fitted by carrying out multiple regression analysis and F-statistics to identify statistically significant term.

Microsoft EXCEL was used to identify non-significant terms. A coefficient is significant if ti>tcrit(v), where v denotes the degrees of freedom of residual variance. The refined model may be used for calculating the residuals or for drawing the contour plot.

Contour Plot

Contour plot is a diagrammatic representation of values of the response and it is helpful in explaining visually the relationship between independent and dependent variables. The reduced model was used to plot two dimension contour plot using demo version of Design Expert 11 software.

Response Surface Plot

Response surface plot is helpful in understanding the main and the interaction effects of variables in the formulation development. The effect of level of independent variable on the response parameter can be understood from the respective response surface plot.

Optimization of SNEDDS formulation overlay plot by Design Expert software

The desirability function approach is a technique for the simultaneous determination of optimum settings of input variables that can determine optimum performance levels for one or more responses.[7] The optimization of SNEDDS formulation was performed using Design Expert software employing overlay plot with desirability approach.

Checkpoint Analysis

A check point analysis was performed to validation the evolved polynomial equations in the formulation development of SNEDDS. Difference of theoretically computed values of GS, PDI, Zeta potential and drug release at 15 minutes for Fenofibrate and Atorvastatin Calcium and then mean values of experimentally obtained GS, PDI, Zeta potential and drug release at 15 minutes for Fenofibrate and Atorvastatin Calcium were compared by using Student's t-test.

Measurement of evaluation parameters of SNEDDS Formulations

Measurement of Globule Size, Polydispersity Index (PDI) and Zeta Potential[8]

Globule size, Polydispersity index (PDI) and zeta potential of SNEDDS were determined using Zetasizer Nano ZS (Malvern Instruments, UK), which follows principle of LASER light diffraction. SNEDDS was added (after suitable dilution with purified water) to the sample cell and put into the sample holder unit and measurement was carried out with the help of software of same instrument.[8]

Drug Content[9]

Fenofibrate and Atorvastatin Calcium from pre-weighed SNEDDS was extracted by dissolving in 25ml methanol. Then methanolic extract was separated out and Fenofibrate and Atorvastatin Calcium content in methanolic extract were analysed HPLC Method at 248nm, against standard methanolic solution of Fenofibrate and Atorvastatin Calcium.

In-Vitro Drug Release Study[7]

In vitro drug release study was carried out for all formulations, marketed product and active drug substance using USP Type II dissolution test apparatus. The dissolution medium (900 ml water) maintained at 37 ± 0.5°C and rotated at 50rpm. Aliquots were collected periodically and replaced with fresh dissolution medium. Aliquots, after filtration through 0.45µ PVDF filter paper, were analysed by HPLC at 248nm for Fenofibrate and Atorvastatin Calcium content.[9,10]

Stability Study of Fenofibrate and Atorvastatin Calcium SNEDDS

Stability of optimized formulation was carried out at 40±2°C/75±5%RH and 25±3°C (room temperature) as per ICH guidelines. SNEDDS was stored in glass vial for 6 months. Samples was withdrawn at 0, 1, 3 and 6 months and analysed periodically.[7,10]

Comparison of in vitro drug release between Optimized SNEDDS formulation, pure drug powder and marketed product

In vitro drug release study was performed as method described above for optimized SNEEDS formulations, marketed product and active drug substance to compare the in vitro drug release profile.

RESULT AND DISCUSSION

Solubility Study

Vehicles should have good solubilizing capacity for the drug substance, which is essential for formulating SNEDDS. Fenofibrate and Atorvastatin Calcium had highest solubility in Capmul MCM Oil, Cremophor RH 40 and Transcutol-P (Table 1). Capmul MCM Oil as oil, Cremophor RH 40 as surfactant and Transcutol-P as co-surfactant were selected for SNEDDS formulation resulting in improved drug loading capability.

Table 1: Solubility of Fenofibrate and Atorvastatin Calcium.

Material	Average (mg/ml) ± SD	
	Fenofibrate	Atorvastatin Calcium
Castor Oil	72.18 ± 0.15	11.60 ± 0.06
Labrafac PG	58.85 ± 0.14	28.14 ± 0.04
Oleic Acid	21.43 ± 0.11	19.40 ± 0.10
Capmul MCM Oil	178.93 ± 0.38	52.97 ± 0.07
Light Liquid Paraffin	25.70 ± 0.12	10.69 ± 0.09
Tween-80	74.80 ± 0.20	40.13 ± 0.04
Span-20	47.22 ± 0.24	26.06 ± 0.07
Labrafac Lipophile WL 1349	63.89 ± 0.22	42.02 ± 0.03
Cremophor EL	61.48 ± 0.18	30.43 ± 0.05
Labrasol	119.93 ± 0.46	74.48 ± 0.08
Capmul GMO-50	36.29 ± 0.14	26.74 ± 0.08
Captex 355	25.19 ± 0.08	14.31 ± 0.08
PEG-400	36.39 ± 0.11	38.67 ± 0.07
Propylene Glycol	34.17 ± 0.11	10.74 ± 0.09
Transcutol-P	177.11 ± 0.43	82.28 ± 0.08
Cremophor RH 40	112.85 ± 0.31	71.32 ± 0.28

Selection of Concentration of Oil, Surfactant and Cosurfactant

Capmul MCM oil and Cremophor RH 40: Transcutol-P mixture (3:1) were used to found their suitable concentration in formulation development of SNEDDS of Fenofibrate and Atorvastatin Calcium.

Table 2: Preliminary trials for development of Fenofibrate and Atorvastatin Calcium SNEDDS.

Preliminary Batches	Oil (ml)	S:Co-S (3:1) (ml)	Dispersion	Dispersion time (Seconds)
P1	0.5	0.5	Turbid	68
P2	0.5	1.0	Clear & Translucent	60
P3	0.5	1.5	Clear & Transparent	44
P4	0.5	2.0	Clear & Transparent	41
P5	0.4	0.5	Clear & Translucent	65
P6	0.4	1.0	Clear & Transparent	64
P7	0.4	1.5	Clear & Translucent	60
P8	0.4	2.0	Clear & Transparent	58
P9	0.6	0.5	Clear & Translucent	71
P10	0.6	1.0	Clear & Translucent	63
P11	0.6	1.5	Clear & Transparent	46
P12	0.6	2.0	Clear & Transparent	45

The preliminary trials were carried out using different concentration of Capmul MCM oil (0.4mL – 0.6mL), and Cremophor RH 40 and Transcutol-P (3:1) (0.5mL – 2.0mL). The

results of dispersion status and dispersion time were presented in Table 2. The result of preliminary trial with 0.5mL of Capmul MCM oil (batch P3) was found satisfactory. Apart from oil concentration, Concentration of S/Cos mixture (3:1) was also important in formulation development of SNEDDS and 1.5mL of Cremophor RH 40: Transcutol-P mixture (3:1) was found appropriate in the preliminary study.

Optimization of SNEDDS of Fenofibrate and Atorvastatin Calcium using factorial design

The concentration of oil and surfactant/Cosurfactant play important role in stable formulation of SNEDDS; hence concentration of Capmul MCM oil (0.5mL) and concentration of Cremophore RH 40:Transcutol-P (3:1) (1.5mL) were selected as independent variables in factorial design on the basis of preliminary trials (Table 2). The 3^2 factorial design was employed using concentration of oil and surfactant/Cosurfactant as independent variable X_1 and X_2 respectively. The GS (Y_1), PDI (Y_2), ZP (Y_3), drug release at 15 minutes of Fenofibrate (Y_4) and Atorvastatin Calcium (Y_5) were selected as dependent variables. The coded and actual value of independent variable were shown in Table 3. The runs and responses for factorial batches were presented in Table 4.

Table 3: Factors and levels of independent variables in 3^2 factorial design for formulation of Fenofibrate and Atorvastatin Calcium SNEDDS.

Independent variables	Level		
	Low (-1)	Medium (0)	High (+1)
Capmul MCM oil conc. (X_1)	0.4	0.5	0.6
Cremophor RH 40: Transcutol-P (3:1) conc. (X_2)	1.2	1.5	1.8

Table 4: Experimental runs and measured responses of 3^2 factorial design for SNEDDS.

Batch	X_1	X_2	Globule size (nm) (Y_1)	PDI (Y_2)	Zeta potential (mV) (Y_3)	Drug release at 15 min for Fenofibrate (Y_4)	Drug release at 15 min for Atorvastatin Calcium (Y_5)
T1	-1	-1	357.0	0.428	-15.12	91.8	92.4
T2	0	-1	64.1	0.283	-16.40	93.9	93.6
T3	1	-1	55.8	0.221	-17.12	93.1	91.6
T4	-1	0	332.0	0.427	-15.68	93.8	92.9
T5	0	0	20.7	0.189	-27.96	96.7	97.4
T6	1	0	44.0	0.233	-17.60	94.1	93.4
T7	-1	1	307.0	0.426	-15.96	93.5	92.3
T8	0	1	26.6	0.195	-24.28	94.5	95.8
T9	1	1	29.2	0.191	-21.12	95.0	96.0

Multiple regression analysis was carried out for the responses using MS Excel. The reduced model was obtained by using significant terms ($p > 0.05$ was considered non-significant and such termed were neglected) for all the responses. The contour and response surface plot were constructed using Design Expert version 11 (Demo version).

Globule size (Y_1)

A full model equation of globule size (Y_{FGS}) was written as Equation 1.

$Y_{FGS} = 31.9889–144.5X_1–19.0167X_2+150.3667X_1^2+7.7167X_2^2+5.85X_1X_2$ …… (Equation 1)

The reduced model for globule size (Y_{RGS}) was presented as Equation 2.

$Y_{RGS} = 37.1333 – 144.5X_1 – 19.0167X_2 + 150.3667X_1^2$ ……. (Equation 2)

Polydispersity index (PDI) (Y_2)

A full model equation of polydispersivity index (Y_{PDI}) was written as Equation 3.

$Y_{FPDI} = 0.2172 – 0.106X_1 – 0.02X_2 + 0.0987X_1^2 + 0.0077X_2^2 – 0.007X_1X_2$ …… (Equation 3)

The reduced model for polydispersity index (Y_{RPDI}) was presented as Equation 4.

$Y_{RPDI} = 0.2223 – 0.1060X_1 – 0.0200X_2 + 0.0987X_1^2$ .....…….. (Equation 4)

Zeta potential (ZP) (Y_3)

A full model equation of zeta potential (Y_{FZP}) was written as Equation 5.

$Y_{FZP} = -24.2667 – 1.5133X_1 – 2.12X_2 + 5.78X_1^2 + 2.08X_2^2 – 0.79X_1X_2$ …… (Equation 5)

The reduced model for zeta potential (Y_{RZP}) was presented as Equation 6.

$Y_{RZP} = -19.0267 – 1.5133X_1 – 2.1200X_2$ ...…….....……. (Equation 6)

Drug release at 15 minutes for Fenofibrate (DRF) (Y_4)

A full model equation of drug release at 15 minutes for Fenofibrate (Y_{FDRF}) was written as Equation 7.

$Y_{FDRF} = 95.8556 + 0.5167X_1 + 0.7X_2 – 1.4833X_1^2 – 1.2333X_2^2 + 0.05X_1X_2$ … (Equation 7)

The reduced model for drug release at 15 minutes for Fenofibrate (Y_{RDRF}) was presented as Equation 8.

$Y_{RDRF} = 94.0444 + 0.5167X_1 + 0.7X_2$ ……...……. (Equation 8).

Drug release at 15 minutes for Atorvastatin Calcium (DRA) (Y_5)

A full model equation of drug release at 15 minutes for Atorvastatin Calcium (Y_{FDRA}) was written as Equation 9.

$Y_{FDRA} = 96.2333 + 0.5667X_1 + 1.0833X_2 – 2.5X_1^2 – 0.95X_2^2 + 1.125X_1X_2$ ….. (Equation 9)

The reduced model for drug release at 15 minutes for Atorvastatin Calcium (Y_{RDRA}) was presented as Equation 10.

$Y_{RDRA} = 95.6000 + 0.5667X_1 + 1.0833X_2 – 2.5X_1^2$ ……..... (Equation 10).

Table 5: ANOVA of full model and reduced model.

Responnse Y1	**Model**	**DF**	**SS**	**MS**	**F**	**R^2**	**F cal**
Regression	FM	5	172927.6	34585.51	360.64	0.9983	1.334
	RM	3	172671.6	57557.19	529.32	0.9968	
Error	FM	3	287.701	95.900			
	RM	5	543.685	108.737			
Responnse Y2	Model	dF	SS	MS	F	R^2	F cal
Regression	FM	5	0.0896	0.01792	14.286	0.9597	1.541
	RM	3	0.0892	0.0297	36.502	0.9563	
Error	FM	3	0.0003	0.0012			
	RM	5	0.0040	0.0008			
Responnse Y3	Model	dF	SS	MS	F	R^2	F cal
Regression	FM	5	118.673	23.734	1.713	0.7407	1.876
	RM	2	40.707	20.353	1.021	0.2541	
Error	FM	3	41.547	13.849			
	RM	6	119.513	19.918			
Responnse Y4	Model	dF	SS	MS	F	R^2	F cal
Regression	FM	5	11.994	2.398	2.892	0.8282	2.996
	RM	2	4.541	2.270	1.370	0.3136	
Error	FM	3	2.487	0.829			
	RM	6	9.940	1.656			
Responnse Y5	Model	dF	SS	MS	F	R^2	F cal
Regression	FM	5	28.335	5.667	5.053	0.8938	3.062
	RM	3	21.468	7.156	3.497	0.6772	
Error	FM	3	3.364	1.121			
	RM	5	10.231	2.046			

Contour Plots and Response Surface Plots

Two dimensional contour plots were constructed for all dependent variables i.e. GS, PDI, ZP and drug release at 15 minutes for Fenofibrate (DRF) and Atorvastatin Calcium (DRA) and shown in Figure 1, 2, 3, 4, and 5. Response surface plots are very helpful in learning about both the main and interaction effects of the independent variables.

Globule size (GS)

Figure 1 showed contour plot for globule size at prefixed values. The contour plot was found to be linear, thus the relationship between independent variables for GS could be linear.

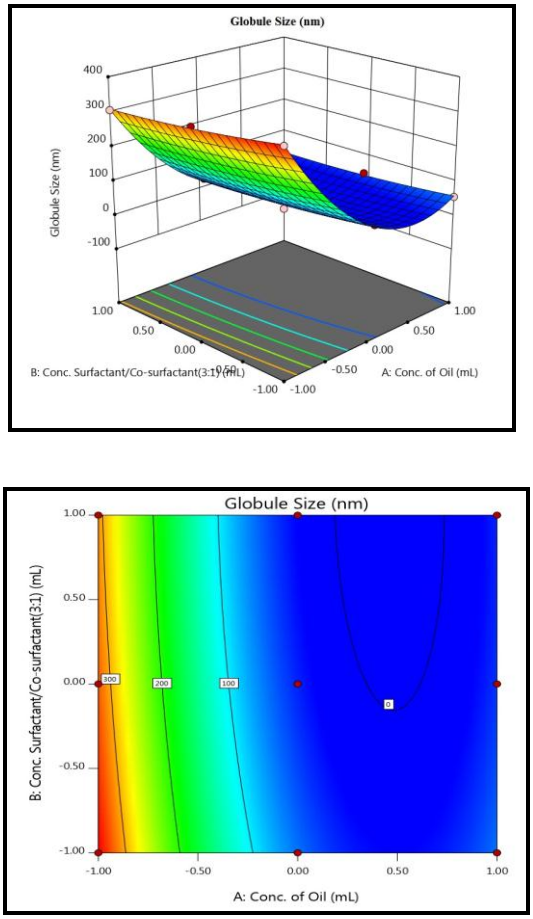
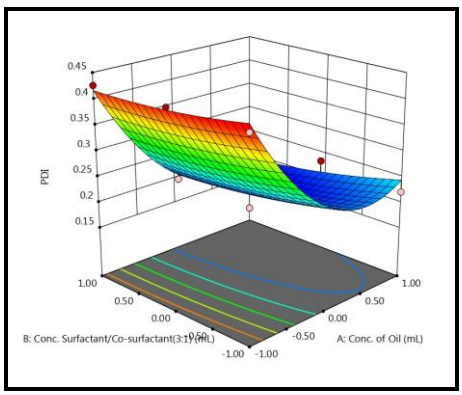


Figure 1: Contour plot and 3D surface plot for the effect on globule size.

The response surface plot showed decrease in globule size with increase in the conc. of Capmul MCM oil and conc. of Cremophor RH 40: Transcutol-P (3:1).

Polydispersity index (PDI)

Figure 2 showed contour plot for polydispersity index at prefixed values. The contour plot was found to be linear. Hence, the relationship between independent variables for polydispersity index could be linear.



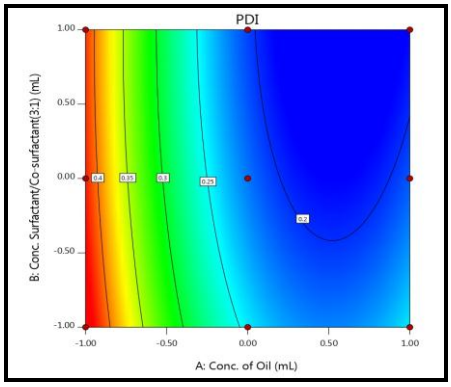
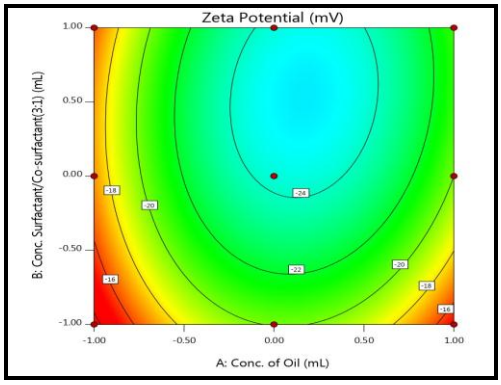


Figure 2: Contour plot and 3D surface plot for the effect on PDI.

The response surface plot showed decrease in polydispersity index with increase in the conc. of Capmul MCM oil and conc. of Cremophor RH 40: Transcutol-P (3:1).

Zeta Potential (ZP)

Figure 3 showed contour plot for zeta potential at prefixed values. The contour plot was found to be non-linear. Hence, the relationship between independent variables for zeta potential could be non-linear.



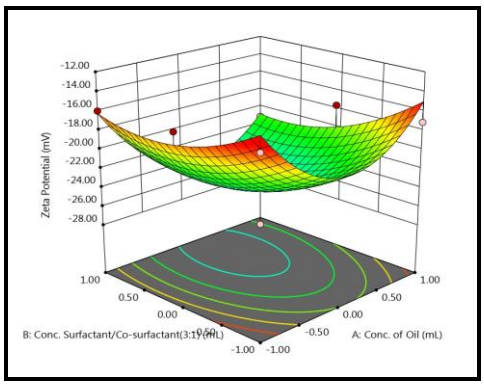


Figure 3: Contour plot and 3D surface plot for the effect on Zeta Potential.

The response surface plot showed decrease in zeta potential with increase in the conc. of Capmul MCM oil and conc. of Cremophor RH 40: Transcutol-P (3:1).

Drug release at 15 minutes for Fenofibrate (DRF)

Figure 4 showed contour plot for drug release at 15 minutes for Fenofibrate (DRF) at prefixed values. The contour plot was found to be non-linear. Hence, the relationship between independent variables for drug release at 15 minutes for Fenofibrate (DRF) could be non-linear.

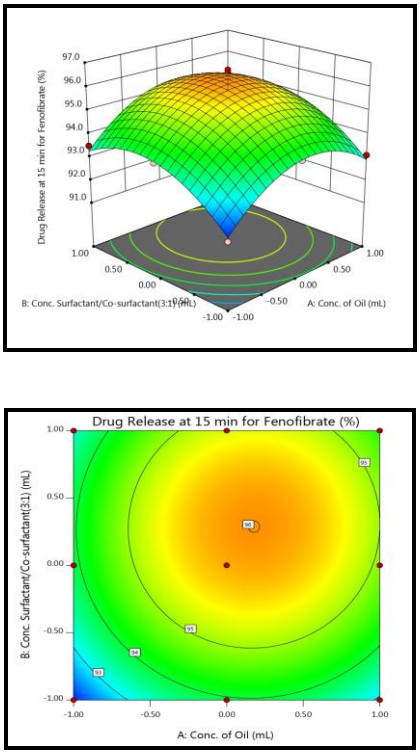


Figure 4: Contour plot and 3D surface plot for the effect on Drug Release at 15 min for Fenofibrate.

The response surface plot showed increase in drug release with increase in the conc. of Capmul MCM oil and conc. of Cremophor RH 40: Transcutol-P (3:1).

Drug release at 15 minutes for Atorvastatin Calcium (DRA)

Figure 5 showed contour plot for drug release at 15 minutes for Atorvastatin Calcium (DRA) at prefixed values of 92.75, 93.75, 94.75, and 95.5. The contour plot was found to be non-linear. Hence, the relationship between independent variables for drug release at 15 minutes for Atorvastatin Calcium (DRA) could be non-linear.

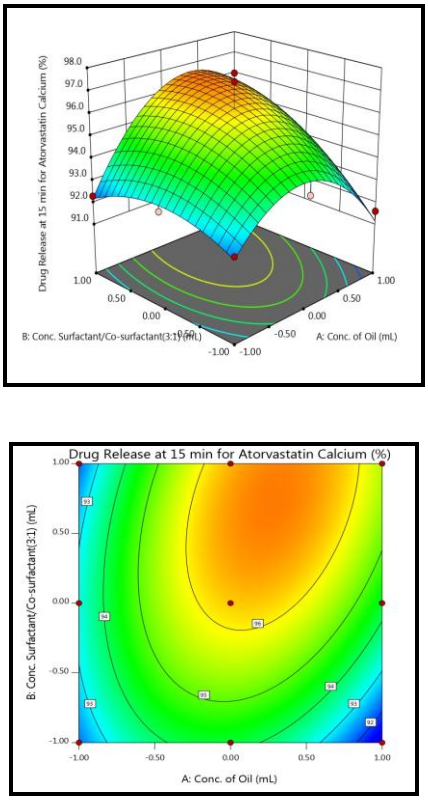


Figure 5: Contour plot and 3D surface plot for the effect on Drug Release at 15 min for Atorvastatin Calcium.

The response surface plot showed increase in drug release with increase in the conc. of Capmul MCM oil and conc. of Cremophor RH 40: Transcutol-P (3:1).

Optimiztion of SNEDDS Formulation

Optimized formulation was selected by arbitrarily fixing the criteria of 20.7 – 357nm of GS (minimize), 0.189 – 0.428 PDI (maximize), -30mV to -21mV ZP (is target = -27), more than 95% drug released at 15 minutes for Fenofibrate and Atorvastatin Calcium. The recommended concentrations of the independent variables were calculated by Design Expert software using overlay plot with desirability approach (Figure 6). The results gave one optmized solution with theoretical target profile characteristics which were shown in Table 6.

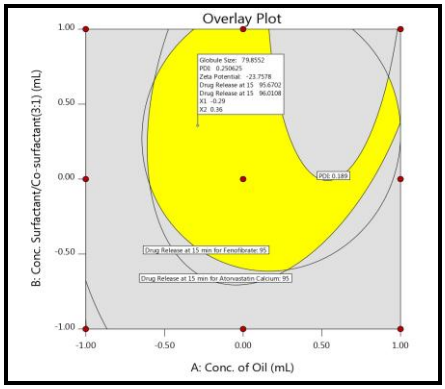


Figure 6: Overlay plot for optimization of SNEDDS formulation.

Table 6: Solution proposed by Design Expert.

Sol. Run	Conc. of oil (mL)	Conc. of S:Co-s (3:1) (mL)	GS (nm)	PDI	ZP (mV)	Drug Release at 15 min for Fenofibrate	Drug Release at 15 min for Atorvastatin Calcium
1	0.471	1.608	79.85	0.250	-23.75	95.67	96.01

Figure 6 showed overlay plot obtained from Design Expert. Grayed area (Shaded areas) on the graphical optimization plot did not meet the selection criteria. Yellow area indicated the area in which the optimized formulation can be formulated. In yellow portion, the values of all variables i.e. GS, PDI, ZP, drug release at 15 minutes for Fenofibrate and Atorvastatin calcium were selected. The point indicating toggle flag showed the value of X_1 and X_2 for optimized formulation.

Table 7: Optimized formulation of Fenofibrate and Atorvastatin Calcium SNEDDS (Batch OP1).

Material used	Quantity per Unit (mL)	Quantity per Unit (%)
Capmul MCM oil	0.471	23.62%
Cremophor RH 40	1.206	57.27%
Transcutol-P	0.402	19.11%

Check point batch analysis

Two different check point batches (C1 and C2) of Fenofibrate and Atorvastatin Calcium SNEDDS were prepared (Table 8). The experimentally and theoretically computed values of GS, PDI, ZP, drug release at 15 minute for fenofibrate and Atorvastatin Calcium were presented and compared using student 't' test, the difference was found to be non-significant ($p<0.05$) in both cases.

Table 8: Composition and results of Check point batches.

Batches	C1		C2	
X_1	0.5		-0.5	
X_2	-0.5		0.5	
Response	**Predicted**	**Experimental**	**Predicted**	**Experimental**
Globule size (nm) [Y_1]	132.39	127.3	7.42	11.56
Polydispersity index [Y_2]	0.288	0.291	0.203	0.194
Zeta potential (mV) [Y_3]	-22.42	-22.15	-21.76	-20.33
Drug release at 15 minutes for Fenofibrate [Y_4]	95.26	95.6	95.06	93.9
Drug release at 15 minutes for Atorvastatin Calcium [Y_5]	95.35	95.8	94.81	93.6

Stability study of optimized SNEDDS formulation

Stability study of optimized batch (OP1) was conducted up to 6 months at room temperature and accelerated condition of 40°C & 75% RH.

The GS, ZP, drug content and drug release at 15 minutes for Fenofibrate and Atorvastatin calcium were analysed at initial and after 1, 3 and 6 months. The results were recorded in Table-9-11.

Table 9: Globule size and Zeta Potential of optimized batch at storage conditions.

	Average of Globule Size (nm)		Zeta Potential (mV)	
Storage Conditions	**Room Temperature**	**Accelerated Conditions**	**Room Temperature**	**Accelerated Conditions**
Initial	78.3	78.3	-23.13	-23.13
1 Month	79.2	79.8	-22.38	-22.45
3 Month	82.1	83.3	-22.24	-22.92
6 Month	82.5	83.9	-21.79	-21.47

Table 10: Drug content of optimized batch at storage conditions.

	% Assay (±) SD			
	(Fenofibrate)		(Atorvastatin Calcium)	
Storage Conditions	**Room Temperature**	**Accelerated Conditions**	**Room Temperature**	**Accelerated Conditions**
Initial	100.2 ± 0.46	100.2 ± 0.46	100.4 ± 0.25	100.4 ± 0.25
1 Month	100.1 ± 0.27	100.0 ± 0.58	99.6 ± 0.46	99.4 ± 0.63
3 Month	99.7 ± 0.52	99.4 ± 0.65	99.3 ± 0.46	99.1 ± 0.82
6 Month	99.4 ± 0.38	99.1 ± 0.62	98.7 ± 0.58	98.3 ± 0.28

Table 11: Drug content of optimized batch at storage conditions.

	% Drug release at 15 minutes			
	(Fenofibrate)		(Atorvastatin Calcium)	
Storage Conditions	**Room Temperature**	**Accelerated Conditions**	**Room Temperature**	**Accelerated Conditions**
Initial	96.2 ± 1.2	96.2 ± 1.2	97.1 ± 1.2	97.1 ± 1.2
1 Month	97.4 ± 1.7	97.1 ± 1.6	97.4 ± 1.7	97.1 ± 1.6
3 Month	97.7 ± 2.4	96.9 ± 2.1	97.7 ± 2.4	96.9 ± 2.1
6 Month	96.4 ± 1.9	96.1 ± 2.5	96.4 ± 1.9	96.1 ± 2.5

The results reveled the absence of any significant change in SNEDDS with respect to GS, ZP, drug content and drug release at 15 minutes for Fenofibrate and Atorvastatin calcium. It indicated that optimized SNEDDS remained stable during the storage conditions.

Comparison of in vitro drug release between optimized batch, pure drug powder, and marketed product

The Fenofibrate and Atorvastatin Calcium release profile of optimized batch was compared with pure drug powder and marketed capsule product. The marketed product was FENOSTAT of Ordain Health Care Global Pvt Ltd. which is a conventional capsule formulation.

Table 12: Comparison of Fenofibrate release profile of batch OP1 with pure drug and Fenostat.

Batches	**% Drug Release (Fenofibrate) (Mean ± SD)**						
	Time (Minutes)						
	0	**10**	**15**	**20**	**30**	**45**	**60**
OP1	0±0	92.1±1.5	96.2±1.2	99.7±1.1	100.1±0.2	99.6±0.1	99.7±0.3
Pure drug	0±0	7.3± 0.7	17.6±0.8	19.1±0.5	27.4±1.1	38.7±0.4	48.2±0.5
Fenostat	0±0	14.1±0.2	22.8±0.7	23.2±0.8	32.4±0.5	47.5±1.4	59.3±1.4

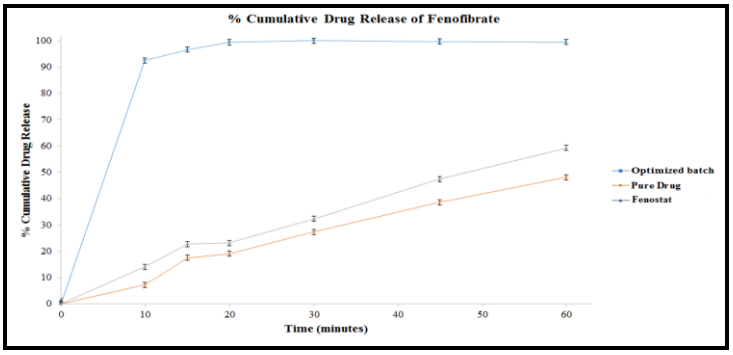


Figure 7: Comparison of drug release profile of optimized batch with pure drug and Fenostat (Fenofibrate).

Table 13: Comparison of Atorvastatin Calcium release profile of batch OP1 with pure drug and Fenostat.

Batches	% Drug Release (Atorvastatin Calcium) (Mean ± SD)						
	Time (Minutes)						
	0	10	15	20	30	45	60
OP1	0±0	92.4±1.5	97.1±1.2	99.3±1.1	100.0±0.2	99.8±0.1	99.7±0.3
Pure drug	0±0	6.3± 1.7	13.3±0.9	18.7±0.8	26.6±1.3	36.7±0.7	46.5±0.5
Fenostat	0±0	13.6±1.2	18.9±0.8	24.7±0.7	31.6±0.5	46.5±1.1	57.6±1.0

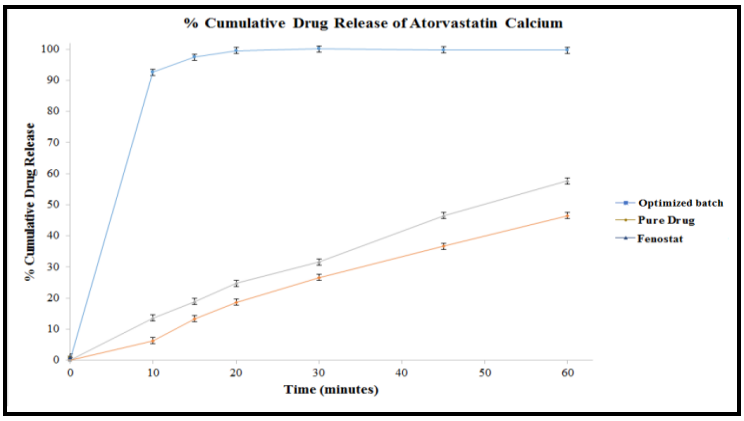


Figure 8: Comparison of drug release profile of optimized batch with pure drug and Fenostat (Atorvastatin Calcium).

Drug release from the optimized batch (OP1) was found to be better as compared with that of pure drug powder and marketed drug formulation (Figure 7, 8). It could be suggested that the optimized SNEDDS batch resulted in spontaneous formation of a nanoemulsion with a small globule size, which permitted a faster drug release into the aqueous phase than that of pure drug powder and marketed formulation. Thus, the greater availability of dissolved Fenofibrate and Atorvastatin Calcium from the SNEDDS formulation could lead to higher absorption and higher oral bioavailability.

CONCLUSION

SNEDDS are isotropic mixtures made up of oil, surfactant and sometimes cosurfactant or cosolvent. In an aqueous environment a homogeneous, transparent (or at least translucent), isotropic and thermodynamically stable dispersion will result up on mild agitation. SNEDDS is best suited for dosage for development of poorly soluble drugs. Fenofibrate and Atorvastatin Calcium are BCS class II drugs having low solubility and high permeability. The present study was aimed to explore stable SNEDDS formulation development using 3^2 factorial design for dissolution improvement compared to marketed formulation of Fenofibrate and Atorvastatin Calcium. On the basis of results of preliminary trials, Capmul

MCM as oil, Cremophor RH 40 as surfactant and Transcutol-P as cosurfactant were used for formulation development of Fenofibrate and Atorvastatin Calcium SNEDDS. The 3^2 factorial design was employed using concentration of Capmul MCM oil and Cremophor RH 40: Transcutol-P (3:1) as independent variables. The Globule size (GS), Zeta potential (ZP), Polydispersity index (PDI) and drug release at 15 minute for Fenofibrate and Atorvastatin Calcium were selected as dependent variables. Multiple regression analysis, contour plot and response surface plot were used to study the main and interaction effects of the variables on the responses. Simple linear equation, or interactive equation or quadratic model was fitted by carrying out multiple regression analysis and F-statistic to identify statistically significant term. The optimized batch was selected on the basis arbitrary criteria using Design Expert employing overlay plot with desirability approach. The batch containing 0.471ml of Capmul MCM oil, 1.608ml of Cremophor RH 40: Transcutol-P (3:1) was selected as optimized SNEDDS formulation. The check point batches were prepared to validate the evolved equations. The optimized formulation was subjected to in vitro dissolution to evaluate drug release as compared to marketed product. The stability study for optimized batch was conducted at room temperature and 40°C & 75% RH. The optimized formulation was found stable and more than 90% drug dissolution was achieved within 15 minutes. The desirable goals can be achieved by systematic formulation approach in shortest possible time with reduced number of experiments for formulation development of Fenofibrate and Atorvastatin Calcium SNEDDS formulation using factorial design.

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