FORMULATION AND DEVELOPMENT OF SELF EMULSIFYING DRUG DELIVERY SYSTEM FOR FEW DRUGS

A THESIS

Submitted to

The Tamil Nadu Dr.M.G.R. Medical University, Chennai

In the partial fulfilment of the requirements for the award of the degree of

DOCTOR OF PHILOSOPHY
In
FACULTY OF PHARMACY

By

D. AKILADEVI, M.Pharm.

(Register no. 141340001)

Under the guidance and supervision of

Dr. M. NAPPINNAI, M.Pharm., Ph.D
Professor, Department of Pharmaceutics,
School of Pharmacy, Surya Group of Institutions,
Vikravandi, Villupuram District 605 652.

January 2017

CHAPTER 1

INTRODUCTION

1.1 DRUG DELIVERY ISSUES

The major, popular route of administering drug from time immemorial is the oral route for both chronic and acute dosage regimen. Unfortunately more than 50 % of drug compounds have unfavorable physicochemical property of which high lipophilicity is major one. Almost 40% of the new drug candidates exhibit low solubility in water which leads to poor bioavailability, high intra and inter -subject variability, lack of dose proportionality. Thus for such compounds, among numerous factors limiting bioavailability is the absorption rate from gastrointestinal lumen. This is in turn is related to dissolution[1]. According to the BCS classification, two classes of drugs show poor aqueous solubility namely BCS II and BCS IV. BCS II drugs possess poor aqueous solubility but have good permeation properties. BCS class IV drugs are poorly water soluble and poorly permeable. A formulation of developing for a class IV drug is nearly impossible.

Various approaches for enhancing bioavailability of poorly soluble drugs[2]

- **Formation of salt:** The drugs in acidic or basic forms have increased solubility because of salt formation. But it's highly significant when the drug exists in salt form.

- **Micronization:** It is one of the methods of reducing the particles to micron size but the approach proved insufficient for enhancement of dissolution rate of the drugs in solid dosage forms.

- **Nanocrystals:** These are developed products for nanoparticle approach in the market with modern technology supplier. They are procured only under license but the secondary step required is to avoid agglomeration of nanocrystals. Nanocrystals by the dense gas technology are used as an alternative method for producing nanocrystals. But it was not marketed and the product was so failed.

- **Solid solutions:** Solid solutions are prepared using novel extrusion technology by the solvent free continuous process and can be easily operated. But this approach may lead to problems which affect the physical stability of the product and crystallization of drug. Numerous methods are adopted with the limitation of reproducibility and stability. The ease of manufacturing and scale-up process is major obstacles in all above mentioned approaches which resulted to put an end to these methods.

Problems associated with methods used for improvement of bioavailability

The improvement of solubility of hydrophobic drugs remains one of the most challenging aspects of drug development. Although salt formation, solubilization and particle size reduction have commonly been used to increase dissolution rate, there are practical limitations for these techniques. The salt formation is not feasible for neutral compounds and the synthesis of appropriate salt forms of drugs that are weakly acidic or weakly basic may often not be practical. The solubilization of drugs in organic solvents or in aqueous media by the use of surfactants and cosolvents leads to liquid formulations that are usually undesirable from the

viewpoints of patient acceptability and commercialization. Although particle size reduction is commonly used to increase dissolution rate, there is a practical limit to how much size reduction can be achieved by such commonly used methods as controlled crystallization, grinding, etc. The use of very fine powders in a dosage form may also be problematic because of improper handling and wetting[3]. To prevail over these limitations, various formulation methods are available like addition of cyclodextrins and permeation enhancers and preparation of nanoparticles and solid dispersions [4].

1.2 LIPID BASED DRUG DELIVERY

One of the popular and recent approaches to improve the oral bioavailability of poorly water soluble compounds are lipid based formulations in which the drug compounds are incorporated into inert lipid vehicles such as oils and surfactant dispersions, self-emulsifying drug formulations and emulsions[5, 6].

1) **Self-dispersing solid** (solution with surfactants): The stearic hindrance to aggregation may build into product and the physical stability of the product is questionable as the drug or polymer may crystallize.

2) **Lipid solution** (LFCS Type I): These are effective for lipophilic drugs and drug is presented in solution avoiding the dissolution step.

3) **Self-emulsifying drug delivery system** (LCFS Type II or Type III lipid systems): It is an effective method to incorporate the drug into self-emulsifying liquid whereby emulsification forms an o/w emulsion spontaneously on mixing with water. For such formulations dispersion leads to rapid absorption and absorption is independent on digestion.

4) **Solid or semi-solid SEDDS**: They are prepared as a free flowing powder or compressed into a tablet form. The surfactant used may be poorly tolerated in chronic use and physical stability of the product is questionable as drug or polymer may crystallize.

5) **Surfactant-cosolvent systems** (LCFS Type IV lipid systems): These formulations have the relatively high solvent capacity for typical APIs. The surfactant used may be poorly tolerated in chronic use. There is the significant threat of drug precipitation on dilution.

Requisites to formulate lipid-based drug delivery systems[7]

1. The lipids should be capable of solubilizing the therapeutic amount of the drug in its final dosage form.

2. The solubility of the drug in lipid vehicles should be maintained under all predicted storage conditions throughout the shelf-life of the drug product.

3. It should not affect the stability of the drug and its formulation ingredients.

4. The inactive approved ingredients added in lipid formulations should be under GRAS (Generally regarded as safe) or any pharmacopoeial category.

5. Lipids should increases or maintains drug solubilization.

6. The absorption of drug from the lipids by intestinal mucosal cells should be optimized.

Tentative drug candidates selected for oral lipid based formulations

The BCS is a scientific framework for classifying a drug candidate based on aqueous solubility and intestinal permeability. It is a prognostic tool for predicting the oral absorption. According to the description of the BCS proposed by Amidon[1] in 1995, both BCS II and IV drugs are promising suitable candidates identified for oral lipid formulations.

The promising drug candidates suitable for oral lipid formulations can be identified based on drug solubility and permeability according to Amidon *et al*[1] BCS which is related with bioavailability. The lipid based formulations which are depicted in Table 1 can potentially improve bioavailability for selected compounds in every BCS category. The BCS Category II compounds possessing poor water solubility and high membrane permeability exhibit substantial enhancements in bioavailability when formulated in solubilizing lipid excipient. Although these compounds are hydrophobic, they possess solubility (50-100mg/ml) in a dietary triglyceride in which drugs can be dispersed and enhances bioavailability by overcoming absorptive barriers of poor aqueous solubility and slow dissolution in the gastrointestinal tract. The enhanced absorption of hydrophobic molecules through lipid formulations involves a mechanism of transfer into the bile salt-mixed micellar phase in which the absorption occurs readily across the intestinal epithelium. The lipids can improve bioavailability through mitigation of intestinal efflux by the p-glycoprotein transporter, which enhances the reduction in first pass metabolism caused by membrane bound cytochrome enzymes and thereby results in changing the permeability of the intestinal membrane. Lipid soluble drugs enter directly into the intestinal lymph in which the drugs enter into the systemic blood circulation, thereby circumventing the potential first pass metabolism. Self-emulsifying drug delivery systems (SEDDS) are a bioavailability enhancer for oral delivery of poorly soluble drugs.

Pouton and Wakerly, the innovators of novel lipid based formulations such as SEDDS disclosed that the self-emulsification process is specific to the nature of the

oil and surfactant pair[8, 9]. The process depends on the oil nature, surfactant concentration and the oil/surfactant ratio and the temperature at which the self-emulsification occurs. For selected compounds under BCS category of lipid based formulations can potentially enhance bioavailability which is illustrated in Table 1. The typical composition of various types of lipid formulations according to Lipid formulation classification system LFCS is indicated in Table 2.

Table 1 : Potential bioavailability improvement of active ingredients categorized by the Biopharmaceutical classification system using oral lipid based formulations[10]

Aqueous Solubility	**Membrane permeability**	**Type**[1]	**Potential formulation Type**	**Potential benefits of the system**
High	High	I	Microemulsion w/o	Stabilization, chemical enzymatic protection against hydrolysis (+efflux).
High	Low	III	Microemulsion w/o	Stabilization, chemical enzymatic protection against hydrolysis (+efflux).
Low	High	II	Self-micro emulsifying drug delivery system (SMEDDS) o/w	Enhancement of dissolution, solubilization, and improved bioavailability
Low	Low	IV	Self-micro emulsifying drug delivery system (SMEDDS) o/w	Enhancement of dissolution, solubilization and improved bioavailability (+efflux).

Table 2 Typical Composition of Lipid formulation classification system by Pouton[10]

Types of excipients in formulation	Percentage content of formulation in weight basis				
	Type I	Type II	Type IIIA	Type IIIB	Type IV
Triglycerides or mixed mono and diglycerides	100	40-80	40-80	<20	–
Water insoluble Surfactants (HLB < 12)	– –	20-60	– –	– –	0-20
Water soluble surfactants (HLB > 12)	– –	– –	20-40	20-50	30-80
Cosolvents (Hydrophilic) (e.g. PEG, propylene, transcutol)	– –	– –	0-40	20-50	0-50

1.3 SELF - EMULSIFYING DRUG DELIVERY SYSTEMS (SEDDS)

SEDDS are considered one of the promising approaches for tackling formulation problems associated with drugs with poor aqueous solubility. SEDDS is an oral lipid dosage form composed of a mixture of oils, surfactant and solvent of hydrophilic capacity and co solvents/surfactants[11]. These formulations disperse freely when they come to contact with gastric fluids and form an o/w emulsion or micro emulsion utilizing mild agitation conditions provided by gastric motility. The lipophilic drug is delivered in liquid form, in small droplets of oil, leading to the elimination of the dissolution which is rate limited process in the absorption of poorly soluble drugs. The bioavailability of SEDDS is thereby improved and the drug content in plasma profile is reproducible in such systems. Fine oil droplets are expected to be emptied rapidly from the stomach and promote a better distribution of the drug throughout the gastrointestinal tract. This can minimize irritation caused by extended contact between drug substance and the gut wall. When compared to conventional oily solutions, SEDDS provide a large interfacial area which enhances drug absorption by increasing the rate of diffusion from oil to the aqueous media of

the gastrointestinal tract. The dispersibility of the administered lipids depends on the extent of drug absorption from lipid vehicles.

The self - emulsifying delivery systems are broadly classified according to their particle size as:

1. **Self-emulsifying drug delivery system (SEDDS):** They have droplets of emulsion size more than 600 nm**.**

2. **Self -micro emulsifying drug delivery system (SMEDDS):** They have droplets of micron size lying between 100-150 nm.

3. **Self-nano emulsifying drug delivery system (SNEDDS):** They have droplets of nanosize lying between 10-100 nm.

Advantages of the system[12]

i. The drug absorption will be more: SEDDS formulations can enhance the bioavailability by increasing the solubility of the drug and minimizes the gastric irritation. In SEDDS, the lipid phase interacts readily with water, forming a fine particulate o/w emulsion. The emulsion droplets will deliver the drug to the gastrointestinal mucosa in the dissolved state readily accessible for absorption.

ii. The drug can be protected from the gut environment: Many drugs are degraded in the physiological system because of acidic pH in the stomach, enzymatic degradation or hydrolytic degradation. Such drugs, when presented in the form of SEDDS, can be well protected against the degradation processes as a liquid crystalline phase in SEDDS might act as the barrier between the degrading environment and the drug[13].

iii. The sensitive drug compounds are protected: SEDDS and SMEDDS are self-emulsified dosage form by agitation in GI tract with the size of droplet less than 50 nm for SMEDDS and for SEDDS between 100 to 300 nm which are physically stable formulations that are easy to manufacture[14].

iv. The oral dose is reduced therefore, the oral bioavailability is enhanced.

v. The efficiency of drug loading is increased and the side effects can be minimized.

vi. They require minimum energy for formation.

vii. They are thermodynamically stable.

viii. The manufacturing and scale-up of the system are easy.

Disadvantage of the system[15]

i. Deficient of reliable *in vitro* models for assessment of the formulations since traditional methods of dissolution cannot be used, because the SEDDS formulations potentially are dependent on digestion prior to the release of the drug.

ii. Lack of suitable *in vivo* animal model for testing lipid based formulations

1.4 CONTENTS OF SEDDS

List of Typical Oil, Fatty and Lipid Compounds used in formulation of SEDDS and SMEDDS

Oils

In the design of formulation of SEDDS, triglyceride oils of long and medium chain with different degrees of saturation are used. Further edible oils/natural oils are preferred in lipid excipient for the development of SEDDS. The oil components used in the SEDDS formulation solubilizing relevant amount of poorly water soluble drug, facilitate self-emulsification and increase the absorption of the drug from the gastro intestinal tract, depending on the molecular nature of the triglyceride (TG)[15]. Some of the fatty acids, salts, and esters are aluminium monostearate, calcium stearate, ethyl oleate, isopropyl myristate, esters of isopropyl alcohol and palmitic acid, magnesium stearate, oleic acid, PEG 40 stearate, propionic acid, sodium stearate and zinc stearate.

Examples of oils and oil esters

Almond oil, castor oil, cod liver oil, corn oil, cotton seed oil, diacetylated monoglyceride, glycerides of behenic acid, glycerol ester of stearic acid,

hydrogenated castor oil, hydrogenated mineral oil, mineral oil, mono and diglycerides, oil soluble vitamins, olive oil, orange flower oil, peanut oil, peppermint oil, oil soluble vitamins persic oil, polyoxyl 40 hydrogenated castor oil, rose oil, safflower oil, sesame oil, soyabean oil, squalene, vitamin E, vitamin E succinate.

Fatty Alcohols

They comprise of benzyl alcohol, butyl alcohol, cetostearyl alcohol, lanolin alcohol octyldodecanol, oleyl alcohol and stearyl alcohol.

Phospholipids: Lecithin and derivatives

Waxes: Carnauba wax, emulsifying wax, hard fat, petrolatum, microcrystalline wax, yellow ointment, white wax and yellow wax.

Triglycerides Vegetable Oils

Generally, vegetable oils containing Long chain triglycerides (LCT) and medium chain triglyceride (MCT) with various degree of saturation are utilzed in the design of SEDDS. MCT's are largely replaced by novel semi-synthetic derivatives, which can be defined as amphiphilic compounds exhibiting surfactant properties. MCT's are also known as fractionated coconut oil which is highly stable and resistant to oxidation mainly to the saturation of medium chain fatty acids such as caproic (C_6), capric (C_{10}), and lauric acid (C_{12}). MCT is rapidly absorbed from the small intestine, hydrolyzed after ingestion and is transported through portal circulation and MCT is therefore considered to facilitate the uptake of lipophilic drugs.

Vegetable oils derivatives

The vegetable oil derivatives popularly used are the vegetable oil (hydrogenated), glycerides (mixed), polyoxyl glycerides, ethoxylated glycerides and esters of fatty acids with various alcohols. The hydrogenations of the unsaturated bonds present in the oil result in a formation of hydrogenated vegetable oils. The chemical stability of the oil is increased by hydrogenation. Examples of such hydrogenated oils are cottonseed oil, palm oil, castor oil and soybean oil.

Mixed partial glycerides

They are the mixture of mono, di, and tri-glycerides formed by partial hydrolysis of triglycerides present in the vegetable oil. The physical state, melt characteristics and the HLB of the partial glycerides depend on the nature of the fatty acid present and the degree of esterification. The saturated long chain fatty acids are used for sustained release purposes[16] and glycerides with a medium chain or unsaturated fatty acids are used for improving bioavailability. Examples of glycerides with the medium chain fatty acids are glyceryl mono capryl caprate (Capmul MCM) and with a long chain, fatty acids are glyceryl monooleate (Peceol) and glyceryl mono linoleate (Maisine 35-1).

Ethoxylated glycerides

They are formed from ethoxylation (etherification) of ricinoleic acid (present in glyceride) of castor oil which makes the oil hydrophilic. Examples of such glycerides are ethoxylated castor oil (Cremophor EL) and ethoxylated hydrogenated castor oil (Cremophor RH40 and Cremophor RH 60). Cremophor's are widely used as surfactants in the formulation of SEDDS because of its amphiphilic nature. Moreover, they can dissolve large quantities of drugs, have good self-emulsification property and their degradation products are similar to those obtained from intestinal digestion[17].

Polyglycolyzed glycerides (PGG)

They differ in HLB value as they possess different diverging fatty acid and polyethylene glycol (PEG) chain. PEG with vegetable oils has been used to solubilizing poorly water soluble drugs for improving their bioavailability.

Polyalcohol esters of fatty acids

These are newer oil derivatives that possess surfactant properties because of its amphiphilic nature and are effective in replacing conventionally used oils. Their composition is based on nature of alcohol used. They can be polyglycerol (Plurol Oleique CC 497) and propylene glycol (Capryol), and polyoxyethylene glycol (Mirj). Recently the emulsification and solubilization properties of polyglycolyzed glyceride based oils, Labrafil M1944 CS (oleoyl macro glycerides), Labrafil M 2125 CS

(linoleoyl macrogol glycerides), and Labrasol (caprylo caproyl macro glycerides) in self - emulsifying formulations have been explored using Tween 80 and Tween 20 as surfactants[18].

Surfactants

The nonionic surfactants with a high lipophilic and hydrophilic balance (HLB) are widely used in designing self - emulsifying drug delivery system. The widely used emulsifiers are ethoxylated polyglycolysed glycerides and polyoxyethylene 20 oleate (Tween 80). Nonionic surfactants are less toxic than ionic surfactants that result in changes in permeability of intestinal lumen which are reversible. The formulation of a stable SEDDS involves the addition of surfactant ranging between 30-60%. For an effective absorption, the precipitation of the drug compound within the lumen should be prevented for a long period of time the site of absorption. When a mixture of a surfactant containing C_8-C_{10} polyglycolyzed glycerides (Labrafac) are used in increase concentration the mean droplet size is smaller in SEDDS.

Examples of Surfactants and co-surfactants

Capmul MCM C8, Capryol 90, Carbitol, Cremophor EL, Cremophor RH 40, Crodamol EO, Crodamol GTCC, Emulsifier OP, Ethoxylated polyglycolysed glycerides, Gelucire® 44/14, Glycerine, Glycerol, Hexanol, Labrafac PG, Labrafil 2609 WL, Lauroglycol FCC, Maisine 35-1 (glyceryl mono linoleate), Octanol, Oleic acid, PEG 200, PEG 400, Pentanol, Plurol Oleique, Plurol Oleique CC 497, Poloxamer 188, Poloxamer 407, Polysorbate 80, Propylene glycol, Solutol HS 15, Span 20, Span 80, Transcutol, Transcutol HP, etc.

Cosolvents used in SEDDS

The solvents like ethanol, propylene glycol and polyethylene glycol (PEG) phase[19] are used as co solvents in SEDDS formulation which aid the dissolution of hydrophilic surfactants of the drug in lipid and used as co-surfactants in SMEDDS. The limitation of these solvents is evaporation through shells of soft or hard gelatin capsules in conventional SEDDS precipitate the drug. Hence alcohol-free formulations are formulated but their lipophilic drug dissolution ability may be restricted.

Additional excipients in SEDDS

To stabilize the oily phase of SEDDS, some excipients such as pH adjusters, flavoring agents and antioxidants like butylated hydroxyl toluene, ascorbyl palmitate, propyl gallate are added to the formulation. The precipitation of drug in the gastrointestinal tract can be prevented by adding a polymeric precipitation inhibitor which results in the supersaturated state of the drug after the micro emulsion is formed. Hydroxy propyl methyl cellulose is incorporated in the formulation act as a precipitation inhibitor for the development of supersaturated SEDDS.

Phase behavior studies in SEDDS

The construction of phase diagram is used to study the phase behavioral studies of oil, water and surfactant components used in the formulation of SEDDS in which the apex of the triangular phase diagram is represented by 100% of that specific component. The pseudo ternary phase diagram consists of two components of drug/oil, surfactant/co surfactant and water/drug representing the apex of the triangle. The delineation of the phase boundary is achieved by increasing the time consuming for the system in equilibrium. The process can be rapid by applying heat and sonicating the system with amphiphilic surfactants. A series of pseudo binary compositions was prepared and titrated with the third component; finally, the mixture was evaluated after each addition. The aid of phase diagram is to capture the relationship between the phase behavior of a mixture and its composition. By using the function of temperature and pressure the compositional variables are studied. The amphiphilic co-surfactants added to the system get separated between the water and oil interface and reduces the interfacial tension.

Biopharmaceutical aspects[20] of SEDDS

The various mechanisms responsible for enhanced drug absorption in SEDDS are as follows:

i. The lipid in the GIT causes the delay in gastric emptying. This process enables the better dissolution of the drug and improves drug absorption.

ii. Lipids in GI tract enhances the production of bile salts (BS), endogenous biliary lipid including phospholipids (PL) and cholesterol (CH) which is responsible for the development of BS/PL/CH intestinal mixed micelles leads to enhancement of solubilization capacity in GIT. Further enhancement of solubilization capacity may be due to intercalation of administered (exogenous) lipids into already developed BS structures either directly (if sufficiently polar) or secondary to digestion causes swelling of micellar structures[21]

iii. The lipids may enhance the lymphatic transport and increase the bioavailability directly or indirectly through the reduction in first pass metabolism. The intestinal lymphatic transport is stimulated.

iv. The physical and biochemical barrier function of the gastrointestinal tract is changed.

v. The oils exhibit a peculiar effect on absorption to the human body: The SEDDS formulation form o/w emulsion on gentle agitation due to gastrointestinal motility. The self - emulsifying formulations can reduce the batch to batch variation of plasma level-time profile. The mechanism of absorption of poorly aqueous soluble drugs on oils includes variation of gastrointestinal motility with enhanced bile flow, mucosal permeability, mesenteric lymph flow and lymphatic absorption which in turn enhances gastrointestinal absorption depending on the molecular nature of the triglyceride. The peculiar effect of oil absorption depends on the lipophilic drug when incorporated into the mixed micelles which are formed on digestion of the oil are thereby incorporated into the fatty acid uptake mechanism and such drugs are taken up by the lymphatic system, while hydrophilic molecules are absorbed into the hepatic portal vein[22].

The schematic diagram for drug absorption pathway from lipid formulations is illustrated in Fig. 1.

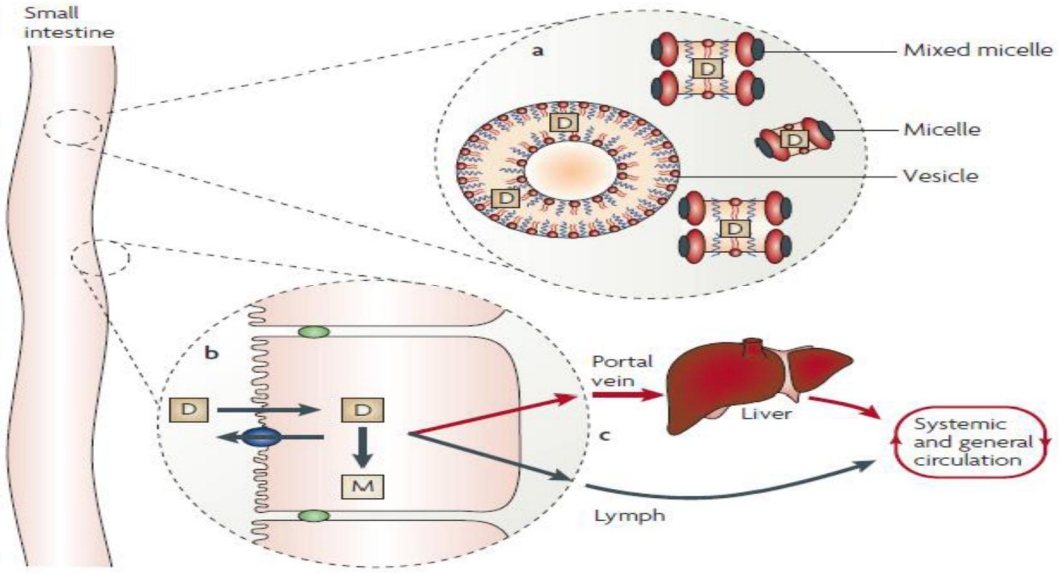


Fig. 1 Pathways for drug absorption from lipid based formulations: a) Change in composition and character in the environment of colloids (Mixed micelles, micelles) by enhancing drug solubilization. b) Lipids interacting with enterocyte based transport and metabolic processes. c) By altering the pathway of transport of the drug, therefore, minimizing the effect of hepatic first pass metabolism. The sustainability and release of the drug (D) are influenced by altered lipid excipients.

Micellar solubilization properties of SEDDS

Table 3 describes the classification and characteristics of four lipid based formulations based on their varying composition. A crude o/w emulsion of droplet size of 1µm is formed in its aqueous dispersion if the solubilization system is stable. The type I SEDDS consists of surfactants with HLB value less than 12 of particle size ranging from 100-250 nm with the solvent capacity very closer to that of lipid solution. The type II SEDDS are prepared with amphiphiles of HLB value more than 12, for dissolving the hydrophobic drugs, sometimes co-surfactants are added to restore the ability of micellar solubilization within SEDDS.

Table 3 : Classification System for Lipid Formulations with Characteristics[10]

Parameters	Lipid Solution	SEDDS type 1	SEDDS type 2	SMEDDS
Oil (%)	100	40-80	40-80	<20
Surfactants (%)	0	20-60 (HLB<12)	20-60 (HLB>12)	20-50 (HLB>11)
Cosolvents (%)	0	0	0-40	50-100
Droplet size (nm)	>1000	100-250	100-250	<100
Ease of self - emulsification	Very Low	Low to medium	Medium to high	High
Solvent capacity	Low	Low to medium	Medium to high	High
Loss of solvent capacity upon dilution	None	None to slight	Slight to moderate	Moderate to high
Digestibility significance on absorption	Crucial	Not crucial, not likely to occur	Not crucial but may be inhibited	Not required not likely to occur.

Mechanism of self-emulsification in SEDDS

In conventional emulsions, the interface between oil and water are stabilized by the presence of an emulsifying agent, which forms a protective film around the dispersed phase of the globule and the excess of free energy depend on the globule size and interfacial tension between two phases. There will be a decrease in surface free energy and interfacial tension if the emulsion is not stabilized by surfactants. But in the case of SEDDS, spontaneous emulsification results and the surface free energy is much minimized. The self-emulsification occurs due to the entry of water in the liquid crystalline phase and it is responsible for droplet formation. The resulting nanoemulsion formed against coalescence[23] is formed due to the stability of the crystalline liquid phase.

1.5 DEVELOPMENT OF SEDDS FORMULATIONS

Solubility Studies

The solubility of the drug in different oils, surfactants and co surfactants are determined by adding the excess amount (150 mg/2ml) of the drug sample to each glass vials containing oil, surfactant, and solubilizer selected for the formulation of SEDDS. The proper mixing of the drug with the vehicles can be performed by sealing the mixture of drug samples in glass vials and kept in a vortex mixer for 10 min. The equilibrium was attained by shaking the samples at 37 ± 1°C for 72 hours in an isothermal shaker. The drug samples are then centrifuged at 5000 rpm for 15 min and a solution is subsequently filtered through 0.22µm of a membrane filter. The supernatant layer of the drug samples are diluted with organic solvent (methanol) and the samples are quantified by a suitable analytical method. Methanol is commonly used because it is relatively inexpensive, lots of compounds can be dissolved and relatively free of regulation compared to ethanol, cost effectiveness and working efficiency.

Pseudo ternary phase diagram

The feasibility of the micro emulsion formed is the first step in formulation development of SEDDS. The delineation of the boundaries in the pseudo ternary phase diagram is accessed from the components shortlisted by the result of solubility studies by water titration method[24]. The different ratios of surfactant to co-surfactant are varied and mixed and (1:1, 2:1 and 3:1) on the weight basis. The different ratios of oil: Smix (surfactant to co-surfactant mixture) are mixed in the ratios varying from 1:9 to 9:1 are prepared and the resultant mixture was added drop wise with water in which the end point is determined by sign of turbidity and the titration is continued until the clear emulsion is formed. The preparation of SEDDS is based on the micro emulsion region clarity which is selected based upon the fact that the solution remains clear on several dilutions.

Preparation of L- SEDDS

A series of SEDDS formulations are generally prepared using different Smix combinations and the oil. In all the formulations, the level of active moiety is kept

constant according to the required dose. The accurately weighed drug is placed in a glass vial and oil, surfactant and co surfactant are added according to their ratios. Then the components are mixed by gentle stirring and vortex mixing and are heated at 40-50ºC on a magnetic stirrer if required until the drug is perfectly dissolved. The mixture is stored at room temperature until further use. The mixture was observed for any signs of turbidity or phase separation for a period of 48 hours. The novel preparation is employed by altering temperature and pressure in SEDDS formulations[25].

Preparation of S-SEDDS

S-SEDDS was prepared by mixing liquid SEDDS containing drug with Aerosil 200 in 1:1 proportion. In brief liquid, SEDDS was added drop wise over Aerosil 200 contained in a broad porcelain dish. After each addition, the mixture was homogenized using a glass rod to ensure uniform distribution of formulation. The resultant damp mass was passed through sieve no. 120 and dried at ambient temperature and stored until further use[26].

Techniques for conversion of liquid SEDDS into solid SEDDS

The liquid formulations of SEDDS are filled in soft or hard capsules of gelatin. Spray drying, adsorption to solid carriers by cross–linked sodium polymethylmethacrylate, encapsulation of liquid and semisolid SEDDS, extrusion spheronization and melt granulation are some of the techniques used for converting liquid SEDDS to solid SEDDS[27].

1.6 EVALUATION TESTS FOR SEDDS

Evaluation tests for L-SEDDS formulations

Self-emulsification and Dispersibility test

The efficiency of self-emulsification of oral micro/nanoemulsion is assessed using a standard USP dissolution apparatus II [28, 29]. One ml of each formulation is added to 500 ml of water at 37 ± 0.5°C. A standard stainless steel dissolution paddle rotating at 50 rpm is used to provide gentle agitation. The *in vitro* performance of the formulations is visually assessed from such dispersion using a suitable grading system

which has been reported to be based on the formation of a micro emulsion (o/w or w/o), micro emulsion gel, emulsion or emulgel is given below:

Grade A: Rapidly forming (within 1 min) nanoemulsion, having a clear or bluish appearance. (Micro emulsion)

Grade B: Rapidly forming, slightly less clear emulsion, having a bluish white appearance. (Micro emulsion gel)

Grade C: Fine milky emulsion that formed within 2 min. (Emulsion)

Grade D: A dull, grayish white emulsion having a slightly oily appearance that is slow to emulsify (longer than 2 min). (Emulgel)

Grade E: Formulation, exhibiting either poor or minimal emulsification with large oil globules present on the surface.

Grade A and Grade B formulation will remain as nanoemulsion when dispersed in GIT, while formulation falling in Grade C could be recommended for SEDDS formulation. The primary means of self-emulsification is the visual evaluation. The effectiveness of self-emulsification of SEDDS can be optimized by its rate of emulsification, droplet size distribution and turbidity measurements.

Turbidity measurement

Turbidity measurement determines an efficiency of self-emulsification by determining the reproducible time after which the dispersion reaches the equilibrium[30]. The dissolution apparatus is connected to the turbidity meter. The apparatus is placed under continuous stirring (50 rpm) on a magnetic plate at ambient temperature and a fixed quantity of self-emulsifying system is added to defined quantity of suitable medium (0.1N hydrochloric acid). After every 15 secs, the optical clarity of the formulations is analyzed to determine the clarity of nano or micro emulsion formed and emulsification time using a turbidimeter. The rate of change of turbidity (rate of emulsification) cannot be monitored because the time required for complete emulsification is too short.

Droplet size measurement

The droplet size and polydispersity index (PDI) of L-SEDDS, S-SEDDS by diluting it 100 times with double distilled water and were determined using a Malvern Zeta Sizer Nano ZS 90 (Malvern Instruments, Malvern, UK). The PDI indicates the width of a particle distribution (e.g. 0.0 for a narrow, 0.5 for a very broad distribution). Prior to the measurement, the samples were diluted with double distilled filtered water to a suitable scattering intensity. All measurements were performed in triplicate. The results are expressed as mean size ± SD.

Zeta Potential measurement (ZP)

The Zeta potential is a measure of the electric charge at the surface of the particles indicating the physical stability of colloidal systems[31]. ZP was measured using a Zeta Sizer Nano ZS 90 (Malvern Instruments, Malvern, UK). Each sample was suitably diluted with double distilled filtered water and placed in a disposable zeta cell. The ZP values were assessed by determining the particle electrophoretic mobility. The electrophoretic mobility was converted to the ZP via the Helmholtz Smoluchowski equation. All measurements were performed in triplicate. The results are expressed as mean ± SD.

Conductance

The type of micro emulsion (o/w or w/o) can be determined by the measure of conductance. It was measured by a conductivity meter. The electro conductivity of the resultant system was measured by an electroconductometer. For the conductivity measurements, the tested micro emulsions were prepared with a 0.01N aqueous solution of sodium chloride instead of distilled water.

Viscosity Determination

SEDDS system is generally administered in soft gelatin or hard gelatin capsules. So it can be easily pourable into capsules and such system should not be too thick to create a problem. The rheological properties of the micro emulsion are evaluated by Brookfield viscometer. The w/o or o/w is confirmed by viscosity

determination. If a system has low viscosity then it is o/w type of the system and if a high viscosity then it is w/o type of the system.

Refractive Index and Percent Transmittance

The refractive index of the system was measured by an Abbe refractometer by placing one drop of solution on the slide and it compared with water (1.333). The percent transmittance of the system was measured by diluting 1 ml of SEDDS formulation to 100 times with double distilled water and analyzed at 650 nm using UV spectrophotometer keeping distilled water as a blank. If the refractive index of the system is similar to the refractive index of water (1.333) and formulation has percent transmittance > 99 percent, then formulation is in transparent nature.

Cloud point measurement

The cloud point is the temperature above which the formulation clarity turns into cloudiness. The cloud point is an essential factor in the SMEDDS consisting of nonionic surfactants and it is responsible for the successful formation of a stable micro emulsion. When the temperature is higher than the cloud point, an irreversible phase separation will occur and the cloudiness of the preparation would have a bad effect on drug absorption, because of the dehydration of the polyethylene oxide moiety[32]. Hence, the cloud point for SMEDDS should be above 37°C, which will avoid phase separation occurring in the gastrointestinal tract.

pH

The SEDDS were diluted with double distilled water and its pH was measured using pH meter.

Dissolution study

In vitro dissolution studies of L-SMEDDS and S-SMEDDS were performed as per the procedure followed in the "Dissolution Methods for Drug Products" guide of Food and Drug Administration[33]. The dissolution tests were performed in triplicate. A graph of percent cumulative drug release against time was plotted. The dissolution profiles were evaluated on the basis of dissolution efficiency (DE) and percentage of drug dissolved (DP) at 5 min and 60 min, time needed to dissolve 50% of drug (t_{50}%),

area under the curve (AUC) and mean dissolution time (MDT). The DE of a pharmaceutical form 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.[33, 34]. It can be calculated by the following equation

$$DE = \frac{\int_0^t y \times dt \times 100\%}{y100 \times t}$$

where y is the percentage of dissolved product.

The Mean dissolution time (MDT), which is a measure of the rate of the dissolution process was calculated using equation [35].

$$MDT = \frac{\sum_{i=1}^{i=n} tmid \times \Delta M}{\sum_{i=1}^{i=n} \Delta M}$$

Assay

The drug from pre-weighed SEDDS is extracted by dissolving in a suitable solvent. The drug content in the solvent extract was analyzed by suitable analytical method against the standard solvent solution of drug using the UV-Visible spectrophotometer.

Stability studies

Robustness to dilution

The robustness of SEDDS to dilution was studied as per Date *et al.,* the method with slight modification[36]. SEDDS were diluted to 10, 100 and 1000 times with various media of water, 0.1N hydrochloric acid and pH 7.4 phosphate buffers. The diluted micro emulsions were stored for 12 hours and observed for any signs of phase separation or drug precipitation.

Thermodynamic stability studies

The objective of thermodynamic stability is to evaluate the phase separation and effect of temperature variation on SEDDS formulations. The SEDDS were diluted to 100 times with double distilled water and centrifuged at 10,000 rpm for 20 minutes and formulations were observed visually for phase separation. To evaluate the effect of temperature, the formulations were subjected to freeze–thaw cycles (- 20°C for 2 days followed by +25°C for 2 days) [37]. At the end of the cycle, the formulations were diluted and centrifuged as described above and phase separation and the change in droplet size were determined.

Physical and chemical stability

Physical and chemical stability was evaluated by storing the L- and S-SMEDDS samples at 4-8°C (refrigerator) and 25°C for up to 6 months. Samples were withdrawn at predetermined time intervals after 1, 2, 3 and 6 months. The clarity, phase separation, particle size and ZP after dilution with double distilled water at 1:100 were measured for physical stability of SEDDS. In addition, chemical stability of SEDDS was determined by UV spectrometric method/ HPLC assay and dissolution method.

***In vitro* permeability studies**

The diffusion studies were executed in a Franz diffusion cell using cellophane membrane as a barrier[38]. The *ex-vivo* studies were carried out by replacing cellophane membrane with goat intestine sac/stomach. The stomach/intestinal part are rinsed with cold ringer's solution to remove the mucous and lumen contents. The SEDDS sample is diluted with 1 ml of distilled water (outside mixing for 1 minute by vortex mixer). The resultant sample (2mg/ml) is injected into the lumen of the stomach/duodenum using a syringe and both the sides of the intestine are tightly closed. Then the tissue is placed in a chamber of organ bath with continuous aeration and a constant temperature of 37 ^{0}C. The receiver compartment is filled with 30 ml of phosphate buffer solution pH 7.4). The aliquots arre collected at periodical intervals of time up to 6 hours. The absorbance is measured using a UV-VIS spectrophotometer at the specific wavelength, keeping the respective blank. The percent diffusion of the drug is

calculated against time and plotted on a graph. The results are compared with that of pure drug.

Everted sac technique

An albino rat (male) was fasted for 24 hours and anesthetized with chloroform. The ileum was removed and transferred into the beaker containing ice cold phosphate buffer pH 7.4 and was aerated. The contents were removed by flushing with buffer using 2 ml glass syringe using a glass rod designed specifically. The intestine was everted, tied at one end. The fresh buffer was filled in the sac and tied at another end. The sac was suspended in the receptor fluid (saline phosphate buffer of pH 7.4) of 200 ml which contains drug solution.

This method can be used to determine kinetic parameters with high reliability and reproducibility. The oxygenated tissue culture media and specific preparation techniques ensure tissue viability for up to 2 hours. The technique can be used to study drug transport across the intestine and into the epithelial cells, provided that sensitive detection methods are employed [39].

Determination of flux

The cumulative amount of drug permeated was plotted against time and the angular coefficient of that curve provides the flux (J) value. The following equation was used to calculate the permeability coefficient (K_p) [40]

$$K_p = J/C$$

where C is the initial concentrations of drug in the SMEDDS formulation.

***In vivo* Pharmacokinetic study**

The relative bioavailability studies performed in SEDDS were compared with marketed formulations and standard drug. The drug plasma concentration values were determined from the calibration curve. The trapezoidal method was employed to calculate the area under the curve (AUC) of plasma concentration as a function of time (t). The Mean residence time (MRT) was calculated as area under the first moment curve (AUMC) divided by AUC. AUMC was determined from the plot of

plasma concentration multiplied by time (C x t) versus time. All the pharmacokinetic parameters were calculated using MS-Excel software. The maximum plasma concentration (Cmax) and the time to reach maximum plasma concentration (t_{max}) were determined by the plasma concentration curve using MS-Excel software. The elimination rate constant (Kel) was calculated by the regression analysis from the slope of the line and the half-life ($t_{1/2}$) of the drug was obtained by 0.693/Kel. Other parameters, clearance (Cl) and volume of distribution at steady state (Vss) were calculated using the following equations: Cl = Dose/AUC and Vss = Dose X AUMC/ (AUC) [41].

Evaluation of S-SEDDS

Flow properties of S-SEDDS

Angle of repose

The angle of repose of S-SEDDS was determined by funnel method. Accurately weighed sample was taken in a funnel. The height of the funnel was adjusted in such a way that the tip of the funnel just touches the apex of the heap of S-SEDDS powder. The powder was allowed to flow through funnel freely onto the surface. The diameter of the powder cone was measured and angle of repose calculated using the following equation[42].

$$\theta = \tan^{-1} h/r$$

where h and r are the height and radius of the heap of powder

Bulk density

Both loose bulk density (LBD) and tapped bulk density (TBD) were determined for S-SEDDS. A quantity of 2 g of S-SEDDS was introduced into a 10 ml measuring cylinder. The initial volume was observed, the cylinder was allowed to fall under its own weight onto a hard surface from a height of 2.5 cm at 2-second intervals. The tapping was continued until no further change in volume was noted. LBD and TBD were calculated using the following formulae

LBD =Weight of powder/Volume of packing

TBD =Weight of powder/Tapped volume of packing.

Compressibility Index

The compressibility of the granules was determined by Carr's Compressibility Index.

Carr's compressibility index (%) = TBD-LBD/TBD× 100.

Hausner ratio

A similar index like compressibility index has been defined by Hausner. Hausner ratio can be calculated by a formula:

Hausner ratio =TBD/LBD

Reconstitution properties of S-SEDDS

Dilution study by visual observation

The dilution study was done to study the effect of dilution on S-SEDDS, because dilution may better mimic the condition of the stomach after oral administration. In this method, S-SMEDDS (100 mg) was introduced into 100 ml of double distilled water in a glass beaker that was maintained at 37ºC and the contents were mixed gently using a magnetic stirrer. The tendency to emulsify spontaneously and progress of emulsion droplets were observed with respect to time. The emulsification ability of S-SMEDDS was judged qualitatively "good" when clear micro emulsion formed and "bad" when there was turbid or milky white emulsion formed after stopping of stirring[43].

Hausner ratio

Pellets (100g) are placed in a 10 ml volumetric cylinder and their volume is determined. The bulk density is calculated as g/cm^3.The cylinder was then tapped 1250 times and the volume is determined again afterward to calculate the tapped density.

Hausners ratio = Tapped density/Bulk Density

Water content

The residual water content present in the pellets after drying was determined by thermo gravimetric or IR-LOD apparatus (Infra Red- Loss on drying) connected to a sample analyzer. The moisture content was determined using IR-LOD apparatus. A specified amount (3 g) of pellets was kept so as to cover the full surface of the pan. The equipment was operated at 105^{o} C for 15 min. After 15 min, the percentage moisture content was recorded from the digital recorder.

Friability

About 5 gm accurately weighed pellets were taken from the modal class fraction of the pellets and placed in a Roche friabilator and tumbled for 200 revolutions at 25 rpm. Twelve steel balls (diameter 6.3 mm, weighing 1.028 gm each) were used as attrition agents. After friability testing, the pellets were sieved through a series of sieves. The weight loss (% F) after friability testing was calculated by formula given below

% Friability = Initial weight –Final Weight/Final weight×100

where the initial weight of the pellets before friability testing and the final weight of pellets retained above the sieve with 0.355 mm aperture size after friability testing were determined.

Disintegration time

The disintegration time of pellets in size fraction mode value was studied in deionised water at 37^{0}C using a disintegration test apparatus. Six pellets from each formulation were evaluated. The end point was taken as the time for the disintegration of the pellets. The mesh size used is 35 (500 μm) in place of 10 (2000 μm) mesh.

DSC analysis

The physical state of the drug in S-SEDDS/SMEDDS was characterized by Differential Scanning Calorimetry). The thermograms of standard drug powder, aerosil 200, their physical mixture (PM) and S-SMEDDS were recorded in order to characterize the physical state of a drug. A heating rate of 10°C/min was employed in

the range of 25-300°C with nitrogen atmosphere supplied at 40 ml/min. Each sample was taken (~4-8 mg) in an aluminum pan, crimped and sealed. An empty aluminum pan was used as a reference.

XRD analysis

XRD diffractograms of standard drug powder, aerosil 200, their physical mixture and S-SMEDDS were obtained using Bruker AXS D8 Advance X-ray diffractometer. Scans were performed between $5º < 2\theta < 80º$.

Scanning electron microscopy

The pellets morphology is evaluated by scanning electron microscopy (SEM). Samples are examined using a scanning electron microscope at 10 kV accelerating voltage using the secondary electron technique.

Stability studies

The optimized self-emulsifying pellet or tablet dosage forms containing the lipid formulation are subjected to accelerated stability studies. Two ounce amber colored glass containers, each containing 5 gm pellets are stored at 4^0C in temperature controlled ovens at 25^0 and 30^0C; light stability chamber at 250C UV irradiation (350 nm) using a 60W black light; and in humidity chambers at 25^0C/60% RH, 30^0C /60% RH, and 40^0C/75% RH. These conditions are selected to facilitate comparison of stability data without strictly adhering to the ICH guidelines which recommend 30^0C/65% RH as the intermediate storage condition. The saturated salt solutions of sodium bromide (for 60% RH) and sodium chloride (for 75% RH) are used to maintain the humidity conditions. An equivalent amount of pellets is removed at each time point (15 days, 1 month, 1.5 months and 4 months) and evaluated for their hardness and dissolution profiles.

1.7 DESIGN OF EXPERIMENTS (DOE) FOR SEDDS

The primary goal of the pharmaceutical investigator is to develop an optimally performing product or process through various techniques such as experimental design, modeling, and optimization strategies with the efficient determination of a set of conditions. There are numerous literature references for solid dosage form

development, relatively little has been published concerning solution or disperse systems[44] using these techniques which are useful for determining rational limits for critical formulation or processing variables, outside of which unacceptable product would be produced. The "design" of an experiment is simply defined as the plan that governs the performance of the experiment. The use of properly designed reduce the procedural errors in data collected. The observations resigned from a designed experiment are examined using analysis of variance (ANOVA) techniques in which the variance associated with a particular independent variable or interaction between independent variables is compared with a variance associated with the random error occurs in the experiment. If there is a difference between the treatment and error variances, then the treatment being tested is considered to have a significant effect on the measured response. The comparisons between variances are used in F test or F distribution test. Orthogonal designs are two perpendicular lines, neither having an effect on the direction of the other. For experimental design, orthogonality is defined as follows

$$\sum_{u=1}^{N} x_{iu}\, x_{ju} = 0 \ (i \neq j)$$ where independent variable levels are coded such that

$$\sum_{u=1}^{N} x_{iu = 0 \text{ for } = 1,2\ldots k.} \quad \text{and} \quad \sum_{u=1}^{N} x^2 iu = N$$

where N is the number of experimental trials and xiu and xju represents the u^{th} level of variables i and j for k variables. For factorial designs, the variable levels are fixed equally spaced and coded at -1(low level), +1(high level) and 0 (midpoint).

In many fields[45] the problem of experimental design or design of experiments (DOE) is encountered. The terms of factors or design variables (X_i, X_2 ...Xn), set at specified levels (predefined values) and response variables of interest or responses (Y_i, Y_2 ...Ym) constitute an experimental design which is represented by the sequence of experiments to be performed. The relationship between design variables and responses is complicated and requires statistically designed experiments in many cases. In DOE ‘n' is the total number of design variables in which each of design variables combination could be visualized as a point in the n-dimensional design

space. An experimental design is a design of experiments in which the particular arrangement of points is designed in the design space. In pharmaceutical development, DOE can be used to underline the relationship between formulation or process variables and their influence in obtaining the optimized formulation. When the responses are influenced by the design variables and the exact relationship is unknown, it is often helpful to approximate this relationship with an empirical model: $Y_i = f(X_i, X_2 ... Xn)$. Usually, the function f(X) is a first- or second-order polynomial. When this empirical model is second order then it is called a response surface model (RS Model), response surface methodology (RSM), or curve fit.

Response surface methodology

A useful tool for developing, improving and optimizing processes[46] is response surface methodology which is termed as a collection of statistical and mathematical techniques useful for building an empirical model and model exploration. To obtain a regression model and to find a suitable approximation for the time functional relationship between the responses and the set of independent variables response surface methodology was devised. The basic idea is to screen several variables and identify a few variables to perform RSM.

Screening

Screening experiments are used to reduce the set of factors to those that are most influential to the responses being investigated, when the number of factors is large or when experimentation is expensive.

First-order experimentation:

A first order polynomial model is employed as a response surface model, when the objective of an experimental design is to identify the most significant design variables, ignoring possible interactions between these variables. The mathematical relationship in this model is,

$$Y_1 = Ao + A_1X_1 + A_2X_2 + ... A_mK_m.$$

where Ao is the intercept and A_1, A_2 and Am are the coefficients of factors X_1, X_2 and X_m.

Second-order experimentation:

A second-order polynomial model is used if there is curvature in the system and the interactions between design variables play a significant role in the underlying relationship between a particular response and the design variables. The mathematical form of the model is,

$$Y_1=A_o + A_1X_1 + A_{11}X_1' + A_2X_2 + A_{22}X_2^2 + A_{12}X_1X_2 +\ldots.$$

where A_{12} is the interaction coefficient of X_1 and X_2.

The experimental runs should be must be greater than or equal to the number of coefficients in the model, when at least three levels of the factors are required to construct this model. The first- or second-order models are utilized to solve all problems.

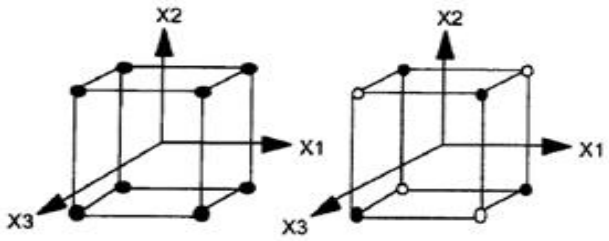
Design of experiments by factorial and fractional factorial designs

The simultaneous main effects and their interactions are estimated by most basic experimental factorial designs. In this design, a polynomial model is used as a response surface model where the coefficients of the polynomial can be estimated using the method of least squares. The RS model, however, should not be used to predict the values of the responses far outside the points that are used to construct this RS model. Therefore, in order to estimate the relationship between the response and the design variables within a multidimensional box in the design space, the box should be defined by the upper and lower limits of the design variables, in this case, the responses are estimated at the vertices of this multidimensional box. The arrangement of points in the design space is called a full factorial experimental design.

It's because design variables are involved in the RS model often are referred to as factors in statistics, the design is called a factorial. The product of the number of levels for each factor is the number of design points dictated by a full factorial design. The most common are 2^n (for evaluating main effects and interactions) and 3^n designs (for evaluating main and quadratic effects and interactions) for n factors at 2 and 3 levels, respectively. Factorial designs are often used in pharmaceutical applications

when design variables or factors are five or less. A factorial design was employed for the evaluation, optimization of the operating parameters and the HLB value of different oil in water emulsions[47]. A full factorial design was also used to evaluate the effect of plasticizer concentration and the volume of coating dispersion on the release of propranolol from pellets coated with Eudragit RS[48]. Lipps and Sakr[49] used a randomized full factorial (3^2) design to investigate the effects of processing conditions on granulation of acetaminophen powder using 5% polyvinyl pyrrolidone as the binder. The 3^2 factorial design was successfully applied to the optimization of process variables for the preparation of ibuprofen coprecipitates[50]. To make the factorial designs more practical, it is possible to estimate the responses, not at all the vertices of the box, but at a subset of the vertices only[51]. The experimental design obtained is called a fractional factorial design and is denoted according to the number of points in the design, $2^{(n-m)}$, where n is the total number of design variables and m is an integer number smaller than n. Fractional factorial designs are used when experiments are costly and many factors are required. The most common fractional factorial designs are $2^{(n-m)}$ designs in which the fraction is $_{1/2}{}^{m}$.

The 2^3 full factorial design illustrated in figure 2 (a) shows the estimation of all main effects (X_1, X_2, X_3), all two factor interactions (X_1X_2, X_1X_3, and X_2X_3) as well as the three factor interaction ($X_1X_2X_3$). The $2^{(3-1)}$ fractional factorial is indicated by the solid dots in figure 2(b), the main effects, however are biased with the two factor interactions. The screening of important factors is identified by using 2^n and $2^{(n-m)}$ designs. The system is assumed to be dominated by main effects and low-order interactions when there are numerous factors. The greatest effects are identified by two level fractional factorial designs which are also used to screen them.



(a) 2^3 Full Factorial (b) 2^{3-1} Fractional Factorial

Fig. 2a 2^3 Full Factorial Fig. 2b 2^{3-1} Fractional Factorial

Statistical software tools for designing SEDDS

Selecting the appropriate design is essential for effective experimentation where the desire is to gain as much information as possible about the response-factor. There are several interactive statistical software packages that support phase of experimental design which include Design Ease (Stat-Ease Inc., Minneapolis, MN), Jass (Joiner Associates, Inc., Madison, WI), X-STAT (Wiley Professional Software, New York, NY) and CADE (International Quality Technology, Ltd Plymouth, MN), ECIP (Expert in a chip Inc., Hockessin, DE) and RS/Discover (BBN Software products Corp., Cambridge, MA) are useful software packages for classic, mixture and optima designs[52].

Commercially available Lipid formulations in market[53]

The currently estimated oral lipid based formulations are 2- 4 % which are commercially available in the market above 20 years.

Sand immune® (Cyclosporine)

Category: Immunosuppressant for organ transplantation.

Molecular weight: 1202.61

Log P: 2.92 (approx)

Dose: 25-700 mg (2-10 mg/kg)

Formulation and ingredients: Soft gel: 25-100 mg cyclosporine in ethanol, corn oil, Labrafil M 2125 CS, gelatin, glycerol. Oral solution: 100 mg/ml in 12.5 % ethanol, olive oil, Labrafil M 1944.

Norvir ® (Ritonavir)

Category: Antiretroviral agent

Molecular weight: 720.9

Log P: 5.28

Dose: 1200 mg (600 mg BID)

Formulation and ingredients: Soft gel: 100 mg Ritonavir, ethanol, oleic acid, polyoxyl 35 castor oil, butylated hydroxytoluene. Oral Solution: 80 mg/ml drug in ethanol (43%w/v), polyoxyl 35 castor oil, propylene glycol, citric acid.

Fortovase ® (Saquinavir)

Category: Antiretroviral agent

Molecular weight: 670.84

Log P: 4.40

Dose: 1200 mg

Formulation and ingredients: Soft gel: 200 mg drug in medium chain mono and diglycerides and povidone.

Aptivus ® (Tipranavir)

Category: Antiretroviral agent

Molecular weight: 602.66

Log P: 7.2

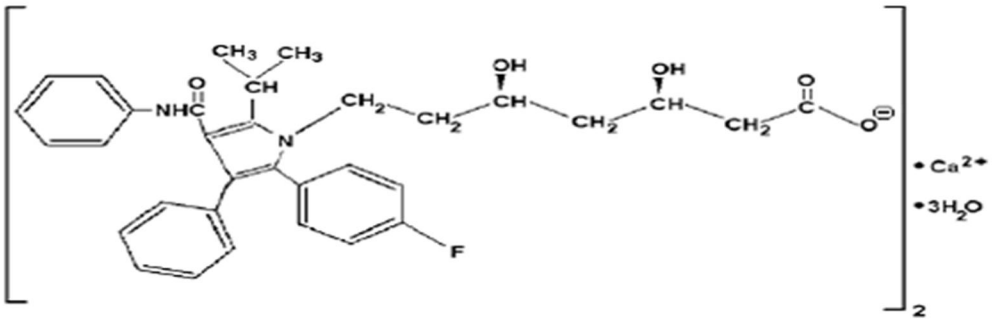
Dose: 1000 mg (with 400 mg Ritonavir)

Formulation and ingredients: Soft gel: 250 mg Tipranavir, polyethylene glycol 400, vitamin E polyethylene glycol succinate, purified water, and propylene glycol

1.8 DRUG PROFILE

Drug I: Atorvastatin Calcium[54-56]

Chemical structure:



Category: Cardiovascular agent and antihyperlipoproteinemic

Chemical name: Atorvastatin calcium is (βR, δR)-2-(p-fluorophenyl) – β,δ-dihydroxy -5-isopropyl-3-phenyl-4-(phenyl carbamoyl) pyrrole-1-heptanoic acid (1:2) trihydrate[2].

Molecular formula: $C_{66}H_{68}CaF_2N_4O_{10}3H_2O$

Molecular weight: 1209.4

BCS Class: Class II (Low solubility high permeability)

Physical state and appearance: White to off-white crystalline powder

Description

Atorvastatin belongs to the drug class known as statins. It is used for lowering cholesterol. Atorvastatin is a competitive inhibitor of hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase which is the rate-determining enzyme in cholesterol biosynthesis through the mevalonate pathway. HMG-CoA reductase catalyzes the conversion of HMG-CoA to mevalonate. Atorvastatin acts primarily in the liver. The decreased hepatic cholesterol levels increase hepatic uptake of cholesterol and reduce plasma cholesterol levels.

Solubility

The drug is freely soluble in methanol, soluble in dimethylsulphoxide (DMSO), dimethyl formamide (DMF) and insoluble in aqueous solution with pH less than 4.0. It is very slightly soluble in distilled water, phosphate buffer (7.4) and acetonitrile. It is slightly soluble in ethanol.

Pka: 4.46

Log p (Octanol/Water): 5.39

Melting point: 159.2-160.7 °C

Mechanism of Action

Atorvastatin lowers plasma cholesterol and lipoprotein levels by inhibiting HMG-CoA reductase and cholesterol synthesis in the liver and by increasing the number of hepatic LDL (low density lipoprotein) receptors on the cell-surface to enhance uptake and catabolism of LDL. Atorvastatin also reduces LDL production and the number of LDL particles.

Absorption

Atorvastatin is rapidly absorbed after oral administration with maximum plasma concentrations achieved in 1 to 2 hours. The absolute bioavailability of atorvastatin (parent drug) is approximately 14% and the systemic availability of HMG-CoA reductase inhibitory activity is approximately 30%. The low systemic bioavailability is due to presystemic clearance by gastrointestinal mucosa and first-pass metabolism in the liver.

Volume of distribution: 381 L.

Protein binding: More than 98% bound to plasma proteins

Metabolism

Atorvastatin is extensively metabolized to ortho- and para-hydroxylated derivatives and various beta-oxidation products. The *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.

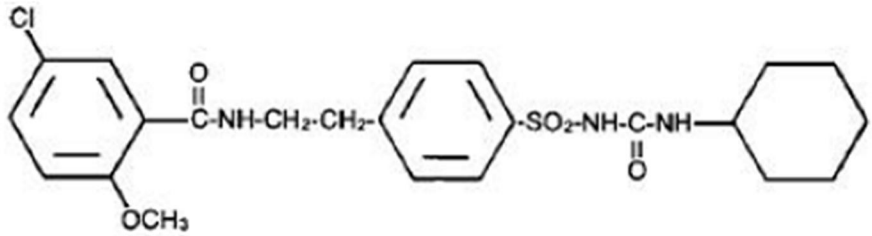
Half-life: The half-life of the drug is 14 hours, but the half-life of HMG-CoA inhibitor activity is 20-30 hours due to longer-lived active metabolites.

Toxicity: The side effects are myalgia, constipation, asthenia, abdominal pain and nausea. The other possible side effects include myotoxicity (myopathy, myositis, and rhabdomyolysis) and hepatotoxicity. The toxicity in Asian patients can be avoided by lowering the doses.

Dose: The starting dose of the drug is 10mg/day and maximum recommended dose is 20mg/day. A dose of 10-80 mg daily is given depending upon the diseased conditions.

Drug II: Glibenclamide [54-58]

Chemical structure



Category: Antidiabetic

Chemical name: Glibenclamide is 1-{4-[2-(5-chloro-2-methoxybenzamido) ethyl} benzenesulphonyl}-3-cyclohexylurea.

Molecular formula: $C_{23}H_{28}ClN_3O_5S$

Molecular weight: 494

BCS Class: Class II (Low solubility high permeability)

Physical state and appearance: A white or almost white crystalline powder

Description: Glibenclamide is an oral anti hyperglycemic agent used for the treatment of noninsulin dependent diabetes mellitus (NIDDM). It belongs to the sulfonylurea class of insulin secretagogues, which act by stimulating β cells of the pancreas to release insulin. Sulfonylureas increase both basal insulin secretion and

meal-stimulated insulin release. Sulfonylureas also increase peripheral glucose utilization, decrease hepatic gluconeogenesis and may increase the number and sensitivity of insulin receptors. Sulfonylureas are associated with weight gain, though less so than insulin.

Solubility: The drug is practically insoluble in water, slightly soluble in alcohol and in methyl alcohol. It is sparingly soluble in dichloromethane.

Pka: 5.3

Log p (Octanol /Water): 4.8

Melting point: 172-174°C

Mechanism of Action: Sulfonylureas such as glibenclamide bind to ATP-sensitive potassium channels on the pancreatic cell surface, reducing potassium conductance and causing depolarization of the membrane. The depolarization stimulates calcium ion influx through voltage sensitive calcium channels, raising intracellular concentrations of calcium ions, which induces the secretion or exocytosis of insulin.

Absorption: The drug absorption is significant within 1 hour and peak plasma levels are reached in 2 to 4 hours and the onset of action occurs within one hour.

Volume of distribution (V_d): Steady state V_d =0.125 L/kg; V_d during elimination phase=0.155 L/kg.

Protein binding: The unchanged drug is approximately 99% bound to serum proteins and 4-trans-hydroxyglyburide is greater than 97% bound to serum proteins.

Metabolism: The metabolism of the drug occurs by the liver (mainly cytochrome P450 3A4). The major metabolite is 4-trans-hydroxy derivative. The retention of major metabolite 4-trans-hydroxyglyburide may prolong the hypoglycemic effect in patients with severe renal impairment.

Half-life: The half-life of the unchanged drug is 1.4-1.8 hours and 10 hours with metabolites included. The duration of effect is 12-24 hours.

Dose: The oral dose of the drug is 2.5 mg-5 mg daily with breakfast. The increments may be of 2.5 – 5 mg daily up to 15 mg daily.

1.9 EXCIPIENT PROFILE

Sunflower oil [59]

Nonproprietary Names:

BP: Refined Sunflower Oil

Ph Eur: Sunflower Oil, Refined

USP-NF: Sunflower Oil

Chemical name: Sunflower oil

Structural Formula: Sunflower oil is classified as the oleic linoleic acid oil. The composition of oil includes linoleic acid (66%), oleic acid (21.3%), palmitic acid (6.4%), arachidic acid (4.0%), stearic acid (1.3%), and behenic acid (0.8%). The USP 32–NF-27 describes sunflower oil as a refined fixed oil obtained from the seeds of Helianthus Annuus Linne (Fam. Asteraceae alt. Compositae). The Ph Eur 6.2 describes sunflower oil as the refined fatty oil obtained from the seeds of *Helianthus Annuus* C. by mechanical expression or by extraction. A suitable antioxidant may be added.

Description: Sunflower oil occurs as a clear, light yellow-colored liquid with a bland, agreeable taste.

Boiling point: 40–60°C

Density: 0.915–0.919 g/cm^3

Hydroxyl value: 14–16

Iodine number: 125–140

Melting point: -18°C

Refractive index: 1.472-1.474

Specific gravity: 0.914-0.924

Saponification value: 180-200

Solubility: Miscible with benzene, chloroform, carbon tetrachloride, diethyl ether and light petroleum; practically insoluble in ethanol (95%) and water.

Functional Category: Diluent, emollient, emulsifying agent, solvent and tablet binder.

Stability and storage conditions: Sunflower oil should be stored in an airtight, well-filled container, protected from light. Stability may be improved by the addition of an antioxidant such as butylated hydroxytoluene.

Coconut Oil [59]

Nonproprietary Names:

BP: Coconut Oil,

JP: Coconut Oil

Ph Eur: Coconut Oil, Refined

USP-NF: Coconut Oil

Chemical name: Coconut oil

Structural Formula: Coconut oil contains triglycerides, the fatty acid constituents of which are mainly lauric and myristic acids with smaller proportions of capric, caproic, caprylic, oleic, palmitic and stearic acids. The PhEur 6.2 and USP32–NF27 state that the fatty acid composition for coconut oil is caproic acid (41.5%), caprylic acid (5.0–11.0%), capric acid (4.0–9.0%), lauric acid (40.0–50.0%), myristic acid (15.0–20.0%), palmitic acid (7.0–12.0%), stearic acid (1.5–5.0%), arachidic acid (40.2%), oleic acid (4.0–10.0%), linoleic acid (1.0–3.0%), linolenic acid (40.2%) and eicosenoic acid (40.2%).

Description: Coconut oil generally occurs as a white to light-yellow mass or colorless or light yellow clear oil, with a slight odor characteristic of coconut and a mild taste.

Boiling point: > 450°C

Density: 0.915–0.919 g/cm^3

Flash point: 216°C

Hydroxyl value: 14–16

Iodine number: 8-9.5

Melting point: 23-26°C

Refractive index: 1.448-1.450

Specific gravity: 0.918-0.923

Saponification value: 180-200

Solubility: Practically insoluble in water; freely soluble in dichloromethane and in light petroleum, soluble in ether, carbon disulfide, and chloroform; soluble at 60°C in 2 parts of ethanol (95%) but less soluble at lower temperatures.

Functional Category: Emollient; ointment base.

Stability and Storage Conditions: Coconut oil remains edible and mild in taste and odor for several years under ordinary storage conditions.

Corn Oil [59]

Nonproprietary Names:

BP: Refined Maize Oil

JP: Corn Oil

PhEur: Maize Oil, Refined

USP-NF: Corn Oil

Chemical name: Corn oil

Structural Formula: Corn oil is composed of fatty acid esters of glycerol known commonly as triglycerides. Typical corn oil produced in the USA contains five major fatty acids such as linoleic 58.9%; oleic 25.8%; palmitic 11.0%; stearic 1.7% and linolenic 1.1%. Corn grown outside the USA yields corn oil with lower linoleic and higher oleic and higher saturated fatty acid levels. Corn oil also contains small quantities of plant sterols. The USP 32 describes corn oil as the refined fixed oil obtained from the embryo of *Zea mays* Linne (Fam. Gramineae).

Description: Clear, light yellow colored, oily liquid with a faint characteristic odor and slightly nutty, sweet taste resembling cooked sweet corn.

Boiling point: > 450°C

Density: 0.915–0.918 g/cm^3

Flash point: 321°C

Hydroxyl value: 8–12

Iodine value: 109-133

Melting point: -18 to -10°C

Refractive index: 1.470-1.474

Specific gravity: 0.914–0.921

Saponification value: 187-193

Solubility: Miscible with benzene, chloroform, dichloromethane, ether, hexane and petroleum ether; practically insoluble in ethanol (95%) and water.

Functional Category: Oleaginous vehicle; solvent.

Stability and Storage Conditions: Corn oil should be stored in an airtight, light-resistant container in a cool, dry place. The exposure to excessive heat should be avoided and prolonged exposure to air leads to thickening and rancidity. Corn oil may be sterilized by dry heat, maintaining it at 150°C for 1 hour[60].

Sesame oil[59]

Nonproprietary Names

BP: Refined Sesame Oil

JP: Sesame oil

PhEur: Sesame oil, Refined

USP-NF: Sesame oil

Chemical name: Sesame oil

Structural Formula: A typical analysis of refined sesame oil indicates the composition of the acids, present as glycerides, to be as arachidic acid 0.8%; linoleic acid 40.4%; oleic acid 45.4%; palmitic acid 9.1% and stearic acid 4.3%. Sesamin, complex cyclic ether, and sesamolin, a glycoside are also present in small amounts. The monographs for Sesame Oil in the USP32 NF27 and Refined Sesame Oil in the PhEur 6.3 specify the acceptable range of eight triglycerides found in sesame oil.

Description: Refined sesame oil is a clear, pale yellow colored liquid with a slight, pleasant odor and a bland taste. It solidifies to a soft mass at about -48°C.

Density: 0.916–0.920 g/cm^3

Flash point: 338°C

Freezing point: -58°C

Refractive index: 1.4650–1.4665

Specific gravity: 0.916–0.921

Iodine value: 103-116.

Saponification value: 188-195.

Solubility: Insoluble in water; practically insoluble in ethanol (95%); miscible with carbon disulfide, chloroform, ether, hexane and light petroleum.

Functional Category: Oleaginous vehicle and solvent.

Stability and Storage Conditions: Sesame oil should be stored in a well filled, airtight, light resistant container, at a temperature not exceeding 40°C. The Ph Eur 6.3 permits the addition of a suitable antioxidant to sesame oil. Sesame oil may be sterilized by aseptic filtration or dry heat. It has been demonstrated that dry heat sterilization of sesame oil at 150°C for 1 hour is sufficient to kill all added bacillus subtilis spores[61].

Mustard oil [62]

Description: The oil is obtained from good quality of mustard cake or from clean and sound seeds of *Brassica Compestris* Linn, *Brassica juncea* Linn, Czern or the mixture of seed all belonging to the family Cruciferae, by a process of solvent extraction or from the mustard seeds by the process of expression.

Types and Grades:

The oil can be classified as following types and grades.

a) Expressed type i) Refined grade ii) Raw grade I iii) Raw grade II

b) Solvent extracted i) Refined, ii) Semi refined iii) Raw grade I iv) Raw grade II.

The refined grade mustard oil of the expressed and solvent extracted types and the raw grades of expressed types are suitable for direct edible consumption.

Refractive Index: 1.4646 – 1.4666

Saponification value: 169-177

Iodine value: 98-110

Acid value:

i) Refined grade mustard oil -0.5

ii) Raw grade I mustard oil -1.5

iii) Raw grade II mustard oil - 60

Storage Conditions: Mustard oil must be stored in well closed container

Rice bran oil [63]

Description: The rice bran oil shall be obtained from the layer around the endosperm of rice obtained from paddy of *Oryza sativa* Linn of family Gramineae and which is removed during the process of rice milling and is generally known as rice bran.

Grades: The oil shall be of the following three grades: a) Refined Grade, b) Raw Grade I, and c) Raw Grade II. The Refined Grade is suitable for edible purposes.

Refractive index: Refined Grade: 1.460 to 1.470

Specific gravity: Refined Grade: 0.910 to 0.920

Saponification value: Refined Grade: 180-195

Iodine value: Refined grade: 90-105.

Acid value: Refined grade: Maximum of 0.5.

Flash point: 250

Storage Conditions: Rice bran oil must be stored in a well closed container.

Olive oil [59]

Nonproprietary Names

BP: Refined Olive Oil

JP: Olive Oil

PhEur: Olive Oil, Refined

USP-NF: Olive Oil

Chemical Name: Olive oil

Structural formula: Olive oil is a mixture of fatty acid glycerides. The analysis of olive oil shows a high proportion of unsaturated fatty acids, and a typical analysis shows that the composition of the fatty acids such as Myristic acid ≤ 40.5%, Palmitic acid of 7.5–20.0%, Palmitoleic acid of 0.3–5.0%, Heptadecanoic acid ≤ 40.3%, Stearic acid - 0.5–5.0%, Oleic acid- 55.0–83.0%, Linoleic acid -3.5–21.0%, Linoleic acid ≤ 40.9%, Arachidic acid ≤ 40.6%, Eicosaenoic acid ≤ 40.4%, Behenic acid ≤ 40.2% and Lignoceric acid ≤ 41.0%. Sterols are also present.

Description: Olive oil is the fixed oil obtained by cold expression or other suitable mechanical means from the ripe drupes of *Olea europaea*. It occurs as a clear, colorless or yellow, transparent oily liquid. It may contain suitable antioxidants. Refined olive oil is obtained by refining crude olive oil such that the glyceride content of the oil is unchanged. A suitable antioxidant may be added.

Flash point: 225°C

Refractive index: 1.4657–1.4893

Smoke point: 160–188°C

Saponification value: 190–195

Iodine value: 79–88

Solubility: Slightly soluble in ethanol (95%), miscible with ether, chloroform, light petroleum (50–70°C) and carbon disulfide.

Specific gravity: 0.910–0.915

Functional Category: Oleaginous vehicle.

Stability and Storage Conditions: When cooled, olive oil becomes cloudy at approximately 10°C and becomes butter like mass at 0°C. Olive oil should be stored in a cool, dry place in a tight, well filled container, protected from light.

Peceol [59]

Source: Gattefosse

Nonproprietary Names

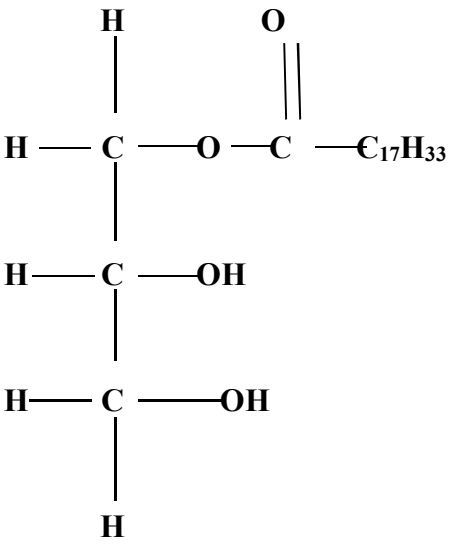
BP: Glycerol Mono oleate

PhEur: Glyceryl Mono oleate

USP-NF: Glyceryl Mono oleate

Chemical Name: 9-Octadecenoic acid (Z), monoester with 1, 2, 3-propanetriol

Structural Formula:



Description: The PhEur 6.3 describes glyceryl monooleate as being a mixture of monoacylglycerols, mainly monooleoylglycerol, together with variable quantities of di and triacylglycerols. They are defined by the nominal content of monoacylglycerols and obtained by partial glycerolysis of vegetable oils mainly containing triacylglycerols of oleic acid or by esterification of glycerol by oleic acid, this fatty acid being of vegetable or animal origin. A suitable antioxidant may be added.

Glyceryl monooleates occur as amber oily liquids, which may be partially solidified at room temperature and have a characteristic odor.

Boiling point: 238–240°C

Density: 0.942 g/cm^3

Flash point: 216°C

HLB value: 1

Melting point: 35°C

Refractive index: 1.4626

Iodine value: 65.0–95.

Saponification value: 150–175

Solubility: Soluble in chloroform, ethanol (95%), ether, mineral oil and vegetable oils; practically insoluble in water. The self - emulsifying grade is dispersible in water.

Functional Category: Bioadhesive material, emollient, emulsifying agent, emulsion stabilizer, gelling agent, mucoadhesive, nonionic surfactant and sustained- release agent.

Stability and Storage Conditions: Glyceryl monooleate should be stored in an airtight container, protected from light in a cool, dry place.

Table 4 : Certificate of analysis for Peceol

Parameters	Specifications	Values
Acid value	< = 3.00 mg KOH/g	0.93mg KOH/g
Saponification value	150-175 mg KOH/g	166 mg KOH/g
Palmitic Acid %	< = 12.0	3.9
Stearic acid (%)	< = 6.0	1.8
Oleic acid (%)	> = 60.0	78.9
Linoleic acid (%)	< = 35.0	12.2
Linolenic acid (%)	< = 2.0	< 2.0
Arachidic acid (%)	< = 2.0	0.2
Eicosenoic acid (%)	< = 2.0	0.7

Labrasol [64-67]

Source: Gattefosse

Non-proprietary name: Caprylo caproyl macrogol glyceride.

Chemical name: Caprylocaproyl macrogol–8 glycerides EP; Caprylocaproyl Polyoxy–8 glycerides NF.

Description: Labrasol is odourless and tasteless white transparent liquid. Labrasol is chemically Caprylocaproyl macrogol glycerides (polyoxyl glycerides) and confirms to EP, USPNF, and FDA IIG specification.

Solubility: Soluble in water, ethyl alcohol, n propyl alcohol and isopropyl alcohol.

HLB value: 12

Functional category: Labrasol improves water solubility of major water insoluble products. Labrasol is an excellent and versatile emulsifying agent. It emulsifies major hydrophobic substances like fatty acids, fatty alcohols, mineral oil. Labrasol improves the bioavailability of liquid and semi-solid lipid systems. It can be incorporated in a number of dosage forms including granules, pellets, tablets and capsules using the conventional equipment. Labrasol in aqueous solutions is stable towards electrolytes e. g. acids and salts, provided that their concentration is not too high. Mercury (II) chloride is an exception and forms a precipitate with the product. Labrasol can be sterilized by heating in an autoclave for 1 hour at 170°C. Acute and chronic toxicity test in animals has shown Labrasol is essentially nontoxic and non- irritant material. Labrasol with the concentration of 0.1 and 1 % was shown to increase the permeability of mannitol by 4.6-fold and 33.8-fold, respectively[68].

Table 5 : Certificate of analysis for labrasol

Parameters	Specifications	Values
Specific gravity at 20°C	1.060 to 1.070	1.064
Refractive index at 20°C	1.450 to 1.470	1.461
Viscosity at 20°C	80-110 mPa.s	89
Acid value	< = 2.00 (mg KOH/g)	0.48
Saponification value (mg KOH/g)	85-105 mg KOH/g	100
Hydroxyl value(mg KOH/g)	170-205 mg KOH/g	189
Caproic acid (%)	< = 2.0	0.1
Caprylic acid (%)	50-80	56.6
Capric acid (%)	20-50	42.3
Lauric acid (%)	< = 3.0	0.4
Myristic acid (%)	< = 1.0	< 0.1

Capryol PGMC

Source: Gattefosse

Chemical name: Propylene glycol monocaprylate (type I) NF

Description: A water insoluble surfactant used in self - emulsifying systems to obtain a coarse dispersion of SEDDS or a fine dispersion of SMEDDS.

Functional Category: It's an oral bioavailability enhancer which inhibits the enterocytic drug metabolizing enzyme CYP3A4.

Physical form: Liquid

HLB value: 6

Formulation techniques and dosage forms: It is suitable for hard and soft gelatin capsules. It can be adsorbed onto neutral carrier powders for use in tablets, capsule filling and sachets. It's used in a formulation of topical ointments, micro emulsions, and emulsions.

Table 6 : Certificate of analysis for Capryol PGMC

Parameters	Specifications	Values
Specific gravity at 20°C	0.930-0.945	0.938
Acid value	< = 0.50 mg KOH/g	0.16
Saponification value	285 to 310 mg KOH/g	296
Caprylic acid (%)	> = 99.0	99.6
Capric acid (%)	< = 3.0	0.1
Lauric acid (%)	< = 3.0	0.1

Labrafil M 1944CS

Source: Gattefosse

Chemical name: Oleoyl macrogol-6 glycerides EP / Oleoyl polyoxyl-6 glycerides NF.

Description: It's a water dispersible surfactant composed of well characterized PEG-esters and a glyceride fraction. It can self-emulsify on contact with aqueous media forming a coarse dispersion and due to the good miscibility with labrasol and Gelucire 44/14, it forms the microemulsion. It's a powerful surface active agent which improves the solubility of drugs for *invitro* and *invivo* studies.

Functional category: It is a bioavailability enhancer. It increases the bioavailability of poorly water soluble drug because of its composition of long chain triglyceride and the highly hydrophobic drugs are selectively absorbed by the lymphatic system which reduces the hepatic first pass metabolism.

Physical form: Liquid

HLB value: 9

Formulation techniques and dosage forms: It is suitable for hard and soft gelatin capsules. It's used in the formulation of topical ointments, micro emulsions, and emulsions.

Table 7 : Certificate of analysis for Labrafil M 1944CS

Parameters	Specifications	Values
Specific gravity at 20°C	0.930-0.945	0.938
Acid value	< = 0.50 mg KOH/g	0.16
Saponification value	285 to 310 mg KOH/g	296
Caprylic acid (%)	> = 99.0	99.6
Capric acid (%)	< = 3.0	0.1
Lauric acid (%)	< = 3.0	0.1

Labrafil M2125 CS

Source: Gattefosse

Chemical name: Linoleoyl macrogol-6 glycerides EP / Linoleoyl polyoxyl-6 glycerides NF

Description: It's a water dispersible surfactant composed of well characterized PEG-esters and a glyceride fraction. It can self-emulsify on contact with aqueous media forming a coarse dispersion and due to the good miscibility with labrasol and Gelucire 44/14, it forms microemulsion. It's a powerful surface active agent which improves the solubility of drugs for *invitro* and *invivo* studies.

Functional category: It is a bioavailability enhancer.

Physical form: Liquid.

HLB value: 9

Table 8 : Certificate of analysis for Labrafil M 2125

Parameters	Specifications	Values
Specific gravity at 20°C	0.935-0.955	0.943
Refractive index at 20°C	1.465-1.475	1.473
Viscosity at 20°C	70-90 mPa.s	83
Acid value	< = 2.00 mg KOH/g	0.73
Hydroxyl value	45-65 mg KOH/g	55
Saponification value	150 to 170 mg KOH/g	166
Palmitic acid (%)	4.0-20.0	11.3
Stearic acid (%)	< = 6.0	1.9
Oleic acid (%)	20-35	30.2
Linoleic acid (%)	50-65	54.3
Linolenic acid (%)	< = 2.0	0.9
Arachidic acid (%)	< = 1.0	0.4
Eicosenoic acid (%)	< = 1.0	0.3

Transcutol HP

Source: Gattefosse

Chemical name: Highly purified diethylene glycol monoethyl ether EP/NF

Description: It is used as a powerful solvent for poorly water soluble pharmaceutical ingredients.

Functional category: Transcutol HP is used as hydrophilic cosolvent for producing self - emulsifying lipid formulations and fine dispersions of the micro emulsion.

Physical form: Liquid

Solubility: Miscibility with water

Boiling point: > 198°C

Flash point: 940°C

Table 9 : Certificate of analysis for Transcutol HP

Parameters	Specifications	Values
Specific gravity at 20°C	0.985-0.991	0.988
Refractive index at 20°C	1.426-1.428	1.427
Acid value	< = 0.10 mg KOH/g	< 0.01
Water content	< = 0.10 %	0.04
2-Methoxy ethanol	< = 20ppm	< 10
2-Ethoxy ethanol	< = 50 ppm	< 10
Ethylene glycol	< = 20ppm	< 10
Diethylene glycol	< = 50 ppm	< 10
Diethylene glycol monoethylether	> = 99.900 %	99.987

CHAPTER 2

AIM AND OBJECTIVES OF THE STUDY

The aim of the proposed research work was to develop a novel o/w self-emulsifying drug delivery system (SEDDS) for poorly soluble BCS system class II drugs of Atorvastatin calcium and Glibenclamide with novel manufactured oils, an assortment of edible natural oils and surfactants/co-surfactants with the utilization of Design of Experiments and factorial designing. The proposed investigated work was selected because of simplicity in the basic procedure of creation and to scale up with the least framework.

The purpose of the present research work was to systematically investigate the interaction, the quadratic effects of formulation variables (independent variables) of SEDDS on desired responses; to develop a model that would yield an optimized SEDDS of Atorvastatin calcium and Glibenclamide. A 13 run factorial design with 2 factors and 3 levels, including 4 replicates at the centre point was used for fitting a second order response surface. The estimation of the coefficients for the second order polynomial model was performed by regression analysis. The model adequacy was checked by an F-test and the determination of correlation coefficient (R^2). All the responses were optimized simultaneously by using desirability function.

The major objectives of the present investigation include

- Formulation of Atorvastatin calcium and Glibenclamide SEDDS by improving their solubility, dissolution characteristics thereby enhancing their relative bioavailability.
- Selection of oil, surfactant, co-surfactant through equilibrium solubility and ternary phase diagram study for the development of SEDDS loaded with Atorvastatin calcium and Glibenclamide
- Optimization of the various formulations by factorial design study.

- Evaluation of prepared formulations for cloud point measurement, emulsification time, particle size, polydispersity index (PDI), zeta potential, viscosity, spectroscopic optical clarity, refractive index, turbidity measurement and percentage drug loading.
- *In vitro* release behaviour studies to be performed and compared with standard drug and marketed formulation for optimized formulations of Atorvastatin calcium and Glibenclamide SEDDS.
- *In vitro* drug diffusion studies and stability studies for optimized formulations.
- To administer the L-SEDDS by filling them in soft gelatin capsules.

CHAPTER 3

REVIEW OF LITERATURE

Abdalla *et al.*,[69] developed a new Progesterone self-emulsifying drug delivery system in which lipid mixtures composed of Solutol HS 15 and optimized their self-emulsifying properties. The liquid SE lipid was mixed with microcrystalline cellulose and transformed into pellets by extrusion/spheronization technique and they were characterized by size, shape, surface characteristics and friability. *In vitro* dissolution and digestion experiments were carried out using physiological dissolution media. The study concluded extrusion/spheronization is a suitable process to produce solid self-emulsifying pellets with up to 40% load of a liquid SE mixture.

Akhter *et al.*, [70] studies considered a solubility enhancing technique of self-emulsifying drug delivery system (SEDDS) for Ibuprofen, a poorly soluble drug using Capmul PG 8 as a co-solvent and hydrophilic surfactant Cremophor EL. In the study, a 3-level factorial design was carried out to optimize the formulation using design expert software trial version 8.0.3.1 and Capmul PG8 and Cremophor EL was used as independent variables and percent drug release as the dependent variable. The study found a possibility of increased release of Ibuprofen by using Capmul PG8 and Cremophor EL.

Albertini B *et al.*, [71] developed a novel preparation approach of solid Self-Emulsifying Drug Delivery System (S-SEDDS) based on spray congealing as potential drug delivery technology for poorly water-soluble drug Glibenclamide with different solid excipients of HLB such as Myverol, Myvatex, Gelucire®50/13 and Gelucire®44/14, Cremophor®EL and Poloxamer 188 as surfactants and PEG 4000 as co-solvent. The screening of the best carrier for S-SEDDS manufacturing revealed that Gelucire®50/13 had greater performance. The dissolution studies showed that the formulation containing Gelucire®50/13 and PEG 4000 increased the drug solubilisation of five times and the microparticles showed self-dispersibility within 60 min and micelles dimensions around 360 nm.

Bachhav YG *et al.*, [72] investigated the development and evaluation of self-micro emulsifying drug delivery system (SMEDDS) for improving the delivery of a BCS class II antidiabetic agent, Glyburide. The solubility of Glyburide in oils, cosurfactants, and surfactants was evaluated to identify the components of the microemulsion. The ternary diagram was plotted to identify the area of microemulsion existence. The *in vitro* dissolution profile of Glyburide SMEDDS was evaluated in comparison to the marketed Glyburide tablet and pure drug in pH 1.2 and pH 7.4 buffers. The chemical stability of Glyburide in SMEDDS was determined as per the International Conference on Harmonisation guidelines. The area of microemulsion existence increased with the increase in the cosurfactant (Transcutol P) concentration. The Glyburide microemulsion exhibited globule size of 133.5 nm and polydispersity index of 0.94. The stability studies indicated that Glyburide undergoes significant degradation in the developed SMEDDS.

Balakrishnan *et al.*, [73] studied prepared a solid form of lipid-based self-emulsifying drug delivery system (SEDDS) by spray drying liquid SEDDS with an inert solid carrier Aerosil 200 to improve the oral bioavailability of poorly water-soluble drug Dexibuprofen. The liquid SEDDS was a system that consisted of Dexibuprofen, Labrasol, Capryol 90 and Labrafil M 1944 CS. The solid SEDDS was characterized by Scanning electron microscopy, Differential scanning calorimetry and X-Ray diffraction studies. The *in vivo* study of solid SEDDS and Dexibuprofen powder in rats at the dose of 10 mg/kg showed that the initial plasma concentrations of drug in solid SEDDS were significantly higher than those of Dexibuprofen powder. The study result suggested that solid SEDDS could be used as an effective oral solid dosage form to enhance rate and extent of absorption of lipophilic drugs.

Bandivadeka *et al.*, [74] studies demonstrated the use of smaller molecular oil (Capmul MCM) for developing self-micro emulsifying drug delivery system with Atorvastatin calcium using the combination of Tween 20 and Labrasol surfactants and proved for better *in vitro* and i*n vivo* performance. The formulations were evaluated for percentage transmittance, droplet size, polydispersity index, Zeta potential, refractive index and cloud point measurement. Of all the oils accessed for drug solubility, Capmul MCM showed better higher solubility capacity for Atorvastatin calcium.

Bandivadekar *et al.*, [75] has studied developed that formulation of a single non-ionic surfactant of Tween 20 based self-nano emulsifying drug delivery system (SNEDDS) enhanced dissolution and absorption of a poorly soluble drug of Atorvastatin calcium using Capmul MCM as an oil phase. The study assessed that none of the formulation showed cytotoxicity and permeation enhancement of the drug.

Bandyopadhyay *et al.*,[76] developed systematically optimized self-nano emulsifying drug delivery systems (SNEDDS) using long chain triglycerides (LCT's) and medium-chain triglycerides (MCT's) of Ezetimibe employing using 3^2 Central Composite design and the optimized formulations located using overlay plot and the *in vitro* and *in vivo* performance were evaluated. The study indicated the successful formulation development of self-nano emulsifying systems with distinctly improved bioavailability potential of Ezetimibe.

Barrable K *et al*., [77] studies used established methodologies to assess the inhibitory effect of the excipients on the Pgp-mediated efflux of the probe, Rh123 and tested the hypothesis that long-term treatment of Caco-2 cells with the lipid excipients, Peceol(c) and Gelucire(c) 44/14 decreased Pgp protein expression. The results suggested a new mechanism which contributed to the improved bioavailability for drugs formulated with lipid-based excipients.

Basalious., [78] studies reported SNEDDS formulations of Lacidipine (LCDP) containing surfactants were bioenhancer for improvement of dissolution and oral absorption. The study applied D-optimal mixture experimental design to optimize an SNEDDS containing a minimum amount of surfactant, a maximum amount of lipid and possesses enhanced emulsification and dissolution rates. The study included three formulation variables; the oil phase X_1 (a mixture of Labrafil /Campus, the surfactant X_2 (a mixture of Cremophor /Tween 80) and the co-surfactant X3, in the design and the systems, were assessed for droplet size, light absorbance, optical clarity, and drug release and emulsification efficiency. The optimized formulation of LCDP showed a significant increase in dissolution rate compared to drug suspension under the same conditions.

Bhattacharya *et al.*, [79] proved the oral bioavailability of Self-emulsifying formulation of Ketoconazole was significantly greater than aqueous suspension of Ketoconazole and mostly unaffected by concurrent administration of Ranitidine and Antacid. The study has concluded that Self-emulsifying formulations can be utilized as an alternative to conventional dosage forms to overcome pH dependent absorption of weakly basic drugs such as Ketoconazole.

Buyukozturk *et al.,* [80] established an initial foundation for relating emulsion function to formulation design and enabled bioavailability optimization across a broad, representative range of SEDDS formulations using surfactants with HLB values ranging from 10 to 15 and three structurally different oils (long chain triglyceride, medium chain triglyceride, and propylene glycol dicaprylate/dicaprate) were combined at three different weight ratios (1:1, 5:1, 9:1). The release coefficients for each emulsion system were calculated and finally the study indicated that incorporation of a long chain triglyceride (Soybean oil) as the oil phase increased the drug release rate constant.

Cirri M *et al.*,[81] studies developed effective fast-dissolving tablet formulations of Glyburide, endowed with improved dissolution and technological properties, investigating the actual effectiveness of the solid-self micro emulsifying drug delivery system (S-SMEDDS) approach. The selected liquid SMEDDS formulations (Capyol 90 as oil, Tween 20 as surfactant and Glycofurol or Transcutol as cosurfactant) were converted into solid-SMEDDS, by adsorbing them onto Neusilin (1:1 and 1:0.8w/w S-SMEDDS: carrier), and fully characterized in terms of solid state (DSC and X-ray powder diffraction), morphological and dissolution properties, particle size and reconstitution ability. Finally, the 1:1 S-SMEDDS containing Glycofurol as CoSurfactant, showed the best performance, was selected to prepare two final tablet formulations. The ratio test and pair-wise procedures (difference (f1) and similarity (f2) factors) highlighted the similarity of the new developed tablets and the marked difference between their drug dissolution profiles and those of formulations based on the micronized drug.

Date *et al.*, [36] employed a method for screening of self-nano emulsifying drug delivery systems (SNEDDS) and selected of various surfactants based on the

emulsification efficiency for selected oily phase of Cefpodoxime proxetil (CFP). The study also helped in rapid screening of the large pool of co-surfactants available for per oral delivery. The study on ternary phase diagrams has indicated that CFP and pH of the dilution medium significantly affected the area of the nanoemulsion formation for the selected system. SNEDDS of CFP accommodated high dose of CFP (130mg) and exhibited rapid release independent pH of dissolution media.

Devani *et al.,* [82] revealed the emulsification properties of Labrafil/Tween based self-emulsifying systems of two hydrophobic drugs Danazol and Mefenamic acid formed the emulsion of small particle size with greater solubilisation. The study also highlighted the dilution of the emulsion resulted in the change in particle size.

Dixit *et al.,* [25] prepared and optimized Valsartan containing self-micro emulsifying drug delivery system (SMEDDS) using Capmul MCM as the oil phase, Tween 80 as a surfactant, and PEG 400 as cosurfactant. The study evaluated *in vitro* parameters like particle size, polydispersity index (PDI) zeta potential, *in vitro* release, and bioavailability. The combination of all three components, oil/surfactant/cosurfactant in the ratio of 10:45:45 was formulated in SMEDDS yield lower particle size 12.3, PDI 0.138 and zeta potential of −0.746. This optimized SMEDDS showed good *in vitro* release which increased more than 90% when compared with marketed formulation and drug suspension. The *in vivo* study has revealed significant improvement in the extent of absorption of Valsartan in rabbit to 1.78-fold compared to with conventional capsule formulation. The study illustrated the potential use of self-microemulsified drug delivery system to dispense lipid-soluble drug by the oral route.

Elnaggar *et al.,* [83] prepared a self- nano emulsifying drug delivery system using capryol 90 and maisine 35-1 as oils, cremophor RH 40 and propylene glycol as surfactant and cosurfactant. Tamoxifen citrate is a highly lipophilic drug and having first-pass metabolism and Pgp pump efflux in an intestine. The prepared SNEDDS showed a significant increase in release rate compared to the drug suspension and anticipated to solve oral problems of Tamoxifen citrate.

Fernandez [et] *al.,* [84] investigated the lipolytic activity of pancreatic extracts and human pancreatic juice on Labrasol taken orally is due to the combined action of

pancreatic lipase-related protein 2 (PLRP2) and carboxyl ester hydrolase (CEH). The study has revealed about the lipolysis of lipidic excipients proceeded along with the gastrointestinal tract and the lipolytic process affecting the physico chemistry of lipid vehicles and the bio-availability of hydrophobic drugs.

Franceschinisa *et al.,* [85] showed the experimental design approach applied to the study of solid pharmaceutical dosage forms, such as the self-emulsifying pellets produced by wet granulation, led to a mathematical model describing the effects of formulation components on the product characteristics. The study has demonstrated the mathematical equations; the response behavior can be predicted over the whole experimental field for the development of a self-emulsifying system for Nimesulide as a poorly water-soluble model drug allowed to find different formulations with improved drug solubility and permeability characteristics.

Gao.P *et al.,* [86] applied Response surface methodology (RSM) and optimized the self-emulsifying drug delivery system (SEDDS) containing 25% (w/w) model drug, with a high lipophilicity and low water solubility. The dispersion experiment results confirmed the prediction identified potential optimal formulations for further development. The work has demonstrated that RSM is an efficient approach for optimization of the SEDDS formulation.

Hashem FM *et al.,* [87] utilized custom fractional factorial design with 14 experimental runs of highly solubilizing SNEDDS components: oleic acid, Tween 80 and propylene glycol and characterized the formulations. Optimized SNEDDS formula exhibited mean globule size of 73.5 nm, a zeta potential magnitude of -24.1 mV and 13.5 μs /cm of electrical conductivity. The release behavior of the optimized SNEDDS formula showed 56.78% of cumulative ATR release after 10 minutes. The bioavailability estimation in Wistar albino rats revealed an augmentation in ATR bioavailability, relative to ATR suspension and the commercial tablets, from optimized ATR SNEDDS was found to be 193.81%. The findings of the work showed that the optimized nanocarriers enhanced the oral delivery and pharmacokinetic profile of ATR.

Kadu PJ *et al.*, [88] formulated a self-emulsifying drug delivery system of Atorvastatin calcium and determined its solubility in various vehicles such as Captex

355, Captex 355 EP/NF, Ethyl oleate, Capmul MCM, Capmul PG-8, Gelucire 44/14, Tween 80, Tween 20 and PEG 400. Prepared formulations were tested for micro emulsifying properties and evaluated for clarity, precipitation, viscosity determination, drug content and *in vitro* dissolution. The optimized formulation was further evaluated for particle size distribution, zeta potential, stability studies and *in vivo* potential. *In vivo* performance of the optimized formulation was evaluated using a Triton-induced hypercholesterolemia model in male Albino Wistar rats. The formulation significantly reduced serum lipid levels as compared with Atorvastatin calcium. The study has illustrated the potential use for the delivery of the hydrophobic drug such as Atorvastatin calcium by the oral route.

Khan *et al.*, [89] studies explained an incorporation of the maximum of 12.5% of the Atorvastatin (ATV) into the formulation of SEDDS with Oleic acid, Tween 80, and PEG 400 oil, enhanced dissolution of the drug. Pseudo-ternary phase diagram construction was generated to the optimized ratio of oil/surfactant/co-surfactant. A 3^2 factorial design without replication was proved to correlate the dependant and independent variables. The study further revealed that the potential possible gastric irritation due to the use of a large amount of surfactants of these formulations for bioavailability enhancement and the study needed to be further evaluated by *in vivo* studies.

Kishore R *et al*., [38] studies enhanced the solubility of Atorvastatin by designing the suitable solid self-micro emulsifying drug delivery systems (S-SMEDDS).The clear and transparent self-micro emulsifying drug delivery system (SMEDDS) were formulated using coconut oil and isopropyl myristate as lipid phases, tween 80 as a surfactant and PEG 400 and glycerin as co-surfactant at 2:1, 3:1, 1:2 and 1:3 ratio. The pseudo-ternary phase diagrams were constructed to identify the micro emulsion region. The SMEDDS were evaluated for zeta potential, poly dispersity index, globule size, pH, viscosity and drug release. The solid SMEDDS were developed by employing adsorption and melt granulation methods. The S-SMEDDS were evaluated for micromeritics, morphology, solid state property, reconstitution ability, drug release and stability. The micro formulations formed with the particle size of 25 nm had shown a 3-folds rise in drug release. The solid SMEDDS had reconstituted to a good micro emulsion rapidly in 1-3 min, with a

release of 94.62% at the end of 30 min and behaved as immediate releasing capsules. Their shelf-life was found to be 1.3 years. The 1:3 ratio SMEDDS had shown more drug release owing to their less particle size. The solid SMEDDS had shown an increased dissolution profiles than Atorvastatin.

Kosnik A *et al.*, [90] designed and characterized liquid and solid self-emulsifying drug delivery systems (SEDDS) for poorly soluble Atorvastatin. The studies demonstrated the possibility of formulating liquid and solid SEDDS as promising carriers of Atorvastatin and SEDDS, with their unique solubilization properties, provided the opportunity to deliver hydrophobic drugs to the gastrointestinal tract in a solubilized state, avoiding dissolution as a restricting factor in absorption rate of BCS Class 2 drugs of Atorvastatin.

Liu *et al.*, [91] focused on the development of an improved formulation screening and optimization method for a Bufalin self-micro emulsifying drug delivery system (SMEDDS). The study concluded on solubility and ternary phase diagrams combined with experimental design may offer a valuable and efficient strategy for developing and optimizing a SMEDDS to obtain optimal formulations with desired characteristics

Mantri SK *et al.*, [92] designed the self-nano emulsifying drug delivery systems (SNEDDS) of Atorvastatin calcium (ATV) using naturally occurring different vegetable oils, various surfactants and co-surfactants and identified ATV solubility for improving the dissolution thereby oral bioavailability and to minimize the gastric degradation. The prepared SNEDDS were evaluated for visual observations, turbidity, the effect of pH of the dispersion media on globule size and zeta potential, robustness to dilution and *in vitro* dissolution study and optimized. The review summarized that FT-IR and DSC study revealed no interaction between drug and excipients and accelerated stability studies showed no significant changes in the mean globule size, zeta potential, drug content and drug release before and after storage of optimized SNEDDS.

Marasini *et al.*,[93] studies prepared solid self-micro emulsifying drug delivery system (SMEDDS) for Flurbiprofen loaded liquid SMEDDS dispersed in dextran by spray-drying at different factorial combinations of processing parameters by using

three-factor, three-level Box–Behnken design. The study results suggested that design of experiments (DOE) was considered the best approach for the production of pharmaceutical products with required quality attributes as a cost-effective approach with the minimum number of experiments.

Miriyala *et al.*,[94] developed an optimized formulation of self-nano emulsifying drug delivery system (SNEDDS) consisting of oleic acid, Tween 80 and Brij 30 offered the advantage of good solubilisation of Atorvastatin. The study confirmed that SNEDDS can be used as a possible alternative to a conventional oral formulation of Atorvastatin. The results further concluded that SNEDDS can be explored as a potential drug carrier for dissolution enhancement of Atorvastatin and other insoluble drugs.

Mohsin *et al.,* [95] showed the effects of different components of lipids and different ratios on the particle size. They studied the effect of lipid surfactant ratio and the presence of cosolvents on the performance of formulations. The report showed that mono and di-glycerides in the formulation systems lead to increase efficiency of emulsification system

Nagarsenker *et al.,* [36] clearly explained about the screening of surfactants and cosurfactants for peroral delivery to achieve optimum emulsification for selected oil. The prepared SNEDDS were tested for robustness to dilution with various media. *In vitro* release of SNEDDS was assessed by filling it into size 4 hard gelatin capsules using type I dissolution apparatus. The prepared SNEDDS were robust to all dilutions and can accommodate high dose of Cefpodoxime proxetil and exhibited rapid release independent of pH of dissolution media

Nekkanti *et al.,* [43] formulated SMEDDS formulation of Candesartan cilexetil a poorly water-soluble drug for direct filling into hard gelatin capsules for oral administration and evaluated their parameters. The study demonstrated the utility of SMEDDS to enhance solubility and dissolution of sparingly soluble compounds like Candesartan which may result in improved therapeutic performance.

Patel *et al* ., [96] studies developed and characterized self-micro emulsifying drug delivery system (SMEDDS) of the poorly water-soluble drug, Glibenclamide

using captex 200P (oil), cremophor RH40 (surfactant), capmul MCM C8 (co-surfactant). The formulations were prepared according to 3^2 full factorial design using two variables oil: Smix (X_1) and S: Cos (X_2) and their effects were evaluated for three responses of droplet size, self-emulsification time and % drug release at 15 min. All the nine batches were assessed for dispersibility, emulsification time, % transmission, viscosity, zeta potential, particle size, electro conductivity, drug content and dissolution studies. *In vitro* drug dissolution was carried out using USP type-II apparatus and compared with that of marketed GBD tablet (Daonil®) and dissolution was found to be increased in the case of SMEDDS than marketed tablet formulation. Effects of various formulation variables on different responses were extracted out by using Design Expert® software. The result of the study proved that X_1 variable negatively affects self-emulsification time while droplet size was negatively affected by X_2 variable.

Patil *et al.*,[97] study formulated a gelled self-emulsifying drug delivery system (SEDDS) containing Ketoprofen of sustained release solid dosage form with Captex 200 (an oil), Tween 80, Capmul MCM (a co-surfactant) and silicon dioxide was used as a gelling agent, aided in solidification and retardation of drug release. The effect of concentrations of co-surfactant and gelling agent on emulsification process and *in vitro* drug diffusion was studied using 3^2 factorial design. Multiple regression analysis data and response surfaces obtained showed that liquid crystal phase viscosity increased significantly with increasing amount of silicon dioxide which in turn caused an increase in average droplet size of resultant emulsion and slower drug diffusion. The study indicated the potential applications of gelled SEDDS for transformation in sustained release solid dosage forms

Poudel *et al.,* [93] study worked on Valsartan self micro emulsifying drug delivery system (SMEDDS) using 3-level, 3-factor level Box-Behnken design (BBD) on the effects of three formulation factors (Labrafil M 2125 CS as oil, Tween 20 as surfactant and Capryol 90 as co-surfactant) showed significant effect on particle size and equilibrium solubility while the amount of co-surfactant exhibited the main effect on dissolution profile after 15 min). The study concluded that the optimized formulation showed significantly increased bioavailability compared to that of Valsartan powder and BBD facilitated in the better understanding of the inherent

relationship of formulation variables with the responses and in the optimization of Valsartan SMEDDS in relatively cost, time and labor effective manner.

Pouton.,[98] described strategies used for the formulation of self-emulsifying drug delivery system (SEDDS), methods used for assessment of efficiency of emulsification and practical consideration regarding the use of SEDDS for enhancement of the bioavailability of drugs from the gastro-intestinal tract.

Prajapathi *et al.*, [99] concluded that self-micro emulsified drug delivery systems (SMEDDS) would be a promising drug delivery system for the poorly water-soluble drug of Olmesartan in various oils, surfactants and co surfactants by the oral route. The study further compared the *in vitro* and *ex vivo* diffusion rate of the drug from the SMEDDS was significantly higher than that of the plain drug suspension.

Selvam *et al.*,[100] developed SNEDDS for lipophilic Efavirenz using labrafac PG(oil), tween 80(surfactant), PEG 200(Co-surfactant) had sufficient drug loading, rapid self-micro-emulsification in aqueous media and forming droplet size in the range of nanoemulsion. The study revealed that drug solubility was achieved and thus overcome dissolution rate-limited absorption of Efavirenz with *in vitro* drug release. The stability study of prepared SNEDDS showed same physicochemical properties as compare to initial SNEDDS after 3 months stored in stability chamber at 40°C and 75%RH.

Sha *et al.*, [101] designed a self micro emulsifying drug delivery system (SMEDDS) of Probucol composed of olive oil, Lauroglycol FCC, Cremophor EL, Tween-80, and PEG-400. The study evaluated and compared the pharmacokinetics and bioavailability of Probucol suspension, oil solution and SMEDDS in rats. The study has concluded that relative bioavailability of SMEDDS was dramatically enhanced in an average of 2.15- and 10.22-fold that of oil solution and suspension.

Shaji *et al.*, [102] studies prepared, evaluated and optimized, self-micro emulsifying drug delivery system of Celecoxib using 3 factor, 3 level factorial with different amounts of Labrafil 2609 WL, Labrasol and Cremophor EL as independent variables. The response variable was selected on particle size (nm) of the droplets after dilution in 0.1N HCl. Mathematical equation and response surface plots were

used to relate the dependent and independent variables. The regression equation was generated for the particle size after dilution. The study has concluded that the observed response was in close agreement with the predicted values of the optimized formulation and demonstrated the reliability of the optimization procedure in predicting particle size of the self micro-emulsifying delivery system for Celecoxib.

Shakeel F *et al.*,[103] studied the impact of various combinations of nonionic surfactants on the self-nano emulsifying performance of two grades of Lauroglycol (Lauroglycol-90 and Lauroglycol-FCC) in Glibenclamide nanoemulsion by spontaneous emulsification method. The results of thermodynamic stability and self-nano emulsification tests were confirmed by further characterization of these formulations in terms of droplet size, viscosity, refractive index and % transmittance. The study revealed that formulations prepared with Labrasol, HCO-60 and Gelucire-44/14 were found to be suitable for self-emulsifying drug delivery system only whereas those prepared with Tween-80 and Cremophor-EL were found to be suitable for self-nano emulsifying or self-micro emulsifying drug delivery system of Glibenclamide with respect to Lauroglycol-90 or Lauroglycol-FCC.

Sharma *et al* .,[104] studies involved preparation and evaluation of self-emulsifying drug delivery system (SEDDS) of Ibuprofen using peanut oil composed of varying concentrations of peanut oil (solvent), tween 80 (surfactant) and span 20 (co-surfactant). The study has investigated the influence of the concentration of surfactant/co-surfactant and globule size on dissolution rate. The dissolution rate of self the emulsifying capsule was found to be significantly faster than that from conventional tablet from the study.

Shen H, *et al.,* [105] successfully prepared self-micro emulsifying drug delivery systems (SMEDDS) of Atorvastatin calcium to improve its bioavailability. The release of Atorvastatin from SMEDDS capsules was studied using the dialysis bag method in 0.1 M HCl and phosphate buffer (pH 7.4), compared with the release of Atorvastatin from a conventional tablet. The study resulted in bioavailability of Atorvastatin SMEDDS capsules was significantly increased when compared with that of the conventional tablet.

Singh SK *et al.*, [106] developed and characterized self-nano emulsifying drug delivery system of the poorly water-soluble drug, Glibenclamide (GBD). The result of the study has indicated that the self-nano emulsifying drug delivery system of GBD, owing to nanosize, has potential to enhance its absorption and without interaction or incompatibility between the ingredients.

Smita *et al.*, [107] explored the multifunctionality of Rice Germ Oil (RGO) in self-micro emulsifying drug delivery system (SMEDDS) formulation of lipophilic drug such as Tacrolimus (TAC). The antioxidant potential and solubilization capacity of RGO was significantly higher than Rice Bran Oil (RBO) The Tacrolimus (TAC SMEDDS formulation using RGO showed significantly higher dissolution profile as well as improvement in oral pharmacokinetic parameters of TAC in comparison with plain TAC and marketed capsule. The study has concluded that gamma-oryzanol-enriched natural RGO is a multifunctional excipient for lipid drug delivery system like SMEDDS.

Subramanian R *et al.*, [108] studied the effects of two lipid excipients, Peceol and Gelucire 44/14 on the *in vitro* pancreatic lipase activity. The results from this study suggested that these lipid excipients inhibit *in vitro* pancreatic lipase activity and should be taken into consideration when developing oral formulations using these agents

Vithlani *et al.*, [109] improved the aqueous solubility and modified *in vitro* dissolution profile of hydrophobic drug using self-emulsifying drug delivery systems (SEDDS) of Cinnarizine using Capmul PG-12, Cremophor RH 40 and Tween 20 at different weight ratios and incorporated with Cinnarizine. The drug incorporation into pre-concentrate, drug solubility in phosphate buffer, (pH 7.2) the mean droplet size and drug release profile of the SEDDS were also determined. The SEDDS with 30% w/w of Capmul PG-12 provided the greatest enhancement in drug solubility in phosphate buffer as well as rapid drug release despite forming larger droplets upon emulsification. The combination of Capmul PG-12, Tween 20 and Cremophor RH 40 can produce SEDDS which can be used as an alternative dosage form for poorly water soluble drug.

Wang *et al.,* [110] developed and evaluated the new solid self-emulsifying (SE) pellets of poorly soluble Nitrendipine (NTD) prepared by extrusion/spheronization technique, using liquid SEDDS (NTD, Miglyol® 812, Cremophor® RH 40, Tween 80, and Transcutol® P), adsorbents (silicon dioxide and crospovidone), microcrystalline cellulose and lactose, and studies further illustrated that extrusion/spheronization technique could be a useful large-scale producing method to prepare the solid SE pellets from liquid SEDDS.

Yeom D W *et al.,* [111] successfully developed an optimized Atorvastatin (ATV) loaded self-micro emulsifying drug delivery system (SMEDDS) formulation by using the D-optimal mixture design containing 7.16% Capmul MCM (oil), 48.25% Tween 20 (surfactant), and 44.59% Tetraglycol (cosurfactant) which significantly enhanced the dissolution rate of ATV in different types of medium, including simulated intestinal fluid, simulated gastric fluid, and distilled water and compared with ATV suspension. The good agreement was observed between predicted and experimental values for mean droplet size and percentage of the drug released in 15 minutes that could potentially be used for improving the oral absorption of poorly water-soluble drugs. The pharmacokinetic studies in rats showed that the optimized SMEDDS formulation considerably enhanced the oral absorption of ATV, with 3.4-fold and 4.3-fold increased in the area was observed under the concentration-time curve and time taken to reach peak plasma concentration when compared with the ATV suspension.

Zhaoa *et al.,* [112] demonstrated the potential utility of SNEDDS for formulation of Zedoary turmeric oil (ZTO) with improved aqueous dispersibility, stability and oral bioavailability. The study illustrated in the formulated SNEDDS, the essential oil ZTO served as a partial lipid phase with the dual advantages of increasing drug loading and minimizing the amount of the inert oils required. The study served as a prototype approach for the formulation development of other essential oils or hydrophobic drugs in liquid form.

Zhong *et al.,* [113] studies developed a self-double-emulsifying drug delivery system (SDEDDS) for a Hydroxysafflor yellow A (HSYA) the main active ingredient of the safflower plant (*Carthamus tinctorius* L) and improved oral absorption through inhibition of p-glycoprotein efflux. The final study demonstrated that SDEDDS were promising technique for improving the oral absorption of drugs with high solubility and low permeability with no significant toxicity *in vitro and in vivo*.

CHAPTER 5

MATERIALS AND METHODS

5.1 LIST OF MATERIALS

S.No.	Name	Source
1.	Atorvastatin calcium	Goodman Pharmaceuticals, Pondicherry.
2.	Glibenclamide	Goodman Pharmaceuticals, Pondicherry.
3.	Capryol PGMC	Gattefosse (Saint-Priest Cedex, France)
4.	Transcutol HP	Gattefosse (Saint-Priest Cedex, France)
5.	Peceol	Gattefosse (Saint-Priest Cedex, France)
6.	Labrasol	Gattefosse (Saint-Priest Cedex, France)
7.	Labrafil M 1944 CS	Gattefosse (Saint-Priest Cedex, France)
8.	Labrafil M 2125 CS	Gattefosse (Saint-Priest Cedex, France)
9.	Virgin sesame oil	Vama oil industries, Coimbatore.
10.	Sunflower oil	Vama oil industries, Coimbatore.
11.	Olive oil	Shaah Enterprises, Chennai.
12.	Mustard oil	Green spice products, Coimbatore.
13.	Virgin coconut oil	Vama oil industries, Coimbatore.
14.	Rice bran oil	Jupiter Manufacturing industry, Chennai.
15.	Corn oil	Arumuga group of industries, TamilNadu.
16.	Potassium dihydrogen ortho phosphate	Loba Chemie Pvt Ltd, Mumbai.
17.	Sodium hydroxide pellets	Loba Chemie Pvt Ltd, Mumbai.
18.	Methanol	Loba Chemie Pvt Ltd, Mumbai.
19.	Dialysis membrane (Molecular mass cut off 12,000-14,000 daltons)	Himedia, Mumbai.
20.	Dialysis membrane clips	Himedia, Mumbai.
21.	Anhydrous potassium bromide	Loba Chemie Pvt Ltd, Mumbai.
22.	Hydrochloric acid	Loba Chemie Pvt Ltd, Mumbai.
23	Potassium dichromate	Loba Chemie Pvt Ltd, Mumbai.
24.	Potassium iodide	Loba Chemie Pvt Ltd, Mumbai.
25.	Iodine Crystals resublimed	Loba Chemie Pvt Ltd, Mumbai.
26.	Starch	Loba Chemie Pvt Ltd, Mumbai.
27.	Mercuric iodide	Loba Chemie Pvt Ltd, Mumbai.
28.	Sodium thiosulphate	Loba Chemie Pvt Ltd, Mumbai.
29.	Iodine monochloride	Loba Chemie Pvt Ltd, Mumbai.
30.	Iodine trichloride	Loba Chemie Pvt Ltd, Mumbai.
31.	Carbon tetra chloride	Loba Chemie Pvt Ltd, Mumbai.
32.	Phenolphthalein indicator	Loba Chemie Pvt Ltd, Mumbai.
33.	Rectified Spirit	Loba Chemie Pvt Ltd, Mumbai.
34.	Ethanol	Loba Chemie Pvt Ltd, Mumbai.
35.	Distilled water	Prepared in laboratory

5.2 LIST OF EQUIPMENTS

S.No.	Instrument/Equipment	Model / Manufacturer
1.	UV-Visible Spectrophotometer	Shimadzu 1700 Pharmaspec, Japan.
2.	Electronic balance	Shimadzu BL-220H, Japan.
3.	Bath sonicator	Sonica 2200MH, Soltech srl, Soluzioni Tecnologirhe, Milano, Italy.
4.	Magnetic stirrer	Remi instruments.
5.	Vortex mixer	Spinix, Japan
6	Humidity chamber	Labtech, Ambhala.
7.	Digital pH meter	Elico scientifics-L1610, Mumbai.
8.	Eppendorff 5415D centrifuge	Marshall scientific, New Hampshire.
9.	Brookfield viscometer	Brookfield engineering Laboratories, USA.
10.	USP dissolution tester	Electrolab, India.
11.	Turbidimeter	Elico D-10-model 331, Japan.
12.	Abbe refractometer	Remi instruments
13.	Malvern Nano Zeta sizer-90	Malvern Instruments Ltd., Malvern, UK
14.	Refrigerator	Samsung
15.	Fourier transform infrared spectrophotometer	FT/IR- 8400S Shimadzu
16.	IR-Hydraulic pellet press	Techno search instruments

5.3 METHODOLOGY

5.3 Development and Evaluation of Atorvastatin calcium and Glibenclamide SEDDS Preformulation Study

Identification test for Atorvastatin calcium and Glibenclamide

5. 3.1 Melting Point

The melting point of Atorvastatin calcium and Glibenclamide were tested by using laboratory melting point apparatus with capillary tube method and the procedure followed as per Indian Pharmacopeia 2007[125].

5.3.2 Fourier transform infrared (FT-IR) spectroscopy of Atorvastatin calcium and Glibenclamide [57]

The drug sample was mixed with anhydrous potassium bromide (KBr) in 1:4 ratio. Briefly about 100 mg of this mixture was made into fine powder using mortar and pestle followed by compression to form transparent KBr pellet using Techno search hydraulic press set at 15 tons pressure. Each KBr pellet was scanned at 4mm/s at a resolution of 2cm over a wave number region from 4000 to 400 cm^{-1} in an FTIR spectrophotometer (8400S Shimadzu, Japan).

5.3.3 Identification test for oils

Specific gravity

The specific gravity of the oils was tested by specific gravity bottle method and the procedure followed as per Bureau of Indian standards IS 548-1[126].

5.3.4 Determination of iodine value by WIJS method [126]

Preparation of potassium iodide solution

About 10 g of potassium iodide free from potassium iodate was dissolved in 90ml of water.

Starch Solution

About 5 g of starch and 0.01 g of mercuric iodide was triturated with 30 ml of cold water and slowly poured it with stirring into 1 litre boiling water. The solution was boiled for three minutes. It was allowed to cool and the supernatant liquid was decanted.

Standardization of sodium thiosulphate solution

About 0·1Normal solution of sodium thiosulphate was prepared by dissolving 24·8 g of sodium thiosulphate crystals in 1 litre of distilled water and made up to 1000ml. About 0.5 g of finely ground potassium dichromate which has been previously dried to a constant weight at105 ± 2°C was weighed accurately and transferred into a 1litre volumetric flask. It was dissolved in distilled water and made up to the mark. About 25 ml of above solution was pipetted into the 250ml conical flask. To the conical flask solution, 5 ml of concentrated hydrochloric acid and 15 ml of a 10% potassium iodide solution was added. It was allowed to stand in the dark for 5 minutes and the resultant mixture was titrated with the 0.1Normal solution of sodium thiosulphate using the starch solution as an indicator towards the end. The end point is the change of blue to green colour.

The normality of the sodium thiosulphate solution was calculated as follows

$$\frac{25W}{49.03V}$$

Where W is weight in g of the potassium dichromate and V is a volume of ml of sodium thiosulphate solution required for the titration.

Preparation of Wijs solution

The solution was prepared by dissolving 13g of iodine in 1litre of acetic acid and the strength of solution was determined by titrating with standard sodium thiosulphate solution. The chlorine gas was introduced into 50 ml of the solution until the characteristic colour change was occurred and the halogen content was nearly doubled as ascertained again by titration.

Procedure

About 0.2 g of the sample was weighed accurately and transferred into a 500 ml iodine flask or well to which 25 ml of carbon tetrachloride have been added and agitated to dissolve the contents. 25 ml of the Wijs solution was added and the glass stopper was replaced after wetting with potassium iodide solution. It was swirled for intimate mixing and allowed to stand in the dark for 30 minutes in the case of non-drying and semi-drying oils and one hour in the case of drying oils. The blank test was carried out under similar experimental conditions. After standing 15 ml of potassium iodide solution and 100 ml of water were added and the liberated iodine was titrated with standard sodium thiosulphate solution. Then 1 ml of starch solution was added to the above solution until the formation of blue colour disappeared after thorough shaking.

Iodine value was calculated as shown below

$$\text{Iodine value} = \frac{12.69\ (B-S)\ N}{W}$$

where

B = volume of ml of standard sodium thiosulphate solution required for the blank

S = volume in ml of standard sodium thiosulphate solution required for the sample

N = normality of the standard sodium thiosulphate solution

W = weight in g of the material taken for the test.

5.3.5 Determination of saponification value[126]

Preparation of alcoholic potassium hydroxide Solution

About 35 to 40 g of potassium hydroxide was dissolved in 20 ml of distilled water and sufficient aldehyde free rectified spirit was added to make up to 1000 ml.

Then the solution was allowed to stand overnight. The clear liquid was decanted and kept in a bottle closed tight with a cork or rubber stopper.

Preparation of 0.5N hydrochloric acid

About 42.5 ml of hydrochloric acid was diluted and made up to 1000 ml of distilled water.

Procedure

About 1 to 2 g of the oil was weighed accurately and transferred in a conical flask. To the flask, 25 ml of the alcoholic potassium hydroxide solution was added and refluxed with air condenser connected to the flask. The flask was heated on a water-bath for not more than one hour. Then the contents of the flask were boiled gently until the sample is completely saponified as indicated by an absence of any oily matter and appearance of the clear solution. The flask was allowed to cool. To the above solution, 1 ml of phenolphthalein indicator was added and titrated with the standard hydrochloric acid.

The saponification value is calculated as follows

$$\text{Saponification value} = \frac{56.1\ (B-S)\ N}{W}$$

where B is the volume in ml of standard hydrochloric acid required for the blank

S is the volume in ml of standard hydrochloric acid required for the sample

N is the normality of the standard hydrochloric acid

W is the weight in g of the material taken for the test.

5.3.6 Determination of acid value[126]

Preparation of 0.1N sodium hydroxide

About 5.611g of potassium hydroxide was dissolved in sufficient water to produce 1000ml.

Procedure

About 1 g of oil was weighed and transferred into 200 ml conical flask. To the solution added 50 ml of freshly neutralized hot ethyl alcohol and 1 ml of phenolphthalein indicator solution. The mixture was boiled for five minutes and solution was titrated with 0.1N sodium hydroxide solution.

The acid value was calculated as given below

$$\text{Acid value} = \frac{5.61\ V\ N}{W}$$

where V = volume of ml of standard sodium hydroxide solution used.

N = normality of standard potassium hydroxide or sodium hydroxide solution.

W = weight in g of the oil taken for the test.

5.4 DEVELOPMENT OF STANDARD CALIBRATION CURVE OF ATORVASTATIN CALCIUM IN METHANOL

UV Spectroscopy (λ max)

The absorption maximum of the standard solution of Atorvastatin calcium was scanned between 200- 400 nm regions on UV- visible spectrophotometer.

5.4.1 Preparation of standard stock solution

An accurately weighed quantity of about 50 mg of Atorvastatin calcium was taken in 50 ml volumetric flask and dissolved in sufficient quantity of methanol followed by sonication[127] in a bath sonicator (Sonica 2200MH) provided with a power supply of 305 Watts during heating at a temperature of 60°C for 10 minutes and finally diluted to 50 ml with methanol to obtain the concentration of 1000 µg/ml. From this solution, 5 ml was pipetted out in a 50 ml volumetric flask and volume was made up with methanol to obtain the concentration of 100 µg /ml.

5.4.2 Preparation of calibration curve

From the stock solution, 2, 4, 6, 8,10 and 12 ml appropriate aliquots were pipetted out from standard stock solution into the series of 100 ml volumetric flask

and the volume was made up to the mark with methanol to get the concentration of 2-12 µg/ml of the drug. The absorbance at various concentrations was measured against methanol as blank at 247 nm using a UV-visible spectrophotometer.

5.5 PREPARATION OF BUFFER SOLUTIONS

5.5.1 Preparation of 0.2M Potassium dihydrogen phosphate

Accurately weighed 27.218g of potassium dihydrogen orthophosphate was dissolved in 1000ml of distilled water.

5.5.2 Preparation of 0.2M sodium hydroxide

Accurately weighed 8.0g of sodium hydroxide was dissolved in 1000 ml of distilled water.

5.5.3 Preparation of Phosphate Buffer pH 6.8

Phosphate buffer pH 6.8 was prepared according to I.P. 2007. A measured quantity of 50 ml of 0.2M potassium dihydrogen phosphate and 22.4 ml of 0.2M sodium hydroxide were taken in 200ml volumetric standard flask and diluted with freshly prepared distilled water to produce the required volume.

5.5.4 Preparation of phosphate buffer pH 7.4

Phosphate buffer pH 7.4 was prepared according to I.P. 2007. A measured quantity of 50 ml of 0.2M potassium dihydrogen phosphate and 39.1 ml of 0.2M sodium hydroxide were added in 200ml volumetric standard flask and diluted with freshly prepared distilled water to produce the required volume.

5.6 DEVELOPMENT OF CALIBRATION CURVE OF ATORVASTATIN CALCIUM IN PHOSPHATE BUFFER pH 6.8

5.6.1 Preparation of standard stock solution

An accurately weighed quantity of about 10 mg of Atorvastatin calcium[128] was taken in 100 ml volumetric flask and dissolved in sufficient quantity of phosphate buffer of pH 6.8 and finally diluted with the same buffer to obtain the concentration of 100 µg/ ml.

5.6.2 Preparation of calibration curve

From the stock solution 2, 4, 6, 8,10 and 12 ml appropriate aliquots were pipetted out from standard stock solution into the series of 100 ml volumetric flask and the volume was made up to the mark with phosphate buffer pH 6.8 to get concentration of 2-12 µg/ml of the drug. The absorbance at various concentrations was measured against blank (phosphate buffer pH 6.8)

5.7 DEVELOPMENT OF STANDARD CALIBRATION CURVE OF GLIBENCLAMIDE IN METHANOL[129]

UV Spectroscopy (λ max)

The absorption maximum of the standard solution of glibenclamide was scanned between 200- 400 nm regions on UV- visible spectrophotometer.

5.7.1 Preparation of standard stock solution

An accurately weighed quantity of about 50 mg of glibenclamide was taken in 50 ml volumetric flask and dissolved in sufficient quantity of methanol to obtain the concentration of 1000 µg/ml. From this solution, 5 ml was pipetted out in a 50 ml volumetric flask and volume was made up with methanol to obtain the concentration of 100 µg /ml.

5.7.2 Preparation of calibration curve

From the stock solution, aliquots 1, 2, 3, 4, 5 and 6 ml appropriate aliquots were pipetted out from standard stock solution into the series of 100 ml volumetric flask and the volume was made up to the mark with methanol to get concentration of 1-6 µg/ml of drug. The absorbance at various concentrations was measured with methanol as blank at 226.5 nm using UV-visible spectrophotometer.

5.8 DEVELOPMENT OF CALIBRATION CURVE OF GLIBENCLAMIDE IN PH PHOSPHATE BUFFER 7.4

5.8.1 Preparation of standard stock solution

An accurately weighed quantity of about 10 mg of Glibenclamide[130, 131] was taken in 100 ml volumetric flask and dissolved in sufficient quantity of phosphate buffer pH 7.4 to obtain the concentration of 100 µg /ml.

5.8.2 Preparation of calibration curve

From the stock solution, aliquots of 2, 4, 6, 8, 10 and 12 ml were pipetted out from standard stock solution into the series of 100 ml volumetric flask and the volume was made up to the mark with phosphate buffer pH 7.4 to get concentration of 2-12 µg/ml of drug. The absorbance at various concentrations was measured with phosphate buffer pH 7.4 as blank at 226.5 nm using UV spectrophotometer.

5.9 SOLUBILITY STUDIES

The solubility for Atorvastatin calcium were determined in aqueous solutions of various pH (pH 4 and 7.4), distilled water, organic solvents such as dimethylsulphoxide and dimethylformamide. Aqueous solution of pH 4.0 and 7.4 was obtained by adding suitable amount of dilute hydrochloric acid and dilute sodium hydroxide. The solubility of Glibenclamide was determined in distilled water, methanol, ethanol and dichloromethane. About 2 ml of each solvent was transferred into 5 ml glass vial and an excess quantity of drug (150 mg) was added to the vial. The solubility of the drug samples were also analyzed by adding excess amount (150mg) of the drug to 2 ml of various oils, surfactants and co-surfactants in screw capped glass vials followed by vortex mixing for 30 secs using vortex mixer (Sphinx, Japan). The mixtures were shaken for 48 h at 30^0C in a thermostatically controlled shaking water bath, followed by equilibrium for 24 hr. The sample mixtures were then centrifuged at 3000 rpm[132] for 10 min and the supernatant liquid was filtered through a millipore membrane filter (0.45µ). Samples were suitably diluted with methanol followed by sonication for 10 min and finally diluted with the same solvent. The final drug concentration was quantified by UV-visible spectrophotometer at 247 nm for

Atorvastatin calcium and 226.5nm for Glibenclamide. The experiment was repeated in triplicates. The results are represented as mean value (mg/ml) ± SD.

5.9.1 Construction of Ternary Phase Diagram: (Following method was used for both the two drugs individually)

Based on the results of saturation solubility studies in Table 12, sunflower oil, labrasol and transcutol HP for Atorvastatin calcium and peceol, labrasol and transcutol HP for Glibenclamide were selected as oil, surfactant and co-surfactant respectively. The percentage limit of surfactant, co-surfactant and oil used herein was selected by considering their acceptable safe dose and decided on the basis of the requirements stated according to the lipid formulation classification system (LFCS) introduced by Pouton[8]. A ternary phase diagram was constructed for the system containing oil-surfactant-co-surfactant by Chemix School software version 3.51. The grading method reported by Craig *et al.*[133] was modified and adopted in this study. A series of self-emulsifying systems were prepared with varying weight percentage of oil, surfactant, and co-surfactant. Since the drug incorporated in the SEDDS may have some effect on self emulsion boundary, every system in the series also consisted of 10% w/w for Atorvastatin calcium and 5% w/w for Glibenclamide. The extreme and middle level of the independent variables consisting of oil, surfactant and co-surfactant were selected for further study. 0.2 ml of each formulation was introduced into 200 ml of water in a glass beaker maintained at 37°C and was mixed gently about 200 rpm with a magnetic stir bar. The tendency to emulsify spontaneously and the progress of emulsion droplets spread were observed. The tendency to form an emulsion was judged as 'good' when droplets spread easily in water and formed a fine milky or slightly bluish emulsion within 1 min[133]. It was judged 'bad' when there was poor, slow or no emulsion formation or when oil droplets coalesced when stirring was stopped or when dull, grayish white emulsion was formed. All studies were repeated thrice.

5.10 PREPARATION OF SEDDS

Optimum ratios of oil and Smix were selected from the phase diagrams. SEDDS formulations were prepared by dissolving the drug in Smix mixtures along with gentle vortexing and sonicating and then by adding oil[134]. The effects of the

formulation variables for different batches were studied by preparing with each batch of SEDDS formulation containing single dose of Atorvastatin and Glibenclamide with varying amounts of oil and Smix using 3^2 factorial designs as illustrated in Table 14a and Table 14b. Then the final formulation was equilibrated in water bath at 37°C for 48 h before carrying out the droplet size, polydispersity index and dissolution. The optimized formulations are prepared by the same method.

5.11 EXPERIMENTAL DESIGN: 3^2 FULL FACTORIAL DESIGN

A 3^2 full factorial design factor was used to explore and optimize the main effects, interaction effects and quadratic effects of the formulation ingredients on the *in-vitro* performance of liquid SEDDS. A total of 13 experimental runs, including 4 replicates at the centre were generated and evaluated by using Design-Expert software (version 10.0.2.0, Stat-Ease Inc., Minneapolis, U.S.A.) which are summarized in Table 14a and Table 14b. The purpose of the replication was to estimate experimental error and increase the precision by computing a model independent estimate of the process standard deviation. The significant response factors studied for assessing the quality of the SEDDS formulation were particle/globule size (Y_1) and drug loading (Y_2).The data obtained after the each response was fitted to quadratic polynomial model explained by the following non-linear equation $Y = \beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_{12}X_1X_2 + \beta_1X_1^2 + \beta_2X_2^2 + E$. where Y is the response of the dependent variables; β_0 to β_2 are the regression coefficients; and X_1, X_2 are independent variables. All the two responses were optimized by using the desirability function approach by fixing the constraints in range and minimizing the particle size (Y_1) and maximizing the drug load (Y_2).

5.12 EVALUATION OF PREPARED SEDDS

5.12.1 Self-emulsification and drug precipitation studies

The efficiency of self-emulsification of oral micro/nanoemulsion is assessed by dispersibility test using a standard USP dissolution apparatus II[25]. One ml of each formulation is added to 500 ml of water at 37 ± 0.5°C. A standard stainless steel dissolution paddle rotating at 50 rpm tends to provide gentle agitation. The *in vitro* performance of the formulations is visually assessed from such dispersion using a

suitable grading system. The grading system has been reported to be based on the formation of a micro emulsion (o/w or w/o), micro emulsion gel, emulsion or emulgel. The drug/excipient precipitation was evaluated by visual inspection of the resultant emulsion after 24 h. The *in vitro* performance of the formulations is visually assessed using the following grading system:

Grade I: Rapidly forming (within 1 min) nano emulsion, having a clear or bluish appearance. (Micro emulsion)

Grade II: Rapidly forming, slightly less clear emulsion, having a bluish white appearance. (Micro emulsion gel)

Grade III: Fine milky emulsion that formed within 2 min. (Emulsion)

Grade IV: A dull grayish white emulsion having slightly oily appearance that is slow to emulsify is formed (longer than 2 min). (Emulgel)

Grade V: Formulation, exhibiting either poor or minimal emulsification with large oil globules present on the surface with phase separation is observed.

Grade VI: The drug is precipitated.

Grade I and Grade II formulation will remain as nano emulsion when dispersed in GIT while formulation falling in Grade III could be recommended for SEDDS formulation. The primary means of self-emulsification is a visual evaluation. The effectiveness of self-emulsification can be optimized by rate of emulsification, droplet size distribution and turbidity measurements.

5.12.2 Phase separation study

The self-emulsifying formulation was diluted with distilled water up to 5 times and the temperature was maintained at 25°C. The mixture was then mixed for 2 min, stored for about 2 hr and visually observed for any phase separation

5.12.3 Determination of emulsification time

The emulsification time (the time for a preconcentrate to form a homogeneous mixture upon dilution) was monitored by visually observing the disappearance of

SEDDS and the final appearance of the emulsion in triplicate. A dissolution apparatus USP II (Electrolab) was employed with 500 ml water and with a paddle speed of 50 rpm at 37°C. The SEDDS (1 ml) was added drop wise to the medium by dropping the pipette and time required for the disappearance of SEDDS was recorded [135].

5.12.4 Spectroscopic characterization of optical clarity

SEDDS formulations disperse in aqueous phase forming the emulsion or micro emulsions and can be detected by the final appearance and droplet size. In practice, the key difference between the emulsion and micro emulsions concerns with their appearance. Emulsions are cloudy while micro emulsions are clear or translucent and the reason for their transparency appearance is due to very small droplet size. The optical clarity may be checked visually. But in order to measure it quantitatively, a UV-visible spectrophotometer was used to measure the amount of light of a given wavelength absorbed by the solution. The cloudier solutions will absorb more of the incident light resulting in higher absorbance values and lower absorbance is obtained with optically clear solutions.

The optical clarity of aqueous dispersions of SEDDS formulations was measured spectroscopically. About 1 ml of SEDDS formulations were diluted to 50 times with double distilled water. The absorbance values of each formulation were measured by a UV-visible spectrophotometer (Shimadzu) at 400 nm[136].

5.12.5 Turbidity measurement

The measurement of turbidity is to analyze whether the dispersion reaches equilibrium rapidly and in a reproducible time. The growth of emulsification is done by nepheloturbidimetric evaluation. The turbidity measurements in nephelometric turbidity unit (NTU) were performed on the resultant emulsion stored in a screw capped sample vials using a turbid meter (Elico D-10-model 331). About 0.5 ml of the SEDDS formulation was introduced into 250 ml of distilled water in 500 ml conical flask under an action of magnetic stirrer rotating at constant speed. The emulsification was done at room temperature[137].

5.12.6 Viscosity determination

The viscosity studies are necessary for SEDDS to characterize the system physically and to control its stability. If the system has low viscosity then, it is o/w type of the system and if a high viscosity then it is w/o type of the system. SEDDS preconcentrate (10 ml) was taken and its viscosity was measured by using Brookfield viscometer (Brookfield engineering Laboratories, USA) using spindle C 16-1 at 25±0.5 ˚C [138] with a shear rate of 50 rpm.

5.12.7 Cloud point measurement

Cloud point temperatures (Tc) was determined by visual observation. 0.5 ml of preconcentrate was diluted to 50 ml with distilling water in a glass beaker. The sample was heated at the rate of about 0.5°C/min. A close observation was made at the appearance of the dispersion with the increase in temperature. The temperature at which the dispersion became turbid was taken as Tc. After the temperature exceeds the cloud point, the sample was cooled below Tc, and then it was heated again to check the reproducibility of the measurements. It mainly insists about the stability of micro emulsion at body temperature[139].

5.12.8 Determination of refractive index

The refractive index, n, of a medium is defined as the ratio of the speed, c, of a wave such as light or sound in a reference medium to the phase speed, vp, of the wave in the medium represented by n=c/vp. It was determined using an Abbes type refractometer[140]. The clarity of micro emulsion could be estimated by measuring the refractive index of the formulations[141].The SEDDS formulations were diluted 100 times with water. The refractive index of the system was measured by an Abbe refractometer by placing 1 drop of solution on the slide and it compares with distilled water.

5.12.9 Droplet size and polydispersity index (PDI) analysis

The droplet size of the micro/nano emulsions is determined by photon correlation spectroscopy (which analyses the fluctuations in light scattering due to a

brownian motion of the particles) using a Zetasizer which can measure sizes between 10 and 5000 nm.

Polydispersity was determined according to the equation:

Polydispersity = D (0.9) – D (0.1) /D (0.5)

where D (0.9) corresponds to particle size immediately above 90% of the sample, D (0.5) corresponds to particle size immediately above 5% of the sample and D (0.1) corresponds to particle size immediately above 10% of the sample. PDI is a measure of particle homogeneity and it varies from 0.0 to 1.0. The closer to zero the PDI value the more homogenous are the particles. The mean droplet size and polydispersity index of formulations were determined by using Malvern Nano Zeta sizer-90. The resultant SEDDS 0.5 ml, was diluted to 100 ml with double distilled water. The samples were loaded into a cuvette placed in a thermostatic chamber and light scattering was monitored at 25^{o}C at a 90^{o} angle[142] after external standardization with spherical polystyrene beads.

Each determination was done in triplicate. The nanometric size range of the particle is retained even after 100 times dilution with water which proves the systems compatibility with excess water[143].

5.12.10 Zeta potential measurement

The zeta potential of prepared SEDDS formulations was determined using a Zeta sizer ZS 90 (Malvern Instruments UK) by using laser Doppler micro-electrophoresis. An electric field is applied to a solution which will cause the particles to move to the electrodes with a velocity related to their zeta potential calculated using Helmholtz–Smoluchowski equation. A suitable amount of the sample (50-100µl) was diluted with 5 ml of distilled water and after sonicating in a bath sonicator to achieve a homogeneous state. Measurements were carried out at 25ºC using disposable polystyrene cuvette with a zeta dip cell. All the measurements were performed in triplicate and the data presented is mean ±SD[144].

Zeta potential determination using following equation

$$\zeta = \frac{V}{E} \quad \frac{\eta}{\varepsilon \cdot \varepsilon 0}$$

where ζ zeta – potential , E electrical intensity, v particle velocity, η viscosity, ε·ε0 dielectric constant

5.12.11 Drug loading efficiency[145]

The drug efficiency was done to investigate the effect of drugs on a self-emulsifying performance of SEDDS. Approximately 10 mg of Atorvastatin calcium was added to 1 ml of boundary formulations of SEDDS and checked for a formation of the clear solution.

5.12.11a Prototype formulation for Atorvastatin calcium

Prototype formulations of Atorvastatin calcium were prepared by varying sunflower oil in 3:1 ratios of the mixture of labrasol and transcutol HP as per the formula composition mentioned in Table 14a. In the first trial, the oil was used at 40% and increased up to 80%. The ratio of surfactant to co-surfactant was maintained at 3:1. Then drug of one dose equivalent of 10 mg atorvastatin calcium was added and stirred for 15 min. The mixture was heated to 30-40°C till the drug was solubilized. The drug loading capacity of each mixture was determined by adding the excess of atorvastatin calcium to each prototype mixture till the clear solution was obtained. The solution was filtered.

The drug content of the SEDDS formulation was determined by diluting the solution in methanol and the volume was made up to 10 ml with methanol (1mg/ml). From the above stock solution, 0.2 ml (200µg/ml) was withdrawn and diluted up to 10 ml with methanol (20 µg /ml). From the above solution 0.2ml (20 µg/ml) diluted up to 10 ml with methanol (2µg/ml) Samples were prepared in triplicate and absorbance was measured at 247 nm using UV-visible Spectrophotometer[146] (Shimadzu UV-1700) using methanol as a reference solution.

5.12.11b Prototype formulation for Glibenclamide

Prototype formulations of glibenclamide were prepared by varying peceol in 1:1 ratios of the mixture of labrasol and transcutol HP as per the formula composition mentioned in Table 14b. In the first trial, the oil was used at 15% with an interval of 5 % and increased up to 25%. The ratio of surfactant to co-surfactant was maintained at 1:1. Then drug of one dose equivalent of 5 mg glibenclamide was added and stirred for 15 min. The mixture was heated to 30-40°C till the drug was solubilized. The drug loading capacity of each mixture was determined by adding the excess of glibenclamide to each prototype mixture till the clear solution was obtained. The solution was filtered.

The drug content was determined by repeating the same procedure as mentioned above with the SEDDS formulation equivalent to 5mg of glibenclamide and the absorbance was measured at 226.5 nm using UV-Visible spectrophotometer (Shimadzu UV-1700).

Drug loading efficiency was calculated by equation

$$\text{Drug loading efficiency} = \frac{\text{Amount of drug in known amount of formulation x 100}}{\text{Initial drug load}}$$

5.12.12 *In vitro* dissolution studies for Atorvastatin calcium

The *in vitro* studies were performed to find out the dissolution rate of SEDDS. The *in vitro* drug release[147] profiles of optimized Atorvastatin of SEDDS, API Atorvastatin calcium and marketed Atorvastatin calcium tablet (Storvas 10mg Ranbaxy Laboratories Ltd) were carried out using USP type II dissolution test apparatus (Electrolab) in 900 ml of Phosphate buffer (pH 6.8). The temperature was maintained at 37 ± 0.5°C and the speed of the paddle was set at 100 rpm. About 120 mg of each optimized SEDDS formulations (AF1, AF5, AF11, AF13 and OPFA) were filled into soft gelatin capsules (size '3') and used for dissolution studies. The capsules were held to the bottom of the vessel using copper sinkers. At predetermined time intervals of 5, 10, 20, 30, 40, 50, 60, 75 and 90 min, an aliquot (5ml) of a sample were collected and filtered through the membrane filter (0.45µm, Whatman). The withdrawn samples were diluted suitably with phosphate buffer (pH 6.8) and analyzed

for the drug content by standard calibration curve method as described in section 5.6.1 and 5.6.2 by UV-visible spectrophotometer (Shimadzu UV-1700) at 247 nm. An equal volume of the dissolution medium was replaced in the vessel after each withdrawal to maintain the sink condition. Each test was performed in triplicate and calculated mean values of cumulative drug release were used while plotting the release curves as illustrated in Fig.16a. The dissolution profile of the API Atorvastatin calcium and marketed tablet (Storvas 10 mg) were assessed by the same method.

5.12.13 *In vitro* dissolution studies for Glibenclamide

The *in vitro* drug release profiles of optimized SEDDS formulations of Glibenclamide, API Glibenclamide and marketed Glibenclamide tablet (Daonil 5mg, Aventis Pharma Ltd) were carried out using USP type II dissolution test apparatus (Electrolab) in 900 ml of phosphate buffer (pH 7.4) as dissolution media[148]. The temperature was maintained at 37 ± 0.5°C and the speed of the paddle was set at 50 rpm. About 70 mg of each optimized SEDDS formulations (GF1, GF2, GF7, GF12 and OPFG) were filled into soft gelatin capsules (size‘3') and used for dissolution studies. The capsules were held to the bottom of the vessel using copper sinkers. At predetermined time intervals of 5, 10, 15, 20 and 30 min, an aliquot (2 ml) of a sample was collected and filtered through the membrane filter (0.45µm, Whatman). The withdrawn samples were diluted suitably with phosphate buffer (pH 7.4) and the drug content was analyzed by standard calibration curve method as described in section 5.8.1 and 5.8.2 by UV-visible spectrophotometer (Shimadzu UV-1700) at 226.5 nm. An equal volume of the dissolution medium was replaced in the vessel after each withdrawal to maintain the sink condition. Each test was performed in triplicate (n = 3) and calculated mean values of cumulative drug release were used while plotting the release curves as illustrated in Fig.16b. The dissolution profile of the API Glibenclamide and marketed tablet (Daonil 5mg) were assessed by the same method.

5.12.14 Kinetic modeling and Mechanism of drug release of optimized formulations

The drug release data of optimized formulations were evaluated for various kinetic models viz. zero order, first order, Higuchi model, Hixson-Crowell model and

Korsmeyer-Peppas model. The study was carried out to determine the mode of drug release from the formulation by using DD Solver software.

The kinetics of *in vitro* drug release

Zero order:

$$C = K_0 t$$

Where K_0 - is the zero-order rate constant expressed in units of concentration/time and t -is the time in h.

First order:

$$\text{Log } C = \text{Log } C_0 - K_1 t / 2.303$$

Where C_0 - is the initial concentration of drug, K_1 - is the first order constant and t - is the time in h

Higuchi:

$$Q_t = Kt1/2$$

Where Q_t - is the amount of the released drug in time t,

K- is the kinetic constant and t- is the time in h.

Korsmeyer-Peppas:

$$Mt / M\infty = Kt\ no$$

where Mt - represents amount of the released drug at time t,

$M\infty$ - is the overall amount of the drug (whole dose) released after 12 h

K is the diffusional characteristic of drug/polymer system constant

n is a diffusional exponent that characterizes the mechanism of release of a drug.

The diffusion exponent and solute release mechanism are given below

Diffusion exponent (n)	Overall solute diffusion mechanism
< 0.5	Quasi-Fickian diffusion
0.5	Fickian diffusion
0.5 < n < 1.0	Anomalous (non-Fickian) diffusion
1.0	Case-II transport
> 1.0	Super case-II transport

5.12.15 *In vitro* diffusion release study for Atorvastatin calcium and Glibenclamide

The conventional dissolution testing can only provide a measure of dispersibility of SEDDS in the dissolution medium of SEDDS. Alternatively, *in vitro* performance of SEDDS can be evaluated by drug diffusion studies using the dialysis technique. The *in vitro* diffusion was performed using the dialysis membrane diffusion technique by a dialysis membrane method. The dialysis membrane of molecular weight cut off 12000 daltons was soaked in distilled water for four hours and then rinsed thoroughly with distilled water. One end of pretreated cellulose dialysis bag (7cm tubing) was sealed firmly with clamp and 0.5 ml of optimized self-emulsifying formulation was introduced in it along with 0.5 ml of dialyzing medium (phosphate buffer pH 6.8).The other end of the bag was also secured with clamp and was allowed to rotate freely. The bags were incubated in beakers containing 500 ml phosphate buffer (pH 6.8) at 37± 0.5°C and shaken at a speed of 100 rpm[149] for atorvastatin calcium and the procedure was repeated with phosphate buffer 7.4 for glibenclamide. About 5 ml of samples were withdrawn individually at 0.5, 1, 2, 4, 6, 8, 10 and 12 hour for Atorvastatin calcium and at 0.5, 1, and 2 hours for Gglibenclamide, which was simultaneously replaced with equal volumes of fresh medium at the same time. The drug content was determined spectrophotometrically at 247 nm as described in section 5.6.1 and 5.6.2 for Atorvastatin calcium and at 227.5 nm under section 5.8.1 and 5.8.2 for glibenclamide. The diffusion of the drug from optimized formulation was compared with the API and marketed tablet.

5.13 STATISTICAL ANALYSIS

The ANOVA provision available in the software was used to establish the statistical validation of the polynomial equations generated by Design Expert. A total of 13 runs for Atorvastatin calcium SEDDS and Glibenclamide SEDDS were generated by optimal design. All the responses were evaluated by a sum of squares, mean of squares, F values and p values. Various feasibility and grid searches were conducted over the experimental domain to find the optimized SEDDS formulations. Three-dimensional response surface plots and 2D contour plots were provided by the Design Expert software 10.0.2.0, where by intensive grid search performed over the whole experimental design.

5.14 STABILITY STUDIES[150]

A pharmaceutical product needs to be physical, chemically, therapeutically toxicologically and microbiologically stable throughout its shelf life. The pharmaceutical companies do stability testing for estimating the shelf life and based on this, the expiry data is given for the product. The real time studies (Long term testing) at recommended storage condition are the ideal method for predicting shelf life. Often the studies are designed to increase the rate of chemical degradation or physical change of pharmaceutical products by using exaggerated storage conditions. This is known as accelerated stability testing. The Pharmaceutical products are subjected to higher temperature and humidity conditions for accelerating the degradation. However, the results of accelerated testing are not always predictive of physical changes and potency. The international Conference on Harmonization (ICH) guidelines for stability testing of new drug substances and products (QIA) describes the stability test requirements for drug registration application in the European Union, Japan and Unites states of America. ICH specifies the length of study and storage conditions as follows

Long term testing: 25°C±2°C at 60% RH± 5% for 12 months.

Accelerated testing: 40°C ± 2°C at 75% RH ± 5% for 6 months.

The pharmacopeia specifies certain storage conditions. The details of the storage condition as specified in Indian pharmacopeia I.P 2007[125] are given below:

Storage condition	Meaning
Cold	Any temperature not exceeding 8ºC (2-8ºC)
Cool	Any temperature not between 8-25ºC
Warm	Any temperature not between 30-40ºC
Excessive heat	Any temperature not above 40ºC

The SEDDS formulations were filled in soft gelatin capsules (size 3) and were subjected to stability studies. The optimized SEDDS was stored under cold condition (4-8°C) at refrigerator and room temperature. The samples were charged at elevated temperature (50±2°C) in stability chamber (Labtech) under ambient humid conditions. After 1 month and 6 months, samples were analyzed for self-emulsification, phase separation, emulsification time, globule size and % drug loading[151]. The % drug loading of the capsules was analyzed using the standard calibration curve method as described under 5.12.11a for Atorvastatin calcium and 5.12.11b for Glibenclamide.

CHAPTER 6

RESULTS AND ANALYSIS

6.1 PREFORMULATION STUDY

6.1.1 Melting point determination

The melting point of Atorvastatin calcium and Glibenclamide determined as per standard IP procedure were found to be 160°C and 173°C, respectively. The results obtained were within the melting point range as mentioned in The Merck's Index[55] (Atorvastatin calcium melting point is 159.2-160.7°C and Glibenclamide melting point is 172-174°C).

6.1.2 FT-IR studies for Atorvastatin calcium

From Figure 3a it was illustrated that the IR spectrum of Atorvastatin calcium showed the characteristic peaks of aromatic N-H stretching at 3364.93 cm^{-1} and the asymmetric stretching of C=O of amide group at 1651.12cm^{1}. However, similar peaks of symmetric C=O stretching were observed at 1579.75 cm^{-1} and O-H stretching at 3566.50 cm^{-1}. The characteristic peaks were observed at the wave numbers 1510.31 cm^{-1} and 1424.48 cm^{-1} due to the C=C ring stretching. The peak found at 1317.43 cm^{-1} was due to CH_3/CH_3 deformation bending vibration at the plane. The two characteristic bands were observed at 3735.28 cm^{-1} and 3055.35 cm^{-1}due to the O-H stretching associated with the hydrogen bond. From the above study, it was inferred that the drug sample was identified as Atorvastatin calcium.

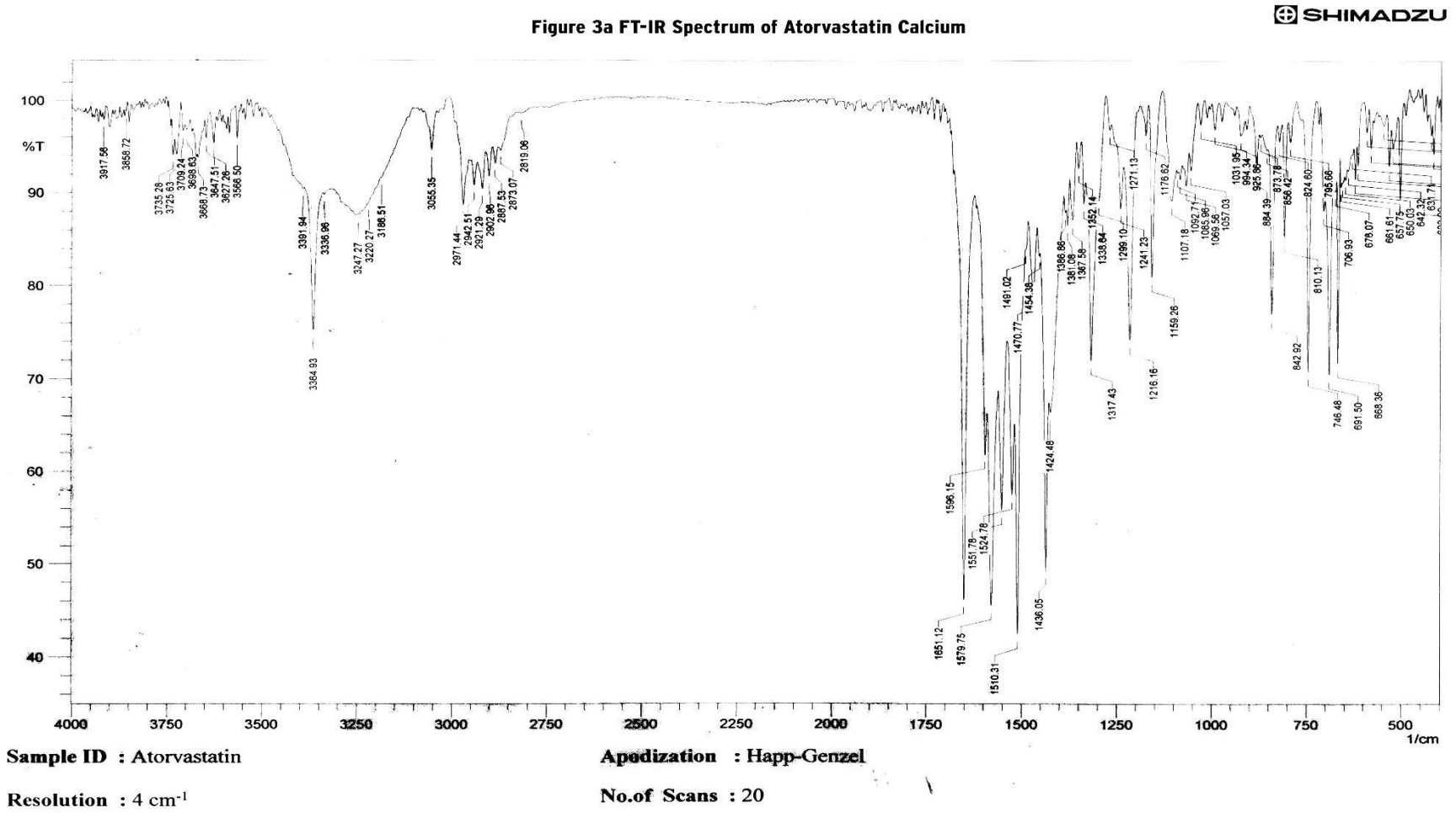


Figure 3a FT-IR spectrum of Atorvastatin calcium

6.1.3 FT-IR studies for Glibenclamide

From the Figure 3b, the IR spectrum of Glibenclamide showed the characteristic bands of N-H stretching of amide group at 3315.41 cm^{-1}. The bands observed at 3033.82 cm^{-1} and 1714.60 cm^{-1} indicated CH=CH stretching and C=O stretching of the spectrum. The characteristic three bands observed at 1593.09 cm^{-1}, 1618.17 cm^{-1}, 1523.66 cm^{-1} were exhibited due to ring C=C stretching, N-H bend scissoring, and N-H bending. A secondary weaker band formed by interaction between the N-H bending and C-N stretching was found to be at 1247.86 cm^{-1}. From the characteristic peaks of IR spectrum, as shown in Figure 3b, it was confirmed that the drug was Glibenclamide.

The melting point and FTIR studies revealed that the drug samples are pure API of Pharmacopoeial standard.

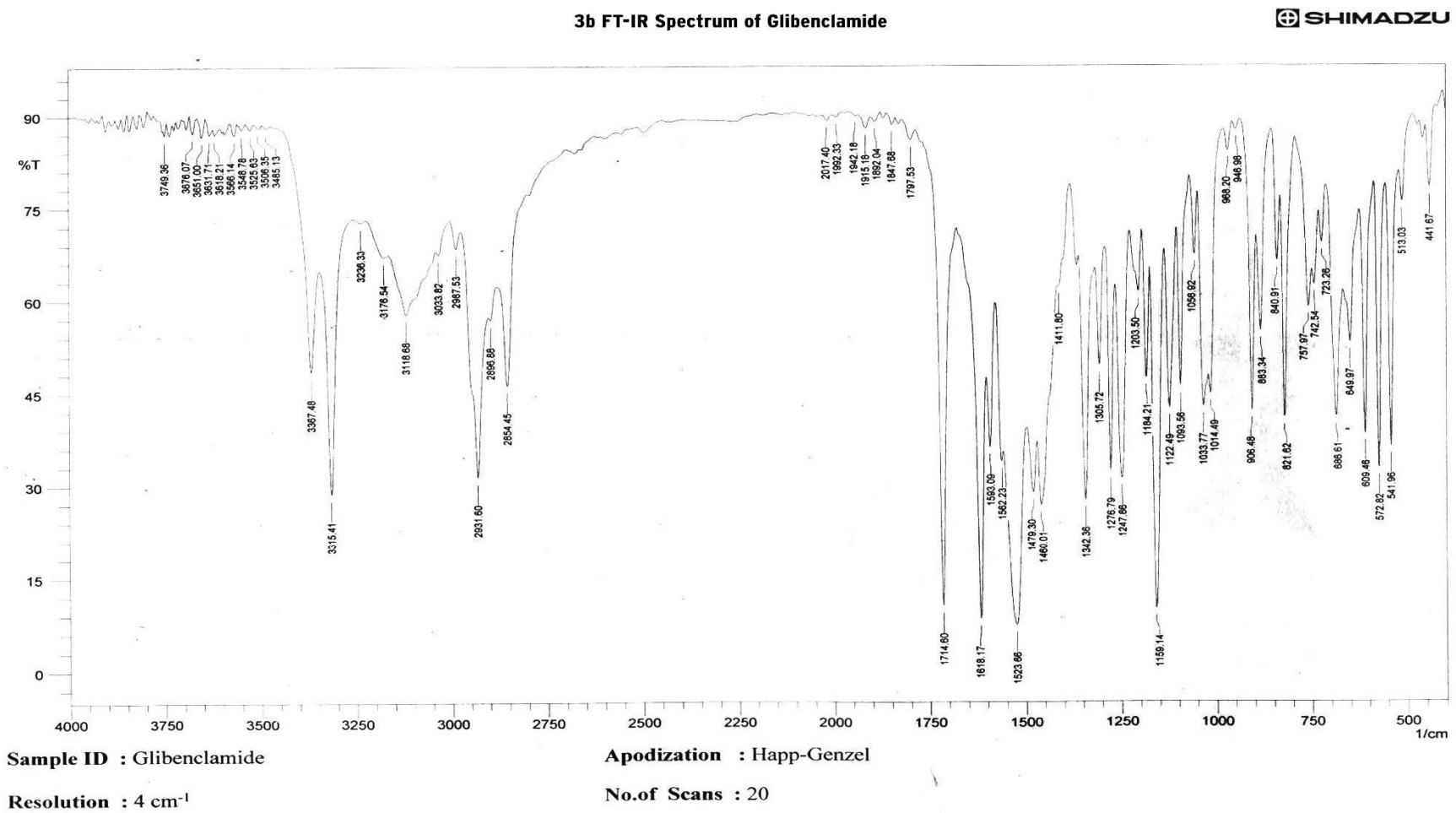


Figure 3b FT-IR spectrum of glibenclamide

Identification test for oils

6.1.4 Specific gravity

The specific gravity of the oils were determined as mentioned in Bureau of Indian standards of Indian standard specification given under the methods of sampling and test for oils and fats IS 548- 1.The results obtained were within specific gravity range are given as follows

Name of oil	Specific gravity	Specific gravity limits as per standards
Virgin sesame oil	0.917	0.916-0.921(Complies as per USP 2009)[152]
Sunflower oil	0.916	0.914-0.924 (Complies as per USP 2009) [152]
Corn oil	0.915	0.914-0.921(Complies as per USP 2009) [152]
Mustard oil	0.920	0.914-0.923 (Complies to USP twelfth revision 1942)[153]
Rice bran oil	0.912	0.910-0.920 (Complies as per BIS IS 3448- 1984)[67]
Olive oil	0.913	0.910-0.915 (Complies as per USP 2009) [152]
Virgin coconut oil	0.918	0.915-0.920 (Complies as per BIS IS 542 1968)[154]
Peceol	0.942	0.942 (Complies as per USP 2009) [152]

6.1.5 Determination of saponification value, Iodine value and acid value for oils

The oils were identified by performing any two assessment tests for oils among saponification value, Iodine value and acid value according to the Bureau of Indian standards for Indian standard specification specified under the methods of sampling and test for oils and fats IS 548-1. The results obtained were given as follows

Virgin sesame oil

Saponification value –191 (complies within the range of 188-195 as per USP 2009) [152]

Iodine value – 110 (complies within the range of 103-116 as per USP 2009) [152]

Virgin coconut oil

Saponification value - 190 (complies within the range of 180-200 as per USP 2009)[152]

Acid value -0.6 (Complies as per BIS IS 542 1968) [154]

Sunflower oil

Saponification value – 192 (complies within the range of 180-200 as per USP 2009)[152]

Iodine value – 110 (complies within the range of 100-140 as per BIS IS 4277-1975)[155]

Corn oil

Saponification value - 189 (complies within the range of 187-193 as per USP 2009)[152]

Iodine value – 110 (complies within the range of 109-133 as per USP 2009) [152]

Mustard oil

Saponification value - 172 (complies within the range of 169-177 as per BIS IS: 546-1975)[62]

Iodine value – 100 (complies within the range of 98-110 as per BIS IS: 546-1975) [62]

Rice bran oil

Saponification value – 188 (complies within the range 180-195 as per BIS IS 3448 1984) [63]

Iodine value – 102 (complies within the range 90-105 as per BIS IS 3448 1984) [63]

Olive oil

Saponification value– 194 (complies within the range of 190-195 as per USP 2009)[152]

Iodine value – 84 (complies within the range of 79-88 as per USP 2009) [152]

Peceol

Saponification value - 164 (complies within the range of 150-175 as per USP 2009)[152]

Iodine value – 75 (complies within the range of 65-95 as per USP 2009) [152]

From the above results, it was analyzed that the natural and synthetic oils used for development of SEDDS are of the pure quality which complies with the tests given under standard specifications.

6.1.6 UV spectroscopic method analysis of Atorvastatin calcium

Linearity and range for calibration curve of Atorvastatin calcium in methanol

The straight line calibration graph was obtained in the concentration of 2-12µg/ml of the Atorvastatin calcium in methanol. The linear regression equation was found to be y=0.045x+0.003 with the correlation co efficient (r^2) of 0.999. The calibration curve was illustrated in Fig. 4a and from the linear regression data (r^2 value) of Table 10a, it can be concluded that the analyzed concentration of the drug solution followed linearity.

Table 10a : Calibration data for Atorvastatin calcium in methanol

S.No.	Concentration (µg/ml)	Absorbance
1.	2	0.0913
2.	4	0.1908
3.	6	0.2836
4.	8	0.3774
5.	10	0.4625
6.	12	0.5465

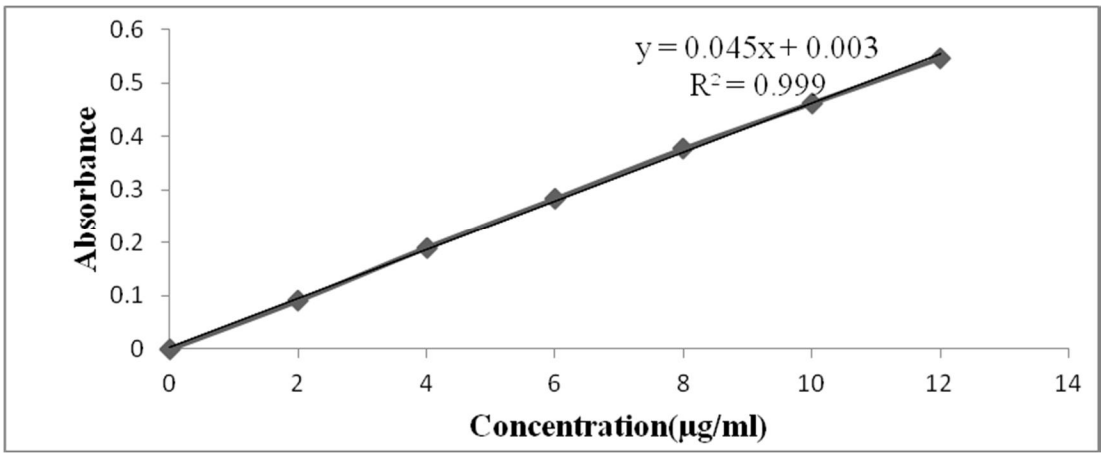


Fig.4a Calibration curve of Atorvastatin calcium in methanol

6.1.7 Linearity and range for calibration curve of Atorvastatin calcium in phosphate buffer pH 6.8

The straight line calibration graph was obtained in the concentration 2-12 µg/ml of the Atorvastatin calcium phosphate buffer pH 6.8.The linear regression equation for Atorvastatin calcium in phosphate buffer pH 6.8 is y=0.012x+0.001 with the correlation co efficient of 0.999. The calibration curve was illustrated in Fig. 4b and from the linear regression data (r^2 value) of Table 10b, it can be concluded that the analyzed concentration of the drug solution followed linearity.

Table 10b : Calibration data for atorvastatin calcium in phosphate buffer pH 6.8

S.No.	Concentration (µg/ml)	Absorbance
1.	2	0.0265
2.	4	0.0529
3.	6	0.0795
4.	8	0.1046
5.	10	0.1279
6.	12	0.1535

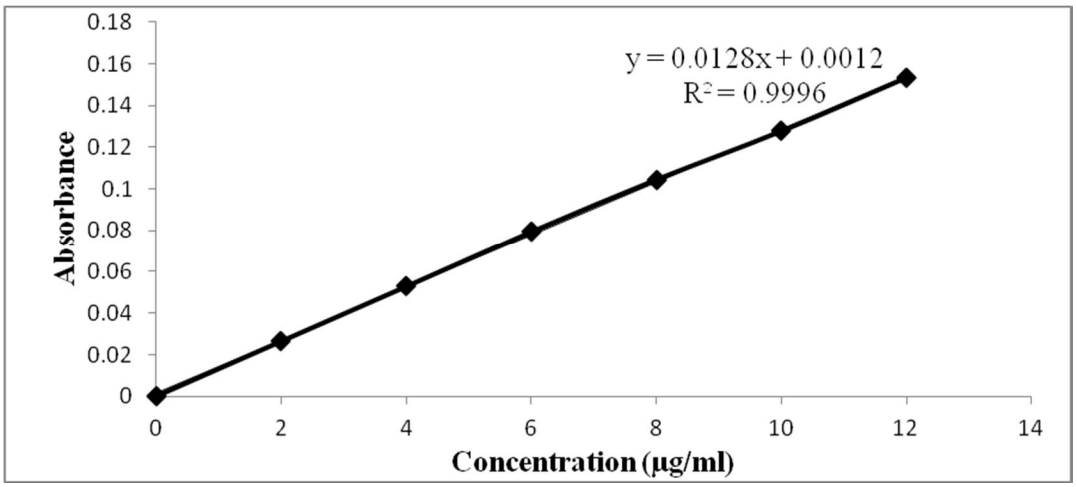


Fig.4b Calibration curve of Atorvastatin calcium in phosphate buffer pH 6.8

6.1.8 Linearity and range for calibration curve of Glibenclamide in methanol

The straight line calibration graph was obtained in the concentration 1-6 µg/ml of the Glibenclamide in methanol. The linear regression equation of Glibenclamide in methanol was y=0.118x+0.002 with the correlation coefficient of 0.999. The calibration curve was illustrated in Fig.5a and from the linear regression data (r^2 value) of Table 11a, it can be concluded that the analyzed concentration of the drug solution followed linearity.

Table 11a : Calibration data for glibenclamide in methanol

S.No.	Concentration (µg/ml)	Absorbance
1.	1	0.1186
2.	2	0.2472
3.	3	0.3558
4.	4	0.4774
5.	5	0.5980
6.	6	0.7116

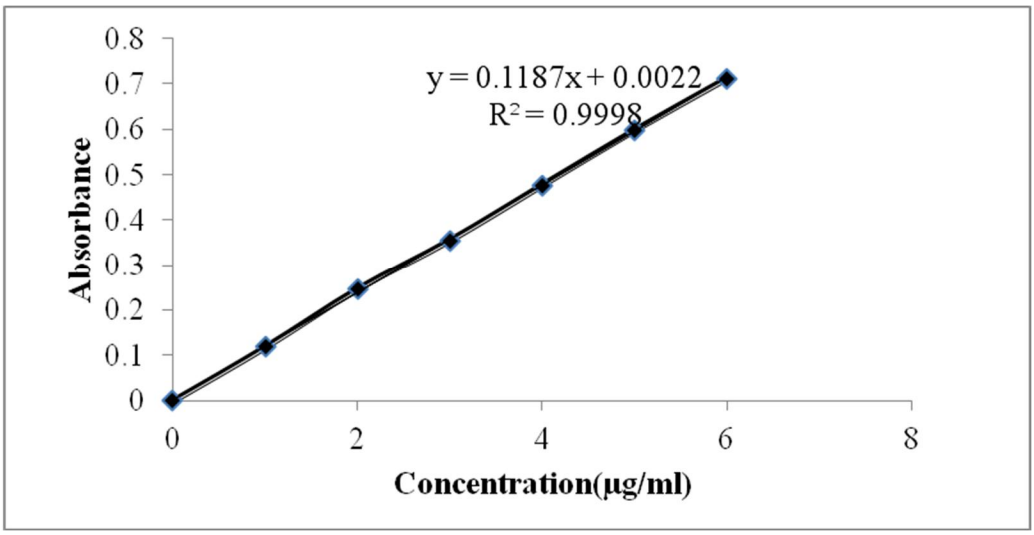


Fig.5a Calibration curve of Glibenclamide in methanol

6.1.9 Linearity and range for calibration curve of Glibenclamide pH phosphate buffer 7.4

The straight line calibration graph was obtained in the concentration 2-12 or 1-6µg/ml of the Glibenclamide in pH phosphate buffer 7.4. The linear regression equation for Glibenclamide in phosphate buffer pH 7.4 is y=0.018x+0.001 with the correlation co efficient of 0.999. The calibration curve was illustrated in Fig.5b and from the linear regression data (r^2 value) of Table 11b, it can be concluded that the analyzed concentration of the drug solution followed linearity.

Table 11b : Calibration data for glibenclamide in phosphate buffer pH 7.4

S.No.	Concentration (µg/ml)	Absorbance
1.	2	0.038
2.	4	0.076
3.	6	0.112
4.	8	0.152
5.	10	0.188
6.	12	0.221

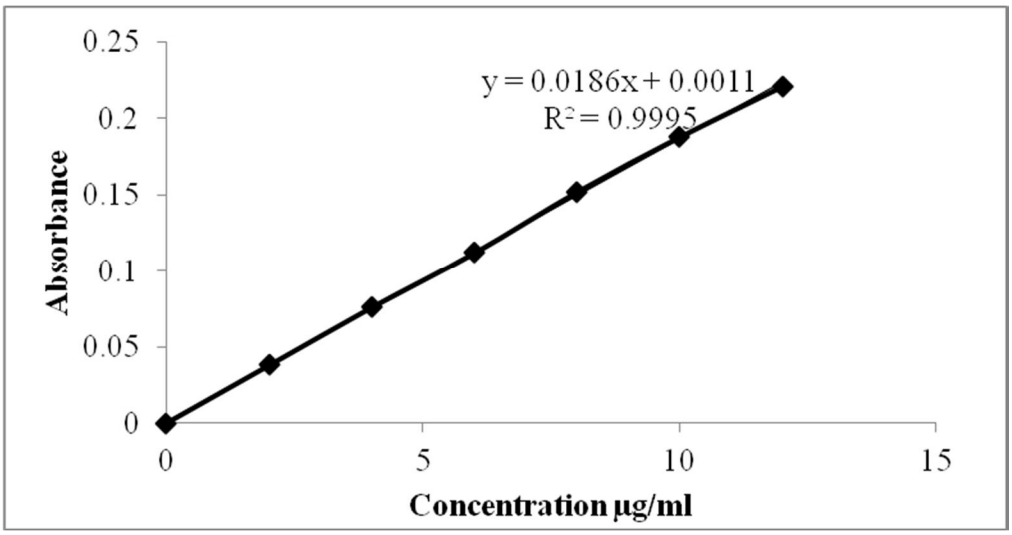


Fig.5b Calibration curve of glibenclamide in phosphate buffer pH 7.4

6.2 SOLUBILITY STUDY

6.2.1 Solubility of Atorvastatin calcium in various excipients

Atorvastatin calcium was found to be insoluble in aqueous acidic solutions of pH 4.0. It was very slightly soluble in water, pH 7.4 phosphate buffer, acetonitrile and ethanol as indicated in Table 12. The drug was found to be freely soluble in methanol, dimethylsulphoxide and dimethylformamide. Further, as Atorvastatin calcium is classified as class II drug of BCS classification it can be considered an ideal candidate for formulation into SEDDS.

The most important criterion for the screening of components for lipid based formulation, is the solubility of the drug in oil, surfactant and cosurfactant. The solubility of a drug in oil is more important because the ability of lipid based formulation to maintain the drug in solubilized form is greatly influenced by the solubility of the drug in the oil phase. At least one dose of the drug should be soluble in the oil phase otherwise it becomes necessary to add the higher amount of surfactant and co-surfactant to dissolve the drug. The drug was found to be more soluble in oils with high HLB value. The function of the oil phase in the self-emulsifying system is to solubilize the hydrophobic/lipophilic active moiety in order to improve both drug loading and bioavailability of the hydrophobic active moiety. Therefore selection of oil plays a vital role in the formulation as it determines the amount of drug that can be

solubilized in the system. A lipid molecule with a large hydrophobic portion compared to hydrophilic portion is desirable as it maximizes the amount of drug that can be solubilized.

The solubility of the Atorvastatin calcium in the various oils, surfactants and co-surfactants were tabulated in Table 12 and the solubility graph was illustrated in Fig.6a. Sunflower oil of solubility of 30.13 mg/ml showed the best solubility for Atorvastatin calcium and it can be seen that one dose of the drug can be successfully dissolved in sunflower oil. The sunflower oil which is a long chain triglyceride consisting of linoleic acid as a major component has been used as a carrier for poorly soluble drugs thus it was selected as oil phase for further study. The hydrophilic surfactants of labrasol tend to show higher solubility for Atorvastatin calcium than the hydrophobic surfactants such as capryol PGMC, labrafil M 1944CS, labrafil M 2125 CS as indicated in Table 12. However, labrasol having caprylic (C8) and capric (C10) fatty acid esters of glycerol in its composition showed the highest solubility of 89.23 mg/ml than the other surfactants. Transcutol HP which showed highest solubilization capacity of 38.62mg/ml was chosen as cosurfactant for further study. The drug content of Atorvastatin calcium was calculated using the Beer Lambert's equation (y = 0.045 × concentration + 0.003).

6.2.2. Solubility of Glibenclamide in various excipients

Glibenclamide was found to be practically insoluble in water as indicated in Table 12. It was found to be slightly soluble in methanol. The drug was found to be sparingly soluble in dichloromethane. It was confirmed that Glibenclamide is classified as class II drug of BCS classification due to poor aqueous solubility and it can be considered as an ideal candidate for formulation into SEDDS. Peceol which is a long chain monoglyceride showed a maximum solubilization capacity of 7.83 mg/ml was selected as oil phase for the drug. The labrasol showed the maximum solubilization capacity of 9.52 mg/ml was selected as surfactant among the other hydrophobic surfactants as indicated in Table 12. The transcutol HP showed the maximum solubilizing capacity of 38.62mg/ml and it was selected as cosurfactant for further study. The drug content of Glibenclamide was calculated using the Beer Lambert's equation (y =0.118× concentration + 0.002).

Table 12 : Solubility of Atorvastatin calcium and Glibenclamide in various excipients

S.No.	Excipients	Atorvastatin calcium Solubility (mg/ml)	Glibenclamide Solubility (mg/ml)
	Oils		
1.	Virgin sesame oil	15.36±0.006	3.5±0.012
2.	Virgin coconut oil	25.37±0.015	6.30±0.007
3.	Sunflower oil	30.13±0.02	2.28±0.001
4.	Corn oil	4.86±0.030	4.89±0.009
5.	Mustard oil	10.35±0.01	2.34±0.002
6.	Rice bran oil	12.29±0.040	4.25±0.001
7.	Olive oil	17.62±0.010	5.65±0.003
8.	Peceol	12.84±0.021	7.83±0.015
	Surfactants		
9.	Labrasol	89.23±0.015	9.52±0.016
10.	Labrafil 1944CS	1.78±0.011	6.24±0.004
11.	Labrafil 2125	1.62±0.012	1.26±0.011
12	Capryol PGMC	2.22±0.006	2.26±0.052
	Co-surfactant		
13.	Transcutol HP	38.62±0.28	18.12±0.018
	Solvents		
14.	Distilled water	0.0096±0.012	0.0001±0.008
15..	Methanol	0.666±0.002	0.0092±0.002
16.	pH Phosphate buffer 7.4	0.0095±0.013	-
17.	Acetonitrile	0.0092±0.003	-
18.	Ethanol	0.0089±0.014	0.0085±0.012
19.	Dimethyl sulphoxide	0.0793±0.022	-
20.	Dimethyl formamide	0.0757±0.003	-
21.	Dichloromethane	-	0.0305±0.004
22.	Aqueous solution of pH 4	0.02± 0.005	-

* Values are mean ±S.D (n=3)

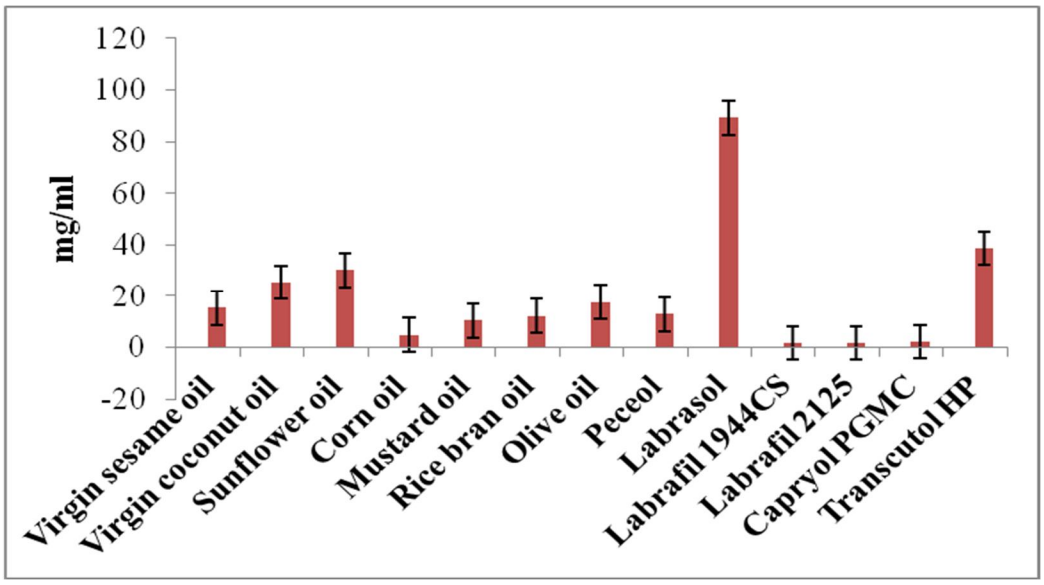


Fig. 6a Solubility profile of Atorvastatin calcium

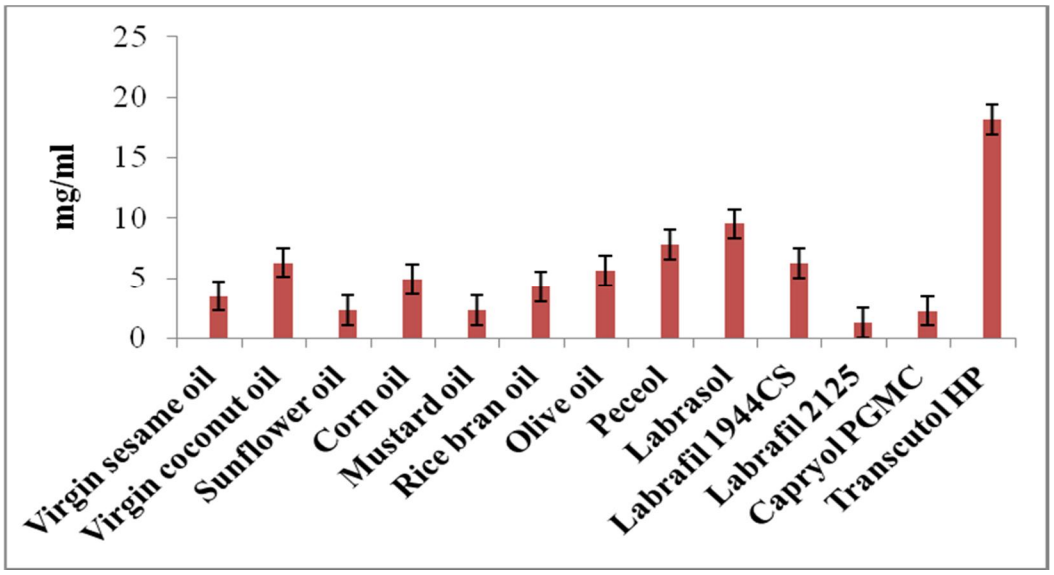


Fig. 6b Solubility profile of Glibenclamide

6.3 CONSTRUCTION OF TERNARY PHASE DIAGRAM

Ternary phase diagrams were designed in order to demonstrate regions of nanoemulsion formation. Variable proportions of oil, surfactant and co-surfactant were tested. The shaded darker region in the ternary phase diagram (Fig.7a and Fig.7b) represents the efficient self-emulsifying region where desired visual

observation characteristics were observed for clarity of the solution, no phase separation, rapidity and spontaneity of the emulsion formation. The range and level for each component (independent variables) was selected as oil (40–80%), surfactant (22.5–52.5%), co-surfactant (7.5–17.5%) for Atorvastatin calcium and oil (15-30%) surfactant (15-25%), cosurfactant (15-25%) for Glibenclamide as shown in Table 13a and Table 13b. The border lines are shown in the Fig. 7a and Fig. 7b which is surrounded by the shaded region indicate the boundary of the level used in the 3^2 factorial design study and the polygonal area bounded by all the border lines indicate the region from which optimum formulation is to be selected. During emulsification, the surfactant molecules migrate to the o/w interface and lower the interfacial tension. By adding cosurfactant, the interfacial tension further decreases and the induction of ideal curvature of an interfacial film takes place. The droplet size decreases and the net outcome is the negative value for the free energy of microemulsion formation which means spontaneous microemulsion formation. As the water is always in considerable abundance and oil volume fraction is low, it was safely supposed that only o/w emulsion was formed and no other dispersed and bicontinuous pseudo phases were formed.

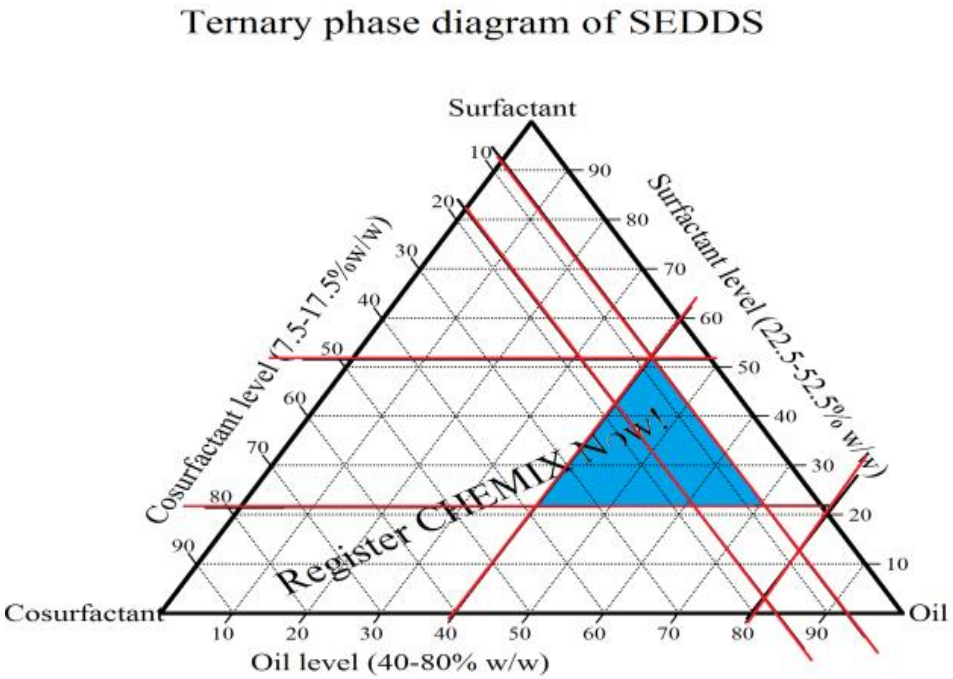


Fig. 7a Ternary phase diagram of Atorvastatin calcium SEDDS

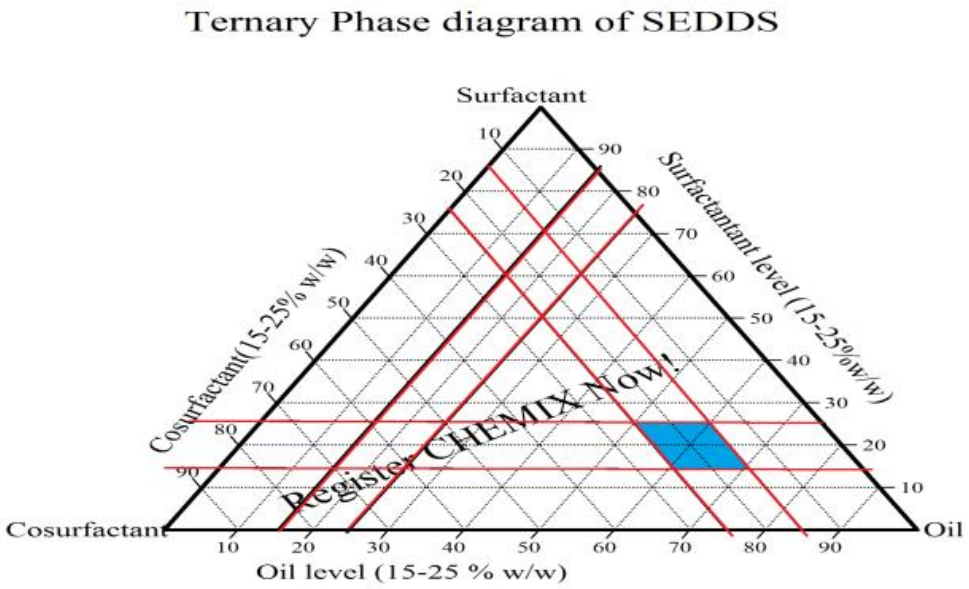


Fig. 7b Ternary phase diagram of Glibenclamide SEDDS

6.3.1 Variables selected for development of Atorvastatin calcium and Glibenclamide SEDDS

Based on the feasibility of micro emulsion formation at extreme values, the range for each component was selected as follows: oil (40-80%), Smix (30-70%) for Atorvastatin calcium and oil (15-25%), Smix (30-50%) for Glibenclamide respectively. The slack variable was taken as water content as it is present in the larger amount in a gastrointestinal tract. The dependent variables which are significant response factors studied for assessing the quality of SEDDS are particle size (Y_1) and % drug loading (Y_2). The optimization of the SEDDS was done using by 3 level 2 factorial design. From the preliminary solubility and ternary phase diagram studies the amount of sunflower oil (X_1) as lipophile and the amount of surfactant mixture (X_2) of labrasol and transcutol HP were selected as the two independent variables for the development of Atorvastatin calcium SEDDS. The amount Peceol (X_1) and surfactant mixture (X_2) of Labrasol and transcutol HP were the two independent variables selected for the formulation of Glibenclamide SEDDS. The three levels of each factor were used to construct experimental design. The levels for sunflower oil (40, 60 80), labrasol and transcutol HP (30, 50, 70) for a formulation of Atorvastatin calcium SEDDS were selected from the preliminary study as depicted in Table 13a. The

levels for peceol (15, 20, 25), labrasol and transcutol HP (30, 40, 50) were chosen for development of Glibenclamide SEDDS as illustrated in Table 13b.

Table 13a : Variables for Atorvastatin calcium in 3^2 full factorial Design

Independent Variables[(a)]	**Levels**		
	Low (-1)	**Middle (0)**	**High (-1)**
X_1: Amount of oil added (mg)	40	60	80
X_2:Amount of Smix in ratio of 3:1 added (mg)	30(22.5:7.5)	50(37.5:12.5)	70(52.5:17.5)
Dependent Variables	**Constraints**		
	Range		**Goal**
Y_1: Particle size (Globule Size in nm)	In the range		Minimize
Y_2: % drug loading	In the range		Maximize

(a) Oil: Sunflower oil; Surfactant: Labrasol; Cosurfactant: Transcutol HP

Table 13b : Variables for Glibenclamide in 3^2 full factorial Design

Independent Variables[(a)]	**Levels**		
	Low (-1)	**Middle (0)**	**High (-1)**
X_1: Amount of oil added (mg)	15	20	25
X_2: Amount of Smix in ratio of 1:1added (mg)	30(15:15)	40(20:20)	50(25:25)
Dependent Variables	**Constraints**		
	Range		**Goal**
Y_1: Particle size(Globule Size in nm)	In the range		Minimize
Y_2: % drug loading	In the range		Maximize

(a) Oil: Peceol; Surfactant: Labrasol; Cosurfactant: Transcutol HP

6.4 STATISTICAL ANALYSIS OF THE DESIGNED EXPERIMENT

The range of oil (X_1), Smix (X_2) were delimited as independent variables; 3^2 full factorial design was performed to optimize SEDDS with constraints on globule size and drug load as the Response Surface methodology (RSM) requires 13

experiments and the observed responses are summarized in Table 14a and Table 14b. All the data were fitted to the second order quadratic model and validation of the model was carried out by analysis of variance (ANOVA) test, lack of fit test and correlation coefficient (R^2). The significance of the ratio of mean square variation due to regression and residual error was tested using analysis of variance (ANOVA). The ANOVA indicated a significant ($p<0.05$) effect of factors on a response. Various statistical evaluations of models for each response are depicted in the Tables 15a, Table 16a and Table 15b, Table 16b for Atorvastatin calcium and Glibenclamide. As shown in Table 15a, 15b at 5% significance level, it was observed that for responses Y_1, and Y_2, quadratic fitting was significant (p-value <0.05). For the Y_1 response of Atorvastatin calcium, the "Lack of Fit F-value" of 32.97 implies the Lack of Fit is significant. There is only a 0.28% chance that a "Lack of Fit F-value" this large could occur due to noise. For the Y_2 response of Atorvastatin calcium response, the lack of fit was The "Lack of Fit F-value" of 1.93 implies the Lack of Fit is not significant relative to the pure error. There is a 26.69% chance that a "Lack of Fit F-value" this large could occur due to noise. For the Y_1 response of Glibenclamide, the "Lack of Fit F-value" of 182.64 implies the Lack of Fit is significant. There is only a 0.01% chance that a "Lack of Fit F-value" this large could occur due to noise. For the Y_2 response of The "Lack of Fit F-value" of 2.02 implies the Lack of Fit is not significant relative to the pure error. There is a 25.33% chance that a "Lack of Fit F-value" this large could occur due to noise. Non-significant lack of fit is good while calculating the correlation coefficient (R^2) for the responses Y_1, and Y_2 the confidence that the regression equations would predict the observed value better than mean were more than 83.22%, 93%, respectively (Table 16a) for Atorvastatin calcium and 79.6%,79.37% for Glibenclamide (Table 6b). The corresponding coefficients which showed the quantitative effects of independent variables (X_1 and X_2) and their interactions on the responses are shown in the Tables 17a and Table 17b. The coefficients (Factor intercepts) ($X_1 \cdot X_2$,) and those with the higher order terms (X_1^2, X_2^2) indicate the interactions and quadratic effects, respectively. For all the models the predicted R^2 value is reasonable agreement with the adjusted R^2 value. Adequate precision values higher than 4 for all responses confirmed that the predicted models can be used to navigate the design space defined by full factorial design. A positive

value represents an effect that favors the optimization and negative value indicates an inverse relationship between the factor and response.

Table 14a : Execution of 3^2 Experimental Design and coding of actual values of independent variables for factorial design with the observed responses for Atorvastatin calcium

Std	Run	Formulation Code (FC)	Oil (mg)	Smix (mg)	Y_1(Particle size) (nm)	Y_2(%Drug Loading)
7	1	AF1	-1(40)	+1 (70)	106.8±4.08	81.8±6.63
4	2	AF2	-1(40)	0 (50)	172±7.5	83.1±4.54
6	3	AF3	+1(80)	0 (50)	290±4.9	91.5±2.78
10*	4	AF4*	0 (60)	0 (50)	112.4±8.5	85.1±2.71
13*	5	AF5*	0 (60)	0 (50)	128.5±5.68	84.3±3.05
9	6	AF6	+1(80)	+1 (70)	285±8.6	87.6±1.65
5	7	AF7	0 (60)	0 (50)	137.9±5.5	88.7±1.1
2	8	AF8	0(60)	-1 (30)	197.6±5.65	75.1±2.75
8	9	AF9	0 (60)	+1 (70)	233.1±3.44	86.1±4.37
3	10	AF10	+1 (80)	-1 (30)	229.7±4.98	89.1±4.53
11*	11	AF11*	0 (60)	0 (50)	140.2±3.0	85.7±4.70
1	12	AF12	-1 (40)	-1 (30)	415±8.7	70.1±2.25
12*	13	AF13*	0 (60)	0 (50)	114.9±7.1	86.9±1.21

Y_1: Particle size; Y_2: Drug Load; *Centre point Formulations

The coded and actual values for the factors used in the 3^2 factorial design for Atorvastatin calcium at three levels are stated as below

Factors		Factor Level used		
		Low level	Mid Value	High Value
Coded value	X_1 & X_2	-1	0	+1
Actual value	X_1	40	60	80
Actual value	X_2	30	50	70

X_1 is the % amount of sunflower oil in mg
X_2 is the % amount of Smix (Labrasol and Transcutol) in mg.

Table 14b : Execution of 3^2 Experimental Design and coding of actual values of independent variables for factorial design with the observed responses for Glibenclamide

Std	Run	Formulation Code	Oil (mg)	Smix (mg)	Y_1 (Particle size) (nm)	Y_2 (% Drug Loading)
10*	1	GF1*	20(0)	40(0)	411.5	87.8 ± 2.25
13*	2	GF2*	20(0)	40(0)	410.3	90 ± 3.7
3	3	GF3	25(+1)	30(-1)	415.2	82 ± 1.9
6	4	GF4	25(+1)	40(0)	233.1	84 ± 4
5	5	GF5	20(0)	40(0)	402.3	91.2 ± 5.4
9	6	GF6	25(+1)	50(+1)	229.7	84.6 ±3.7
12*	7	GF7*	20(0)	40(0)	421.6	89 ± 4.16
2	8	GF8	20(0)	30(-1)	464.1	85.6 ± 2.64
8	9	GF9	20(0)	50(+1)	616.3	92.3 ± 4.12
7	10	GF10	15(-1)	50(+1)	169.7	87.6 ± 1.75
4	11	GF11	15(-1)	40(0)	222.2	92.3 ± 3.26
11*	12	GF12*	20(0)	40(0)	401	92 ± 5.26
1	13	GF13	15(-1)	30(-1)	284.2	88.8 ± 2.38

The coded and actual values for the factors used in the 3^2 factorial design for glibenclamide at three levels are stated as below

Factors		Factor Level used		
		Low level	Mid value	High value
Coded value	X_1 & X_2	-1	0	+1
Actual value	X_1	15	20	25
Actual value	X_2	30	40	50

X_1 is the % Amount of Peceol in mg

X_2 is the %Amount of Smix (Labrasol and Transcutol) in mg

Analysis of Variation and Regression[156, 157]

The observations resulting from a designed experiment are often examined using analysis of variance (ANOVA) techniques in which the variance associated with a particular independent variable or interactions between the variable or interactions between independent variables is compared with the variance associated with the random error that occurs in the experiment. If there are a difference variable and error variances, then the treatment being tested is considered to have a significant effect on the measured response. The comparisons between the variances are made typically using an F test or F distribution. The ANOVA was performed to check the adequacy of suggested models and identify the significant factors. In statistically the mathematical models were evaluated for each response by means of multiple linear regression analysis. The regression analysis is a technique to determine the relationship exists between experimental variables and a response variable. The specific relationship defined for a response variable (Y) and the experimental or independent variables (X) is known as the regression model. The modeling was stated with a quadratic model, including linear, squared and interaction term. A linear polynomial regression model can be used to approximate the relationship between the response variable and independent variables The linear first order polynomial is represented as by $Y = \beta_0 + \beta_1 X + \varepsilon$ where Y is the response, X_1, X_2 are the independent variables and ε is the random error term at which the standard deviation is zero. The β_0 and β_1 are the regression model coefficients that estimate the linear or main effects of the independent variables. The two level factorial design is suitable for linear first order model. For estimation of interaction and quadratic effects the second order polynomial model which involve three levels for each variable are chosen in the experimental design. The least square method of analysis is employed to fit a mathematical model to the data. The estimates of the regression coefficients and the predicted values are unbiased with least variance if the following statements are true

a) The X values are fixed and not random variables
b) The deviations (ε_i) are independent, uncorrelated and have a mean of zero.
c) The variance of the deviations are constant
d) The deviations are normally distributed

The significant term in the model was found by analysis of variance (ANOVA) for each response. The significance was judged by determining the probability level that the p- statistics calculated from the data is less than 5%. The model accuracy was checked by R^2, adjusted R^2 and Predicted R^2 and prediction error sum of squares (PRESS). A good model will have larger predicted R^2 and low press values. The total data variance is divided into two main contributions, the sum of squares explained by the regression, $SS_{R,}$ and the residual sum of squares $SS_{r.}$ Both summations are taken over all the experimental design levels, j = 1,2… m and all the replicates performed at each level, j =1,2……..$n_{i..}$ SS_R is a sum of squares of differences between values predicted by the regression and the grand average of all the response values and has p-1 degrees of freedom where p is the number of coefficients in the model. SS_r is a sum squares of differences or residuals between all the experimental values and the predicted values from the model. It has n-p degrees of freedom where n is the total number of experimental data used to determine the model. The large SS_R and small SS_r values tend to occur for models that accurately describe the experimental data. The total sum of squares of differences between the experimental values and the grand average of the data set happen when their sum is equal to SS_T. The sum has n-1 degrees of freedom since it represents the total variance in the data. The SS_R/SS_T ratio represents the fraction of explained variation and is commonly represented as R^2, the coefficient of determination which varies between 0 and 1. If the pure error exists, it is impossible for R^2 to actually attain 1. Although the coefficients is a measure of how close the model fits the data and it cannot be used to judge the model lack of fit because it does not take in to account the numbers of a degree of freedom for model determination. A real statistic $R^2_a = [1-(1-R^2) \{(n-1)/ (n-p)\}]$ makes an adjustment for the varying numbers of a degree of freedom in the models being compared. The model quality can only be rigorously judged if the SS_r is decomposed into two contributions, the lack of fit and the pure error sums of squares, SS_{lof} and SS_{pe}. The SS_{pe} is a sum of squares of differences between all the individual experimental values and the average of the experimental values at the same level. It has n−m degrees of freedom where m is the number of distinct levels in the experimental design. The SS_{lof} is a sum of squares of differences between the values predicted at each level and the average experimental value at that level and has m−p

degrees of freedom. The regression lack of fit is determined to perform an F-test by comparing the SS_{lof} /SSpe ratio with the tabled F value for m−p and n−m degrees of freedom at the desired confidence level, usually 95%. If the calculated quotient is greater than the tabled value there is evidence of model lack of fit and the model must be discarded. The model can be accepted at this confidence level as providing an adequate representation of the data. The regression significance can be tested by comparing the calculated SS_R/SSr value with the tabled F-distribution value for p−1 and n−p degrees of freedom. The regression is significant if the calculated value is greater than the tabled one. The F-test is only valid for models for which there is no evidence of lack of fit. The regression model failed to explain experimental error and the maximum % of explainable variation is given by $[(SS_T - SSpe)/SS_T] \times 100\%$. The terminology of the following terms used to calculate the accuracy of the model is

Standard deviation: (Root MSE) square root of the residual mean square is used to be an estimate the standard deviation.

Mean: The overall average of the all response data

Co-efficient of variation: It is the standard deviation expressed as the % of mean. It is calculated by dividing the standard deviation by the mean and multiplying by 100.

PRESS: It is the predicted residual error sum of squares and basically it is a measure of how well model from this experiment is likely to predict the response in a new experiment. The smallest of PRESS are values are desirable.

Adjusted R^2: It is a measure of the amount of variation around the mean explained by the model adjusted for the number of terms in the model. The adjusted R^2 decreases the number of terms in the model increases if those additional terms are not added value to the model.

Predicted R^2: It is a measure of the amount of variation in new data explained by model.

Adequate precision: It is basically a measure of S/N ratio (Signal to noise). It is explained by a factor to judge the model if it is adequate to navigate through the design space and can able to predict the response. The desired values should be > 4.0.

The Analysis of Variance for the least squares fit of a model which is linear in its parameters are illustrated below

Source of variation	Sum of squares	Degrees of freedom	Mean square
Regression	$SS_R = \sum_{i}^{m}\sum_{j}^{n_i} (\hat{y}_i - \bar{y})^2$	p-1	$MS_R = [SS_R / (p-1)]$
Residual	$SS_R = \sum_{i}^{m}\sum_{j}^{n_i} (y_{ij} - \hat{y}_i)^2$	n-p	$MS_r = [SS_r / (n-p)]$
Lack of fit	$SS_{lof} = \sum_{i}^{m}\sum_{j}^{n_i} (\hat{y}_i - \bar{y})^2$	m-p	$MS_{lof} = [SS_{lof} / (m-p)]$
Pure error	$SS_{pe} = \sum_{i}^{m}\sum_{j}^{n_i} (y_{ij} - \bar{y})^2$	n-m	$MS_{pe} = [SS_{pe} / (n-m)]$
Total	$SS_R = \sum_{i}^{m}\sum_{j}^{n_i} (y_{ij} - \bar{y}_i)^2$	n-1	

n_i: number of replicates at the i^{th} level; m: number of distinct levels of the independent variables; $n = \sum ni$ = total number of observations; p: number of parameters in the model.

The adequacy of the regression model which is fitted to the experimental data is tested by F test (test for significance of the regression) is provided by ANOVA. The test is comparison of two estimates of the variance. The mean square for error (MSE) yields one unbiased estimate s^2 of the population variance σ^2 if the regression coefficients are zero. The regression coefficient which differs significantly from zero is tested by F test ratio which is given by $F = MSR/s^2$.If the F value is greater than the tabulated critical F value it is indicated that the regression is significant. If the F value is less than the critical F it is suggested that both MSR and s^2 provided reasonable unbiased estimates of σ^2 and the regression is nonsignificant.

A literature search revealed an exhaustive number of publications characterizing the self-emulsified drug delivery system[158]. The reported studies used different methods for *in vitro* evaluation such as self-emulsification time, cumulative percent release, low frequency dielectric spectroscopy, zeta potential measurement

and surface tensiometry. The particle size and drug loading are critical formulation parameters used to help maximize the pharmacokinetics of small molecules[159]. In this present work the particle size of SEDDS after dilution was chosen as Y_1 variable was selected as criteria for *in vitro* evaluation. If the particle size of SEDDS is less, the release of drug will be more resulting with better bioavailability in the formulation of SEDDS. The particle size of around 20 nm gives total transparent system upon dilution, which acts as a solution. Drug loading is a critical parameter which affects the therapeutic efficacy, pharmacokinetics and toxicity of the drug. Higher drug loading is preferable because less non-active excipients are used to produce the same quantity of API in the SEDDS formulation. At a higher drug loading, lower quantity of oils and surfactants (non active ingredients) need to be manufactured to deliver an equivalent dose of API. The benefit of maximum drug loading is that the quantity of surfactants incorporated in the SEDDS formulation can be reduced which in turn reduces irritation on GIT due to large quantity of the surfactants. It can also reduce the manufacturing and processing time, raw material usage. So, particle size was selected as criteria for the optimization. The % drug loading was selected as Y_2 variable because the higher the drug loading in SEDDS formulation reduces final dose of the drug and improves patient compliance with minimum GIT irritation and side effects.

Evaluation of responses in ANOVA [156]

In the design of experiments, it is essential to test the quality of results prior to evaluation. The coefficient of variation (CV= standard deviation/mean) for each dependent variable should be calculated and if the results are below 10 %, they might be considered excellent while values up to 20% are considered acceptable. Once the mathematical model has been selected it is important to determine its significance by means of a variance analysis (ANOVA). The standard deviations of the main and the interactions effects of the selected factors are calculated by ANOVA. If the standard deviations present a lower value than the mean values it is possible to assume that the mathematical model is significant. If it is not possible, the experimental data should be evaluated in order to not presume that the effect is not significant. In the evaluation of experimental designs, a mathematical model is provided to relate the response variable with the factor effects. In this regard, the goodness of fit of the model needs an assessment and the following criteria are analyzed as follows

- Standard deviation of the estimated parameters and model
- Statistical significance of the estimated parameters
- Regression coefficient
- Value of the objective function
- Significance of the regression (ANOVA)
- Analysis of the residuals.

It is considered a good fit to the experimental data when the standard deviation of the parameter presents a lower value than the correspondent effect indicating that the standard deviation of the proposed mathematical model is low and the parameters of a model need to be significant otherwise they will not contribute to the model. It is considered hypothetically that if the model presents a regression coefficient (R^2) above 90% then it is considered excellent. It is only one criterion to evaluate the model goodness of fit. If a regression coefficient is low (<70%), the mathematical model is not good and on the other hand if its value is high (>90%), it means that other statistical criteria such as t and F test are used. By using an appropriate estimation method applied to the chosen model, the regression coefficients will be obtained and the estimated response can be easily calculated. For verification of the model adequacy, several techniques are used like residual analysis, scaling residuals, prediction of error sum of squares residuals and tests of lack of fit. The lack of fit is a measure of a model failure in representing data in the experimental domain significant lack of fit as indicated by a low probability value ($p < 0.05$) and the response predictor is discarded. The overall predictive capability of the model is commonly explained by the regression coefficient (R^2) but this coefficient alone was not used to measure the model accuracy. R^2 is defined as the ratio of the explained variation to the total variation and is a measure of the degree of fit. The regression coefficient values were used to determine whether the mathematical models can be considered good and it is suggested that for a good model fit R^2 should be at least 80%.

In this present work, the R^2 values for both the drugs were found to be above 80% and % CV below 20% as indicated in Table 16a and Table 16b.Therefore it was clearly analyzed that quadratic model for both the Atorvastatin calcium and Glibenclamide were found to be a good mathematical model.

Table 15a : Analysis of Variance in the regression models for Atorvastatin calcium

Source		DF	Sum of Squares	Mean Square	F Value	p-Value	
Y_1(Globule Size in nm)	Model	5	83517.68	16703.54	6.94	0.0122*	Significant
	A-Oil	1	2049.80	2049.80	0.85	0.3867	
	B-Smix	1	7877.13	7877.13	3.27	0.1133	
	AB	1	33033.06	33033.06	13.73	0.0076**	Significant
	A^2	1	15552.15	15552.15	6.46	0.0385*	Significant
	B^2	1	9741.60	9741.60	4.05	0.0841	
	Residual	7	16841.75	2405.96			
	Lack of Fit	3	16187.12	5395.71	32.97	0.0028**	Significant
	Pure Error	4	654.63	163.66			
	Cor Total	12	1.004E+005				
Y_2 (Drug Loading in %)	Model	5	382.82	76.56	18.59	0.0006**	Significant
	A-Oil	1	183.71	183.71	44.60	0.0003**	Significant
	B-Smix	1	74.91	74.91	18.19	0.0037**	Significant
	AB	1	43.56	43.560	10.58	0.0140*	Significant
	A^2	1	5.02	5.02	1.22	0.3031	
	B^2	1	79.10	79.10	19.20	0.0032**	Significant
	Residual	7	28.83	4.12			
	Lack of Fit	3	17.04	5.68	1.93	0.2669	Insignificant
	Pure Error	4	11.79	2.95			
	Cor Total	12	411.65				

Table 16a : Correlation Coefficients for Two Responses for Atorvastatin calcium

Quadratic model	R^2	Adjusted R^2	Predicted R^2	Adequate precision	SD	%CV
Y1	0.8322	0.7123	-0.5672	7.629	49.05	24.88
Y2	0.9300	0.8799	0.5375	16.864	2.03	2.41

Table 17a : Factor coefficients and their corresponding p-values for Atorvastatin calcium

Factors	Y_1		Y_2	
	Regression Coefficient	Probability value (p-value)	Regression Coefficient	Probability value (p-value)
Intercept	135.117		86.0862	
X_1	18.4833	0.3867	5.53333	0.0003**
X_2	-36.2333	0.1133	3.53333	0.0037**
$X_1.X_2$	90.875	0.0076**	-3.3	0.0140*
X_1^2	75.0397	0.0385*	1.34828	0.3061
X_2^2	59.3897	0.0841	-5.35172	0.0032**

Significant model terms at: ** $p<0.01$, * $p<0.05$.

Table 15b : Analysis of Variance in the regression models for Glibenclamide

Source		DF	Sum of Squares	Mean Square	F Value	p-Value	
Y_1(Globule Size in nm)	Model	5	1.481E+005	29620.87	5.46	0.0230*	Significant
	A-Oil	1	6793.93	6793.93	1.25	0.2999	
	B-Smix	1	3640.81	3640.81	0.67	0.4395	
	AB	1	1260.25	1260.25	0.23	0.6444	
	A^2	1	1.363E+005	1.363E+005	25.14	0.0015**	Significant
	B^2	1	22570.73	22570.73	4.16	0.0807	
	Residual	7	37946.56	5420.94			
	Lack of Fit	3	37671.55	12557.18	182.64	<0.0001	Significant
	Pure Error	4	275.01	68.75			
	Cor Total	12	1.861E+005				
Y_2 (Drug Loading in %)	Model	5	109.20	21.84	5.39	0.0238	Significant
	A-Oil	1	54.60	54.60	13.46	0.0080**	Significant
	B-Smix	1	10.93	10.93	2.70	0.1446	
	AB	1	3.61	3.61	0.89	0.3769	
	A^2	1	17.29	17.29	4.26	0.0778	
	B^2	1	8.00	8.00	1.97	0.2030	
	Residual	7	28.39	4.06			
	Lack of Fit	3	17.11	5.70	2.02	0.2533	Insignificant
	Pure Error	4	11.28	2.82			
	Cor Total	12	137.9				

Table 16b : Correlation coefficients for two responses for Glibenclamide

Quadratic model	R^2	Adjusted R^2	Predicted R^2	Adequate precision	SD	%CV
Y_1	0.7960	0.6504	-0.9761	7.414	73.63	20.45
Y_2	0.7937	0.6463	-0.3551	7.335	2.01	2.28

Table 17b : Factor coefficients and their corresponding p-values for Glibenclamide

Factors	Y_1		Y_2	
	Regression Coefficient	p Value	Regression Coefficient	Probability value (p value)
Intercept	420.9		90.1862	
X_1	33.65	0.2999	-3.01667	0.0080**
X_2	-24.6333	0.4395	1.35	0.1446
$X_1.X_2$	-17.75	0.6444	0.95	0.3769
X_1^2	-222.15	0.0015**	-2.50172	0.0778
X_2^2	90.4	0.0807	-1.70172	0.2030

Significant model terms at: ** $p<0.01$, * $p<0.05$.

6.5 ANALYSIS OF VARIANCE FOR PARTICLE SIZE (Y_1) AND % DRUG LOAD (Y_2)

Atorvastatin calcium SEDDS

The observed values of particle size for 13 formulations as shown in Table 14a varied from 106.8 nm to 415 nm and % drug load varied from 70.1% to 91.5% for Atorvastatin calcium. Two-way analysis of variance (ANOVA) can be applied to determine statistical significance of each model coefficient and least significant difference as post hoc test was performed.

Effect of formulation variables on particle size (Y_1)

The polynomial equation derived for particle size for Atorvastatin calcium is given by

$Y_1 = 135.12+18.48*X_1-36.23*X_2 +90.88*X_1X_2 + 75.04*X_1^2 +59.39 *X_2^2$-Equation 1. with R^2= 0.8322, adjusted R^2= 0.7123 and % CV= 24.88.

For the particle size, the model F value of 6.94 with a low probability value of (p value<0.05) implies a high significance for the full regression model which is shown in Table 15a. R^2 values of full models are 0.8322 indicating the excellent correlation between the independent variables in the models. The adjusted R^2 value was 0.7123 for the full model indicating a better model as illustrated in Table 16a. An increase in % CV shows moderate precision and reliability of the conducted experiments. The large SS_R and small SS_r values tend to occur for models that accurately describe the experimental data as shown in Table 15a. A significant (p=0.0076) synergistic interaction between oil and Smix was observed which as illustrated in Table 15a and equation 1. The quadratic regression coefficient of A^2 was statistically significant. The quadratic effect of oil showed significant synergistic effect (p=0.0385) influence on particle size of Atorvastatin calcium SEDDS. The % CV was found to be 24.88 which were considered to be a high value for the response Y_1 variable of particle size. It was concluded that the interaction between Smix and oil increases the particle size and hence both the factors are highly significant.

Effect of formulation variables on % drug load (Y_2)

The second order polynomial equation derived for % drug load of Atorvastatin calcium is given by

$Y_2= 86.09+5.53*X_1+3.53*X_2-3.30X_1X_2+1.35* X_1^2-5.35* X_2^2$- Equation 2

with R^2= 0.9300, adjusted R^2= 0.8799 and % CV= 2.41.

The model coefficients estimated by quadratic model are shown in Table 17a. In the regression model for % drug loading the model F values are 18.59 implying that the model is highly significant with the p-value of 0.0006 as shown in Table 15a. The R^2 value (0.9300) and adjusted R^2 **(**0.8799) was found to be more than 80 %

indicating a good correlation with the independent variable was well correlated with the response variable as shown in Table 16a. A significant antagonistic (p=0.0140) interaction between oil and Smix was observed which was illustrated in Table 15a and equation 2. The lower interaction between oil and Smix showed the higher drug loading. The quadratic effect of B^2 was statistically significant. The quadratic effect of Smix showed significant effect antagonistic (p=0.0385) influence on drug loading of Atorvastatin calcium SEDDS was shown in Table 17a. The % CV was found to be 2.41 below 10 % which was considered to be an excellent value for the response Y_2 variable of % drug load. It was concluded that the interaction between Smix and oil increases the particle size and hence both the factors are highly significant.

Analysis of variance for particle size (Y_1) and % drug load (Y_2)

Glibenclamide SEDDS

Effect of formulation variables on particle size (Y_1)

The measured values of particle size for 13 formulations as given in Table 14b ranged from 169.7nm to 616.3 nm.

The polynomial regression equation derived for particle size for Glibenclamide is

$$Y_1 = 420.90+33.65*X_1-24.63*X_2-17.75*X_1X_2-222.15*X_1^2+90.40*X_2^2$$ - Equation 3.

For the particle size, the model F value of 5.46 with a low probability value of (p value=0.0230) implies a high significance for the full regression model which is shown in Table 15b. R^2 values of a full model were 0.7960 indicating good correlation between the independent variables in the models. The adjusted R^2 value was 0.6504 for the full model as illustrated in Table 16b. An increase in % CV of 20.45% showed moderate precision and reliability of the conducted experiments. The large SS_R and small SS_r values tend to occur for models that accurately describe the experimental data as shown in Table 15b. The quadratic regression coefficient of A^2 was statistically significant. The quadratic effect of oil showed significant effect antagonistic (p=0.0015) influence on particle size of Glibenclamide SEDDS as indicated in Table 15b. The % CV was found to be 20.45 which were considered to be a high value for the response Y_1 variable of particle size.

Effect of formulation variables on % drug load (Y_2)

The observed values of % drug load for 13 formulations as given in Table 14b ranged from 82% to 92.3%. The equations derived for % drug load for glibenclamide is

$$Y_2= 90.19+3.02* X_1+1.35*X_2+0.95* X_1X_2-2.5* X_1^2-1.7 X_2^2$$ - Equation 4.

The values of the coefficient X_1, X_2 were substituted in the equation to obtain the theoretical values of Y.

The model coefficients estimated by quadratic model are shown in Table 17b. In the regression model for % drug loading the model F values are 5.39 implying that the model is significant with the p-value of 0.0238 as shown in Table 15b. The R^2 value (0.7937) was found to be more than 80 % indicating a good correlation with the independent variable was well correlated with the response variable as shown in Table 16b. There was no significant or quadratic effects of the formulation variables observed as shown in Table 17b. The % CV was found to be 2.28 below 10 % which was considered to be an excellent value for the response Y_2 variable of % drug load.

The above equations 1 and 3 indicate as the factor X_2 increases the response Y_1 decreases and is indicated by negative coefficient value of dependant variable in which the Smix concentration is increased the particle size is decreased. The possible explanation is that when the lower concentration of oil and higher concentration of Smix added to facilitate the increase in water penetration and the mixture becomes hydrophilic causing decrease in particle size. The positive coefficient for independent value X_1 indicates the positive effect on dependent variable Y_1 that increase in concentration of oil increases the particle size from above equations 1 and 3. In equations 2 and 4, the positive coefficient for independent value X_1 indicates the positive effect on dependent variable Y_2 that increase in concentration of oil increases the % drug load.

6.5.1 Linear regression and residual plot analysis

The residual analysis is one method to check model adequacy. After model fitting was performed residual analysis was conducted to validate the assumptions

used in ANOVA. The residual analysis includes case statistics to identify examine diagnostic plots such as normal probability of studentized residuals, a distribution plot of studentized residuals against the predicted values, an outlier T plot and a Box cox plot. For the normal probability plots of the studentized residuals, the number of standard deviations of the actual values from their respective predictive values, a straight line is created indicating no abnormalities or significant deviation from the linearity. The normal probability plot of the residuals depicted in Fig.8a, Fig. 8c for Atorvastatin calcium and Fig. 9a, Fig.9c for Glibenclamide revealed that the systematic deviations from the expectations. In residuals plot where the residuals are plotted against the normal values of the model depicted that the points are nearby to a diagonal line which implied that the errors are normally dispersed and are individually independently depicting a homogenous error variances indicating a well fitted model. Residuals from the fitted model are normally distributed therefore all the major assumptions of the model have been validated. The plots are shown in Fig. 8b, Fig. 8d for Atorvastatin calcium and in Fig.9b, Fig.9d for Glibenclamide depicted an agreeable correlation between the predicted and actual values of responses. In this study, the normality is satisfactory as all residual plots are distributed along a straight line. It is inferred that the confidences for the fitness of the regression equations to the observed values are more than 95% for all responses.

6.5.2 Contour plots and response surface analysis

A polynomial model describing relationship between response and factors of a response surface is known as response surface analysis. A model is graphically visualized by drawing 2D contour plots or 3D response plots. The 2D contour plots show the isoresponse lines as a function of two factors. The 3D response represents the response in 3D dimension. Contour plots and surface response plots are diagrammatic representation of the values of the response. These plots are useful to project the magnitude of effects for each variable and interactions. It can also explain the relationship between independent variables and dependent responses. Response surface methodology provides a mathematical trend that can find optimum level of experimental factors required for a given response. The two dimensional contour plot and the three-dimensional response surface plots are graphical representations of the regression equation and express two independent variables at once against the for Y_1

and Y_2 responses (Fig. 10a, Fig.10b, Fig.10c and Fig.10d for Atorvastatin calcium and Fig.11a, Fig.11b, Fig.11c and Fig.11d for Glibenclamide) which are useful to study the effect of the factors on the responses. With the increasing surfactant (coefficient is negative) in the formulation, droplet size is decreased. In Table 17a for Atorvastatin calcium, it can be seen that all independent variables showed significant main effects interaction effects and the quadratic effect of X_1 ($p < 0.05$) for % drug load; the most prominent effect being the amount of oil (X_1) added ($p = 0.0003$). For particle size, the interaction effect was found to be X_1X_2 being the amount of oil and Smix added ($p = 0.0076$) and the quadratic effect of X_1 was found to be significant ($p=0.0385$). In Table 17b the independent variable X_1 was found to be significant ($p=0.0080$) and the X_2 was found to have the p-value of 0.1446 for Glibenclamide. From Fig 10b, Fig 11b it was clearly observed when the level of Smix concentration was increased from low to high the response Y_1 (particle size) was decreased. From Fig.10d, Fig. 11d it was illustrated that when the level of oil concentration was increased from low to high the response Y_1 (% drug load) was increased. The contour plot of Atorvastatin calcium showed that the denser central optimum area with good average particle size between 150-200nm as shown in Fig.10a.The contour plot Y_2 of % drug loading showed denser region between 85% and 90% as illustrated in Fig.10c. Both the responses Y_1 and Y_2 are thus analyzed by the diagrammatic contour plots. The contour plot of Glibenclamide showed the denser region of particle size below 200nm as illustrated in Fig. 11a. The contour plot Y_2 of % drug loading showed denser region between 80% and 90% as illustrated in Fig.11c.

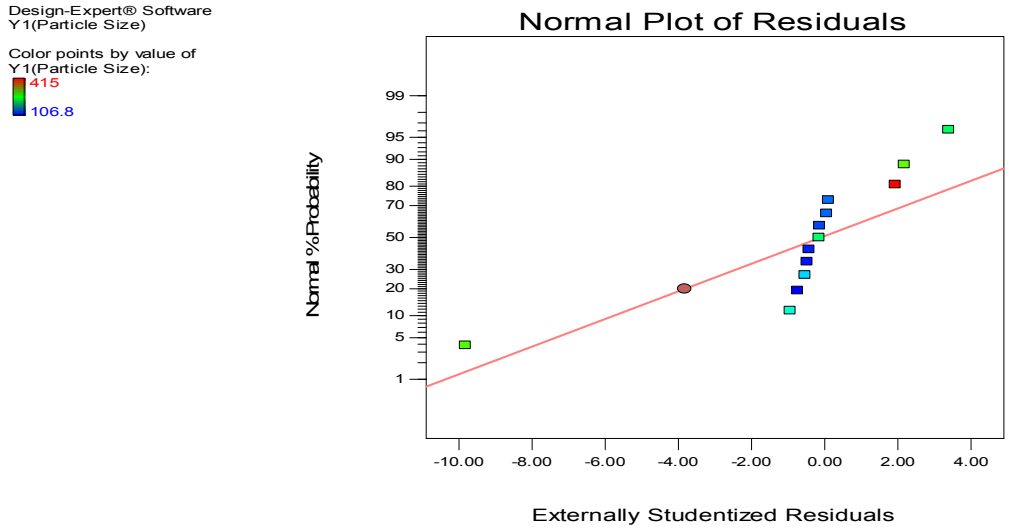


Fig. 8a Normal Residual plot Y_1 of Atorvastatin calcium

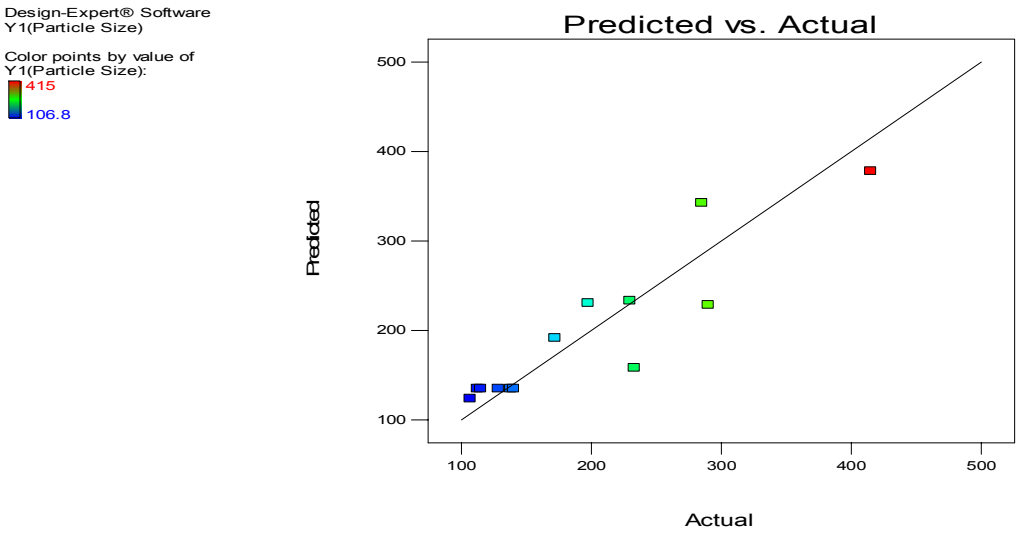


Fig. 8b Linear correlation plot of Y_1 of Atorvastatin calcium

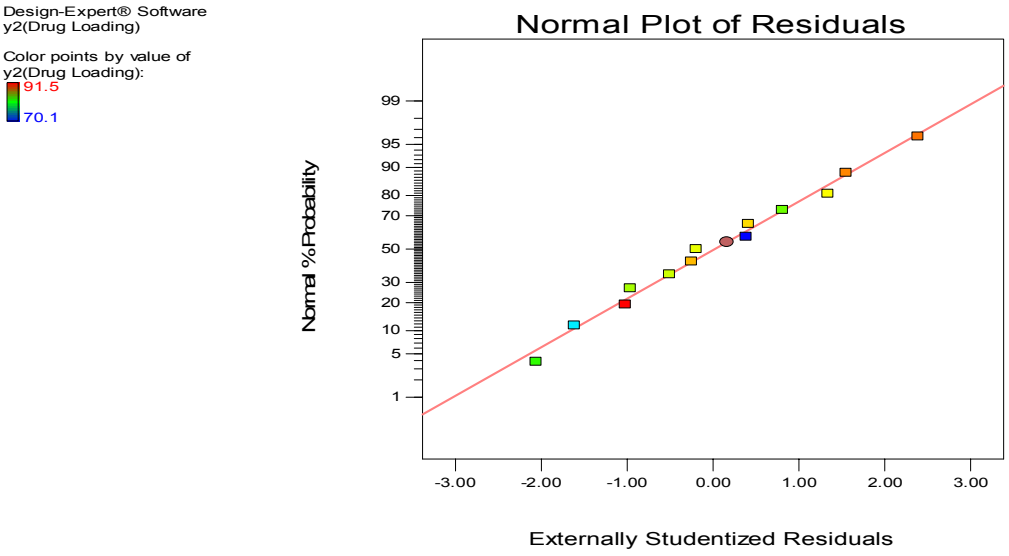


Fig. 8c Normal Residual plot Y_2 of Atorvastatin calcium

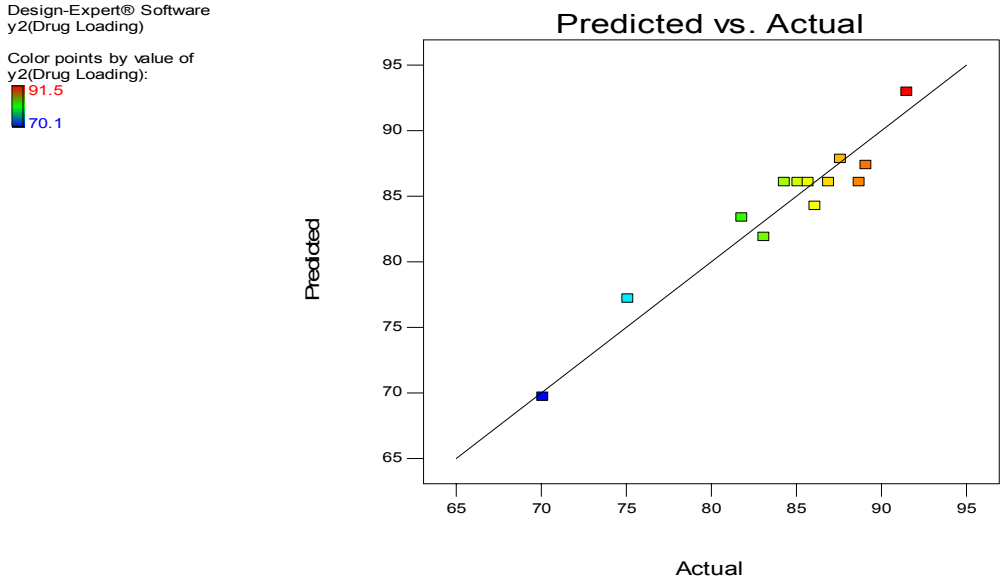


Fig. 8d Linear correlation plot Y_2 of Atorvastatin calcium

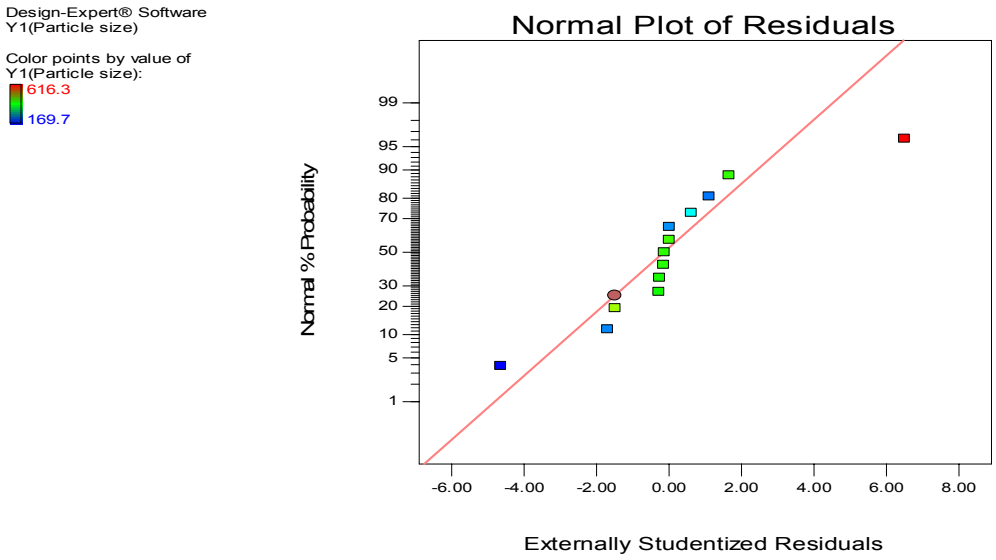


Fig. 9a Normal Residual plot Y_1 of Glibenclamide

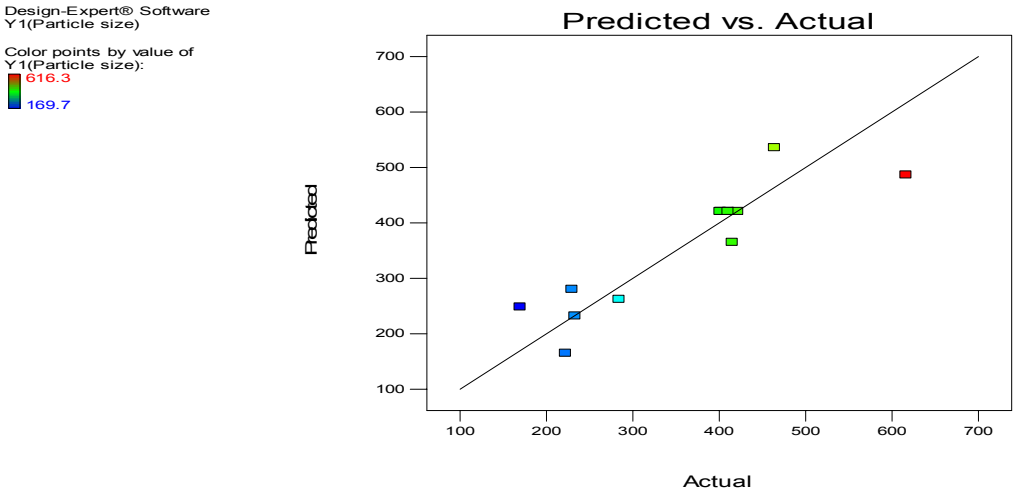


Fig. 9b Linear correlation plot Y_1 of Glibenclamide

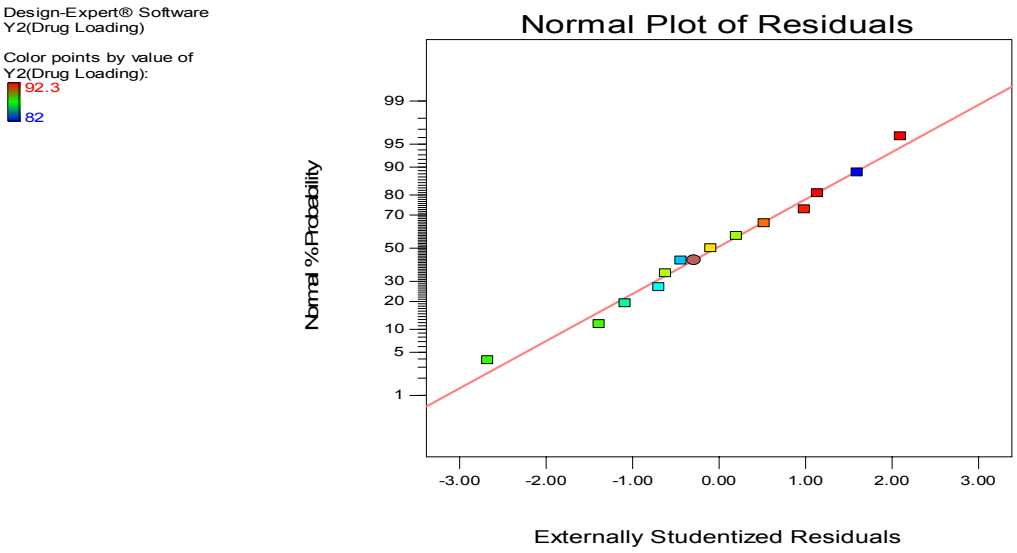


Fig. 9c Normal Residual plot Y_2 of Glibenclamide

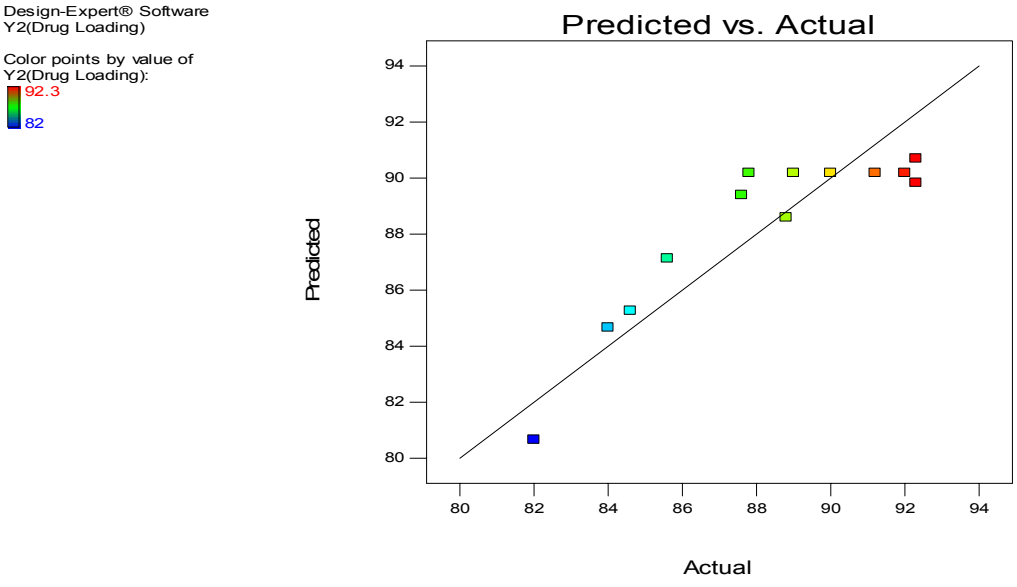


Fig. 9d Linear correlation plot Y_2 of Glibenclamide

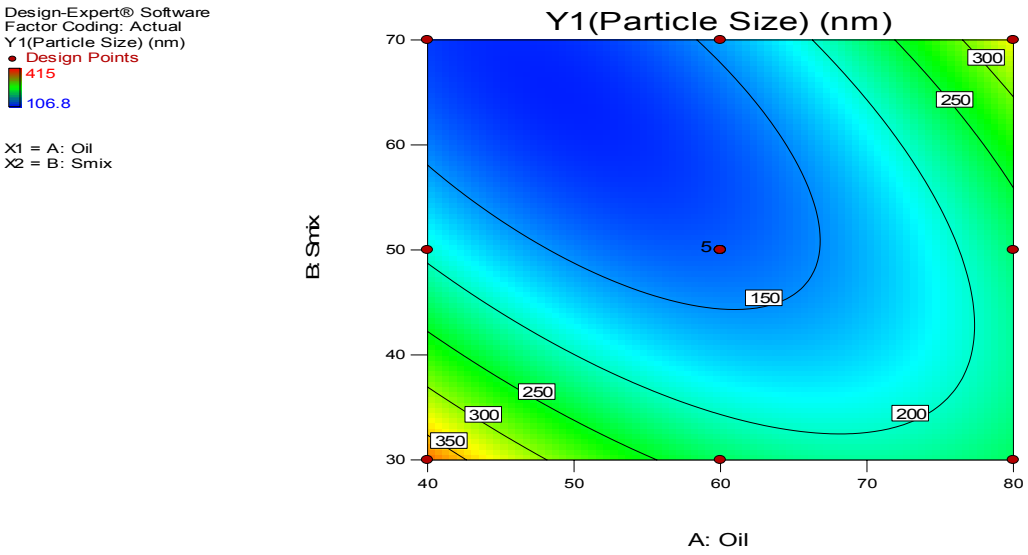


Fig 10a Contour plot Y_1 of Atorvastatin calcium

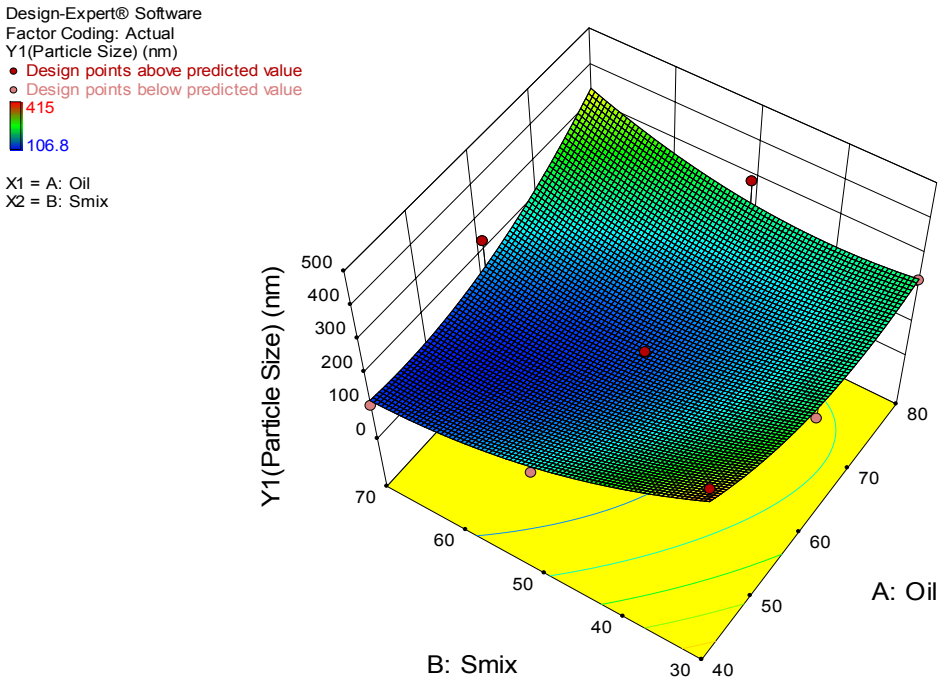


Fig 10b Response surface plot Y_1 of Atorvastatin calcium

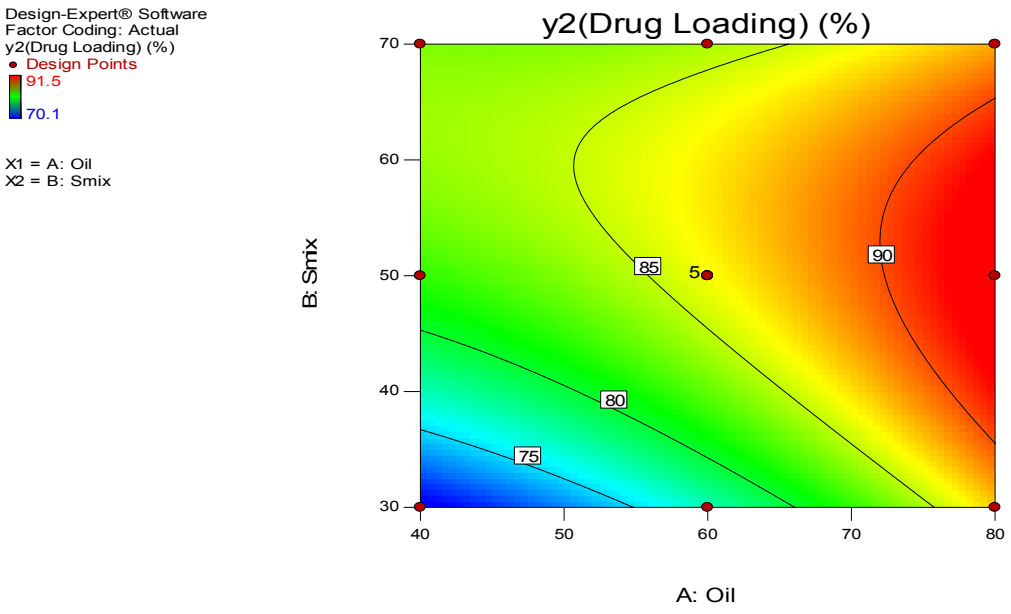


Fig 10c Contour plot Y_2 of Atorvastatin calcium

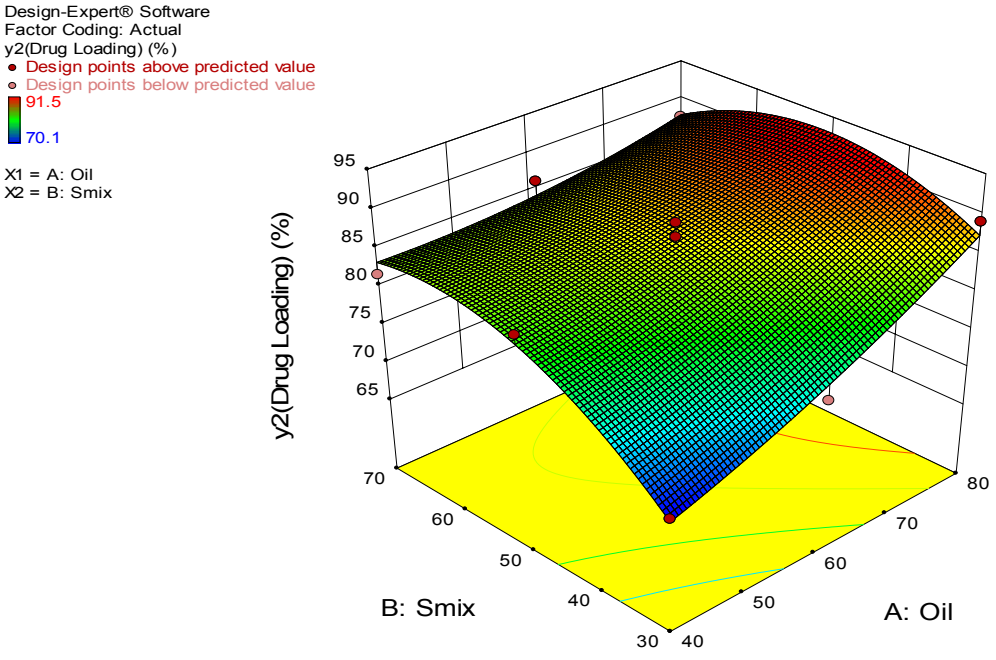


Fig. 10d Response surface Y_2 of Atorvastatin calcium

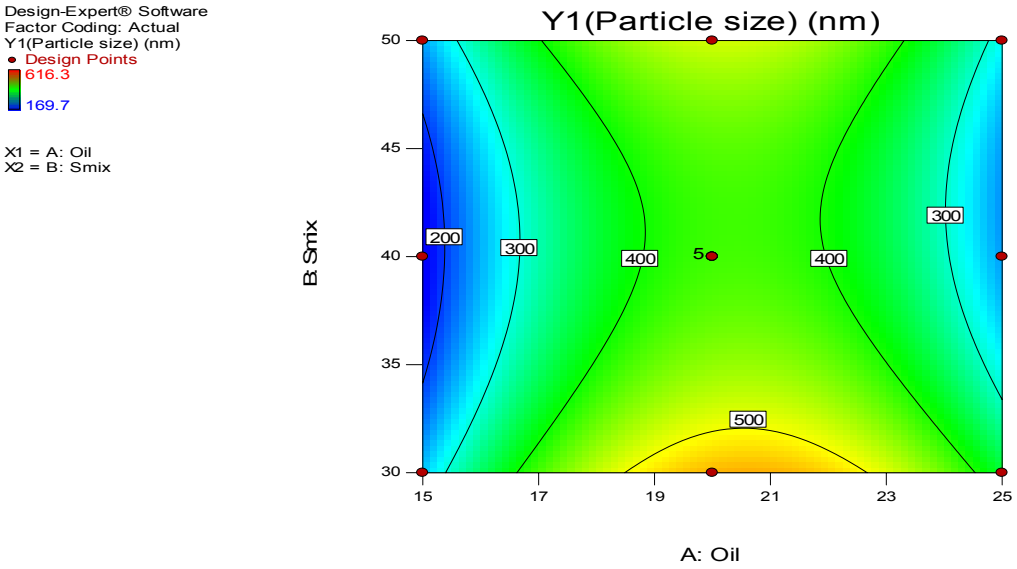


Fig. 11a Contour plot Y_1 of Glibenclamide

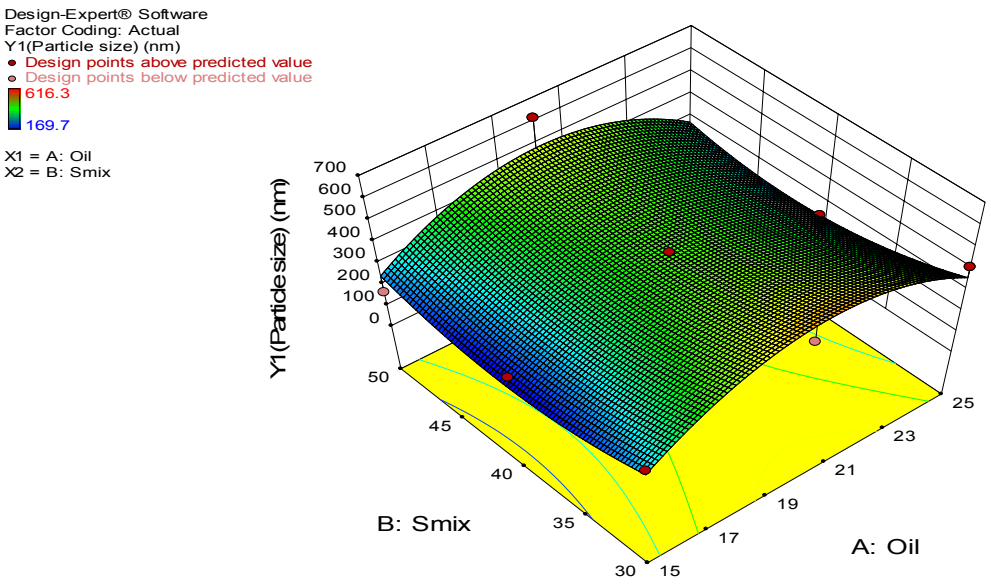


Fig. 11b Response surface plot Y_1 of Glibenclamide

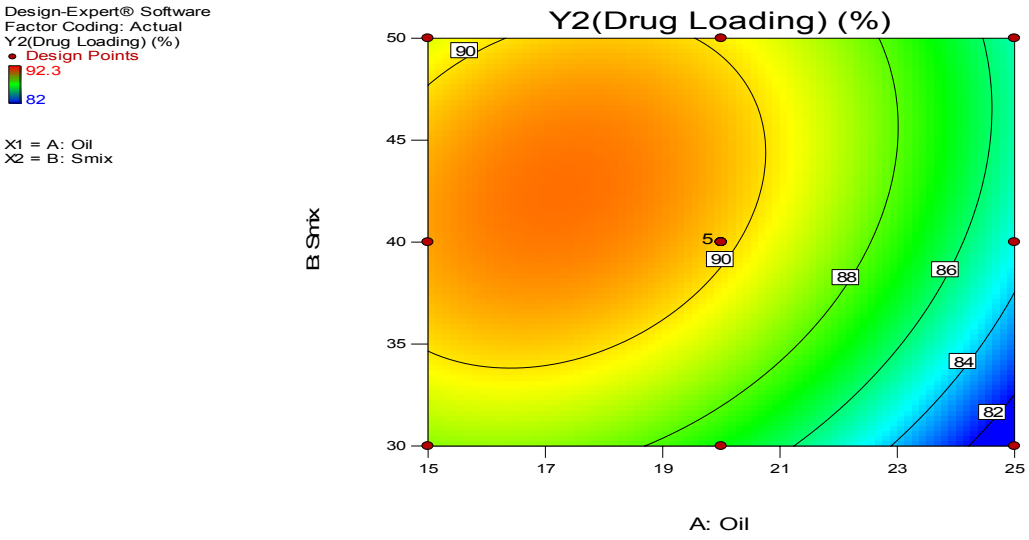


Fig. 11c Contour plot Y_2 of Glibenclamide

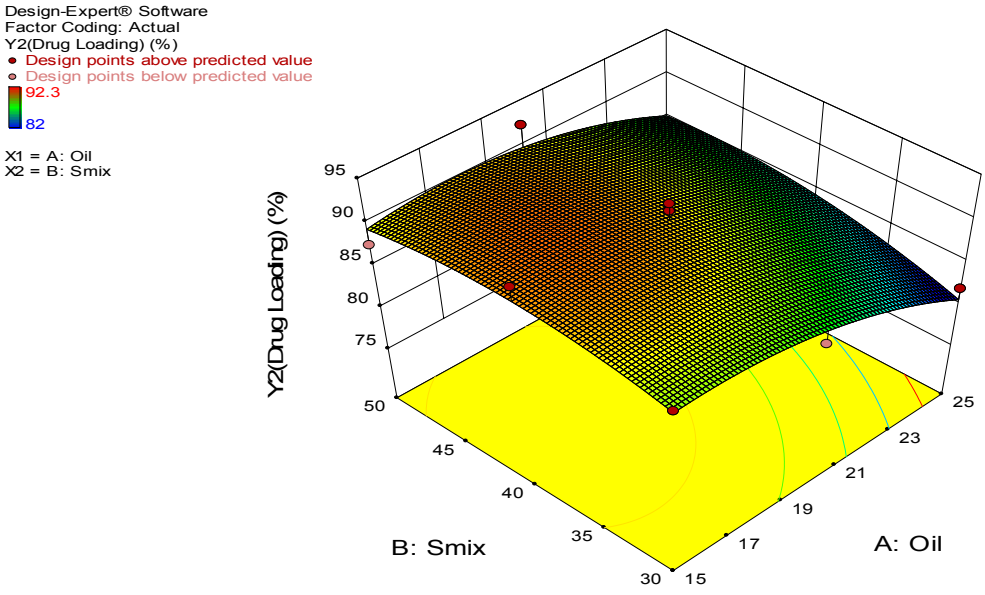


Fig 11d Response surface plot Y_2 of Glibenclamide

6.5.3 Optimization by using Desirability Function

The two responses were optimized by using the desirability function approach (multiple response optimization techniques) introduced by Derringer and Suich[160]. Each response is associated with its partial desirability function (di) scaled from 0 (furthest from the target value) to 1 (closest to the assigned target) and a utility function was computed to provide the overall or global desirability. For the response to be maximized the desirability functions can be defined as $di = (Y_i\text{-}Ymin) / (Ymax\text{-}Ymin)$ where di is individual desirability of the response to be maximized where Y_i is the experimental result and Ymin and Ymax represent the minimum and maximum possible value. If Y_i is equal to or less than Ymin, then, di=0, if Y_i is higher or equal to Ymax, di=1. For the response to be minimized, the desirability function is defined as $di= (Ymax\text{-}Y_i) / (Ymax\text{-}Ymin)$. If Y_i is higher than or greater than Ymax then di=0 and if Y_i is less than or below minimum then di=1. Here lower and upper limits for the responses were set from the highest and lower limits of the observed responses. After obtaining the individual desirability value for each response, the results were combined together usually together to give overall desirable function (D) as the geometric mean which is given by the following equation

$$D = (d1.d2.d3.d4….dn)^{1/n}$$

The optimum formulation was selected based on the criteria of attaining the constraints of variables responses. The total desirability is defined as a geometric mean of the individual desirability for particle size (PS) and drug loading (DL). It can be calculated by the formula

$$D = (dPS \times dDL)^{1/2}.$$

where D is the total desirability, dPS and dDL are individual desirabilities for PS and DL. If both the quality characteristics reach their ideal values, the individual desirability is 1 for both. Consequently, the total desirability is also 1. The independent variables were simultaneously optimized for all the responses by using the desirability function.

The optimization criteria included minimum particle size and maximum drug loading in the range. The global desirability value was calculated by combining all the

individual desirability functions as the geometric mean by using extensive grid and feasibility search over the domain. The suggested optimized formulation for Atorvastatin calcium consisted of 67.586% oil, 52.529% Smix with the corresponding desirability (D) value of 0.856 and the predicted response as Y_1=153.651nm, Y_2= 88.582. Further the suggested optimized formulation for Glibenclamide consisted of 15.046% oil, 41.047% Smix with the corresponding desirability value of 0.921with the predicted response Y_1= 169.678nm, Y_2=90.743. The desirability function plots are illustrated in Figures 13a and 13b for Atorvastatin calcium and Glibenclamide.

Four batches of the optimized formulations were prepared to validate the model adequacy for the prediction, and all the responses were evaluated for each formulation as indicated in Table 18. It can be concluded that the experimental values were in close agreement with predicted values, indicating the success of the design to evaluate and optimize the SEDDS formulation.

Table 18 : Predicted and measured values of responses and corresponding biasness

Atorvastatin calcium responses				**Glibenclamide responses**			
FC	**Particle size (nm)**			**FC**	**Particle size (nm)**		
	Predicted value	**Measured value**	**Biasness %**		**Predicted value**	**Measured value**	**Biasness %**
AF4	153.650	169.2±3.23	10.12	GF1	169.699	173±2.29	1.94
AF5	153.646	169.4±1.97	10.25	GF2	169.695	172.5±1.56	1.65
AF11	153.649	168.9±4.23	9.93	GF7	169.699	172.1±2.04	1.36
AF13	153.636	169.8±1.36	10.52	GF12	169.685	173±2.70	1.95
OPFA	153.651	169.7±3.2	10.45	OPFG	169.678	172±1.12	1.36
% drug loading					% drug loading		
AF4	88.572	87.2±1.23	1.55	GF1	90.743	89.1±2.1	1.81
AF5	88.571	87±2.18	1.77	GF2	90.738	89±2.01	1.92
AF11	88.584	86.9±3.24	1.90	GF7	90.687	88.9±1.98	1.97
AF13	88.586	87.1±2.27	1.68	GF12	90.607	89.2±1.97	1.55
OPFA	88.582	87.2±2.25	1.57	OPFG	90.743	89.3±2.23	1.59

Biasness %= (predicted value-measured value) ×100/predicted value.

The canonical analysis in the Design Expert software is a mathematical tool for simplifying a second-order polynomial model and simultaneously observing the extreme values of several response surface models. Overlaid contour plots of SEDDS were constructed by two independent variables. The overlaid plots for two response values are illustrated in Fig 12a and Fig 12b for Atorvastatin calcium and Glibenclamide. According to the criteria in present study higher drug loading and lower particle size of the optimized formulation of Atorvastatin SEDDS containing oil and Smix were selected at 67.5761% and 52.5328 %. The particle size and % drug loading of the optimized formulation for Atorvastatin calcium were predicted to be 153.597nm and 88.5782 % as illustrated in Fig.12a. The particle size and % drug loading of the optimized formulation for Glibenclamide containing oil and Smix were selected at 15.0457% and 41.0467% respectively. The particle size and % drug loading of the optimized formulation for Glibenclamide were predicted to be 169.678nm and 90.7431 % as illustrated in Fig.12b.

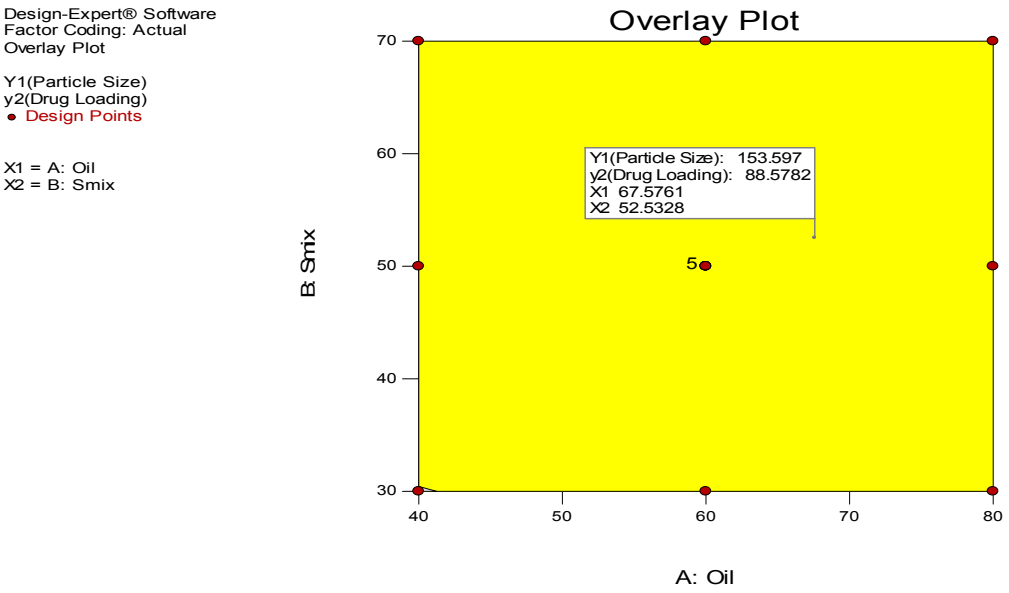


Fig 12a Overlay plot for Atorvastatin calcium SEDDS

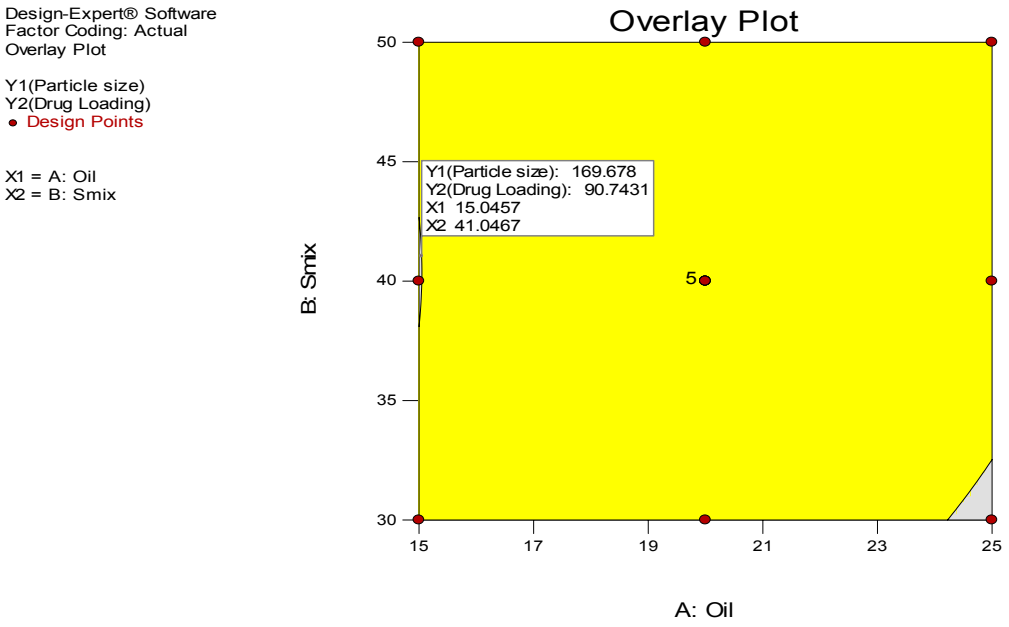


Fig. 12b Overlay plot for Glibenclamide SEDDS

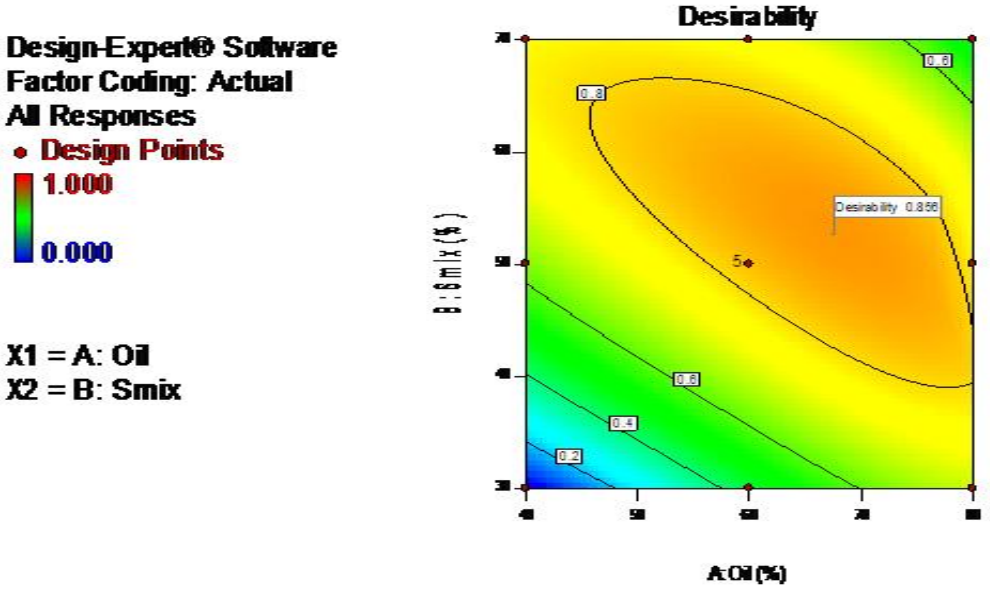


Fig.13a : Desirability plot of Atorvastatin calcium SEDDS optimized formulation OPFA

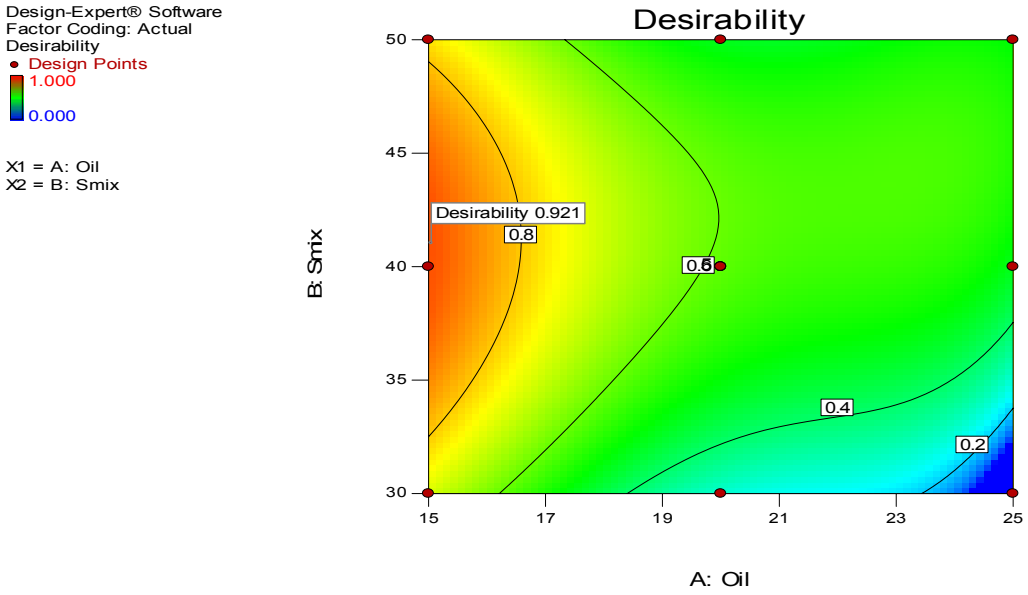


Fig.13b. Desirability plot of Glibenclamide SEDDS optimized formulation OPFG

6.6 Self-emulsification, drug precipitation and phase separation studies

For all the SEDDS formulations the visual observation of self-emulsification study was recorded and evaluated on visibility grades[142] as explained in section 5.12.1. The results of graded formulations were shown in 19a and Table 19b.

In this study, formulations AF4, AF5, AF11, AF13, OPFA (optimized formulations) for Atorvastatin calcium, GF1, GF2, GF7, GF12, OPFG (optimized formulations) for Glibenclamide good stability without any signs drug/excipient precipitation or phase separation were found and the results are shown in Table 19a and Table 19b.

Table 19a : Self -emulsification and drug precipitation of Atorvastatin calcium SEDDS

Formulation Code	Visibility grade	Phase separation	Precipitation
AF1	IV	+	++
AF2	III	+	++
AF3	IV	+	++
AF4*	I	X	XX
AF5*	II	X	XX
AF6	III	+	++
AF7	IV	X	++
AF8	V	+	++
AF9	III	+	++
AF10	IV	+	++
AF11*	I	X	XX
AF12	III	+	++
AF13*	II	X	XX
OPFA	I	X	XX

X -- No phase separation, XX -- No precipitation, + -- phase separation and ++ -- precipitation.

Table 19b : Self- emulsification and drug precipitation of Glibenclamide SEDDS

Formulation Code (FC)	Visibility grade	Phase separation	Precipitation
GF1*	I	X	XX
GF2*	II	X	XX
GF3	IV	+	++
GG4	V	+	++
GF5	II	X	XX
GF6	III	+	++
GF7*	I	X	XX
GF8	V	+	++
GF9	III	+	++
GF10	III	+	++
GF11	IV	+	++
GF12*	I	X	XX
GF13	III	+	++
OPFG	I	X	XX

X -- No phase separation, XX -- No precipitation, + -- phase separation and ++ -- precipitation.

6.7 ASSESSMENT OF EMULSIFICATION TIME STUDIES

The ease of emulsification was suggested to be related to the ease of water penetration into the colloidal or gel phases formed on the surface of the droplet. The emulsification time studies as shown in Table 20a and Table 20b indicated the spontaneous emulsification for all formulations.

Table 20a : Refractive index, Turbidity, Optical clarity, Polydispersity index, Viscosity, Cloud point measurement and Emulsification time of SEDDS formulations of Atorvastatin calcium

FC	Refractive Index ± SD (n=3)	Turbidity (NTU)	Absorbance	Polydispersity index ±SD (n=3)	Viscosity (cps) ±SD(n=3)	Cloud point measurement (°C) ± SD(n=3)	Emulsification time (sec)
AF1	1.3343±0.0006	132	0.402	0.171±0.01	253±2.65	78±3.46	132
AF2	1.3352±0.0003	146	0.487	0.244±0.005	262±2.66	73±3.61	119
AF3	1.3366±0.0005	210	0.529	1.097±0.2	264±1.73	75±5.57	121
AF4*	1.3331±0.0002	90	0.455	0.381±0.03	280±2.31	77±3.46	138
AF5*	1.3334±0.0002	94	0.432	0.377±0.06	291±3.51	74±3.46	126
AF6	1.3345±0.0003	168	0.517	0.148±0.012	272±4.58	78±5.20	112
AF7	1.3363±0.0006	320	0.456	0.379±0.06	269±2.89	75±3.61	95
AF8	1.3358±0.0004	357	0.493	0.292±0.03	254±2.66	75±4.36	82
AF9	1.3349±0.0004	92	0.501	0.128±0.04	249±2.08	79±4.58	75
AF10	1.3347±0.0006	96	0.497	0.386±0.04	263±0.56	77±5.20	62
AF11*	1.3330±0.0003	91	0.466	0.343±0.065	259±1.53	75±3.61	64
AF12	1.3352±0.0002	93	0.629	0.224±0.005	266±4.04	76±2.65	67
AF13*	1.3333±0.0002	95	0.452	0.333±0.005	260±3.56	75±1.73	69
OPFA	1.3330±0.0002	92	0.425	0.2±0.013	258±2.23	72±1.28	61

Table 20b : Refractive index, Turbidity, Optical clarity, Polydispersity index (PDI), Viscosity, Cloud point measurement and Emulsification time of SEDDS formulations of Glibenclamide

FC	Refractive Index ± SD (n=3)	Turbidity (NTU)	Absorbance	PDI ±SD (n=3)	Viscosity (cps) ±SD(n=3)	Cloud point measurement (°C) ±SD (n=3)	Emulsification time(sec)
GF1*	1.3333±0.0006	98	0.583	0.204±0.017	211±2.22	68±2.16	23
GF2*	1.3332±0.0003	93	0.578	0.315±0.21	215±2.36	69±3.61	32
GF3	1.3356±0.0005	127	0.598	0.2±0.01	324±3.24	72±2.23	24
GF4	1.3331±0.0002	134	0.488	0.284±0.01	356±1.72	77±2.46	25
GF5	1.3334±0.0002	142	0.571	0.438±0.01	318±2.34	71±2.76	27
GF6	1.3335±0.0003	132	0.472	0.224±0.02	365±3.24	79±4.20	20
GF7*	1.3333±0.0006	95	0.614	0.282±0.018	232±2.45	65±2.51	28
GF8	1.3358±0.0004	125	0.687	0.423±0.06	348±1.89	73±2.36	30
GF9	1.3349±0.0004	132	0.708	0.328±0.015	298±2.97	78±4.28	22
GF10	1.3347±0.0006	133	0.419	0.418±0.06	288±3.68	76±4.29	24
GF11	1.3330±0.0003	128	0.448	0.327±0.02	272±2.65	76±2.61	26
GF12*	1.3352±0.0002	93	0.523	0.217±0.03	223±1.98	66±2.65	25
GF13	1.3333±0.0002	124	0.506	0.324±0.02	294±2.54	76±2.73	24
OPFG	1.3330±0.0002	92	0.494	0.244±0.04	200±1.95	62±2.25	21

6.8 SPECTROSCOPIC CHARACTERIZATION OF OPTICAL CLARITY

As shown in Table 20a and Table 20b the absorbance of the studied aqueous dispersion of Atorvastatin calcium SEDDS ranged between 0.402 to 0.529 and 0.419 to 0.708 for Glibenclamide which indicates that optically clear and oil droplets formed are to be in a state of finer dispersion.

6.9 TURBIDITY MEASUREMENT

The turbidity of SEDDS was performed determined as per procedure and turbidity for all optimized formulations were found to below 100NTU which shows the stability of SEDDS and the results were shown in Table 20a and Table 20b.

6.10 VISCOSITY DETERMINATION

From viscosity determination, it was observed that as the concentration of oil increased, viscosity of formulations decreased as shown in Table 20a and Table 20b. Overall, the viscosity of the undiluted liquid SNEDDS was found less than 10,000 cps which imply that the developed SEDDS can be filled in soft gelatin capsules.

6.11 CLOUD POINT MEASUREMENT

For all the formulations the cloud point was found to be below 80°C and the results were shown in Table 20a and Table 20b. From the above result, it can be concluded that a stable micro emulsion of SEDDS can be formed at physiological temperature *in- vivo*.

6.12 DETERMINATION OF REFRACTIVE INDEX (RI)

The RI of the prepared formulations was determined using Abbe refractometer. It is indicated from the results that the isotropic nature of the formulations was found to be in range of 1.3330±0.0002 to 1.3366 ± 0.0005 for Atorvastatin calcium and 1.3330±0.0002 to 1.3358±0.0004 for Glibenclamide as shown in Table 20a and Table 20b. It can be seen from the Table 20a and Table 20b the refractive index of the majority of the prepared formulations have similar values as that of distilled water (1.3330±0.0002 n.d.) at 28 ± 0.5°C were found to be clear as water[141]. The closure of the formulations RI value to water indicated the transparency property of the formulations. The results indicated that RI values increased with increase in concentration of oil and corresponding decrease in aqueous content. AF3 exhibited the highest RI value of 1.3366 ± 0.0005 for Atorvastatin calcium in which the oil concentration was 80% as indicated in Table 20a. The Glibenclamide SEDDS formulations of GF3, GF8 exhibited a higher RI value of 1.3356±0.0005 and 1.3358±0.0004 since their oil concentration was more than 20% as indicated in Table 20b.

6.13 DROPLET SIZE, ZETA POTENTIAL AND POLYDISPERSITY INDEX (PDI) ANALYSIS

The PDI for all the formulations were less than 0.5 (AF3-1.097) and the formulations with Smix showed lower PDI values thus indicating the uniform size distribution. The results of PDI were shown in Table 20a and Table 20b**.** After drug addition there was no significant difference in PDI values indicating no interference of the drug with the performance of the spontaneous emulsification.

Among the formulations the optimized Atorvastatin calcium SEDDS (OPFA) was found to have a mean globule size of 169.7nm with a PDI 0.2, and zeta potential -31.8mV as shown in Fig. 14a and Fig. 14b.The selected optimized formulation of Glibenclamide SEDDS (OPFG) was found to have a mean globule size of 172nm with a PDI 0.244, and zeta potential -24.8mV as shown in Fig 15a and Fig 15b.The higher (above +30 or −30 mV) Zeta potential of optimized SEDDS indicates that microemulsion was stable.

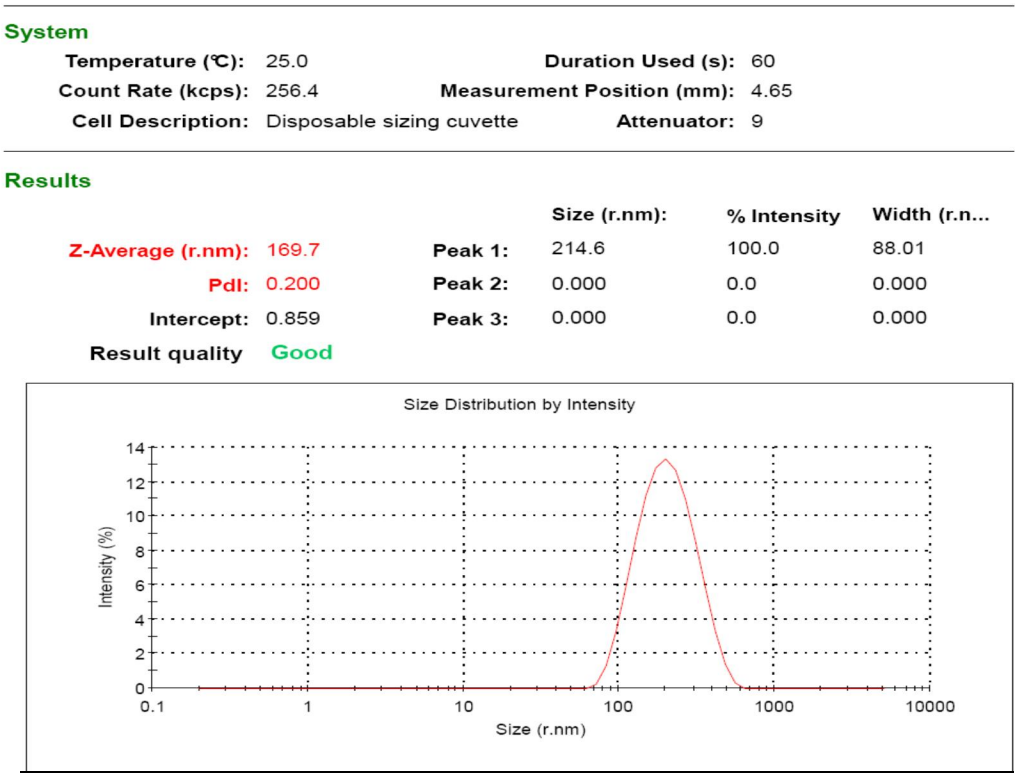


Fig. 14a Particle size of optimized formulation OPFA for Atorvastatin calcium SEDDS

System

Temperature (°C):	25.0	Zeta Runs:	14
Count Rate (kcps):	178.6	Measurement Position (mm):	4.50
Cell Description:	Zeta dip cell	Attenuator:	8

Results

		Mean (mV)	Area (%)	Width (mV)
Zeta Potential (mV):	-31.8	Peak 1: -31.8	100.0	13.2
Zeta Deviation (mV):	13.2	Peak 2: 0.00	0.0	0.00
Conductivity (mS/cm):	2.97	Peak 3: 0.00	0.0	0.00
Result quality :	Good			

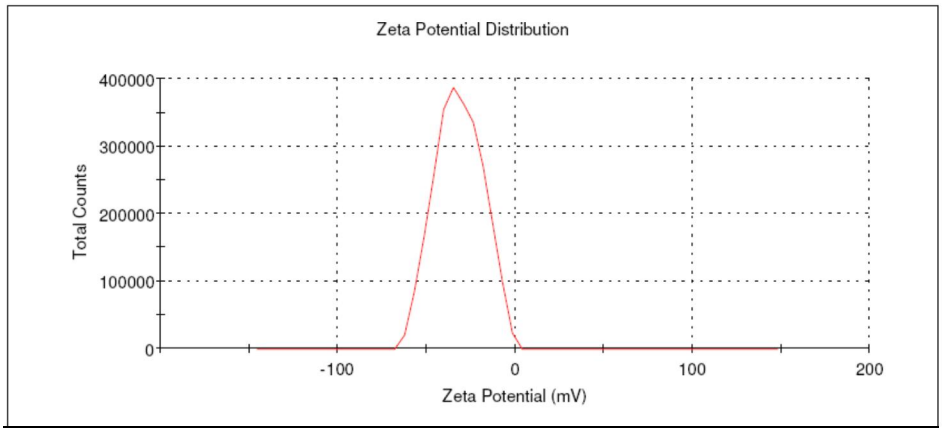


Fig. 14b Zeta potential of optimized formulation OPFA for Atorvastatin calcium SEDDS

System

Temperature (°C):	25.0	Duration Used (s):	60
Count Rate (kcps):	313.0	Measurement Position (mm):	4.65
Cell Description:	Disposable sizing cuvette	Attenuator:	9

Results

			Size (r.nm):	% Intensity	Width (r.n...
Z-Average (r.nm):	172.0	Peak 1:	161.8	97.8	41.88
PdI:	0.244	Peak 2:	2690	2.2	161.4
Intercept:	0.931	Peak 3:	0.000	0.0	0.000
Result quality	Good				

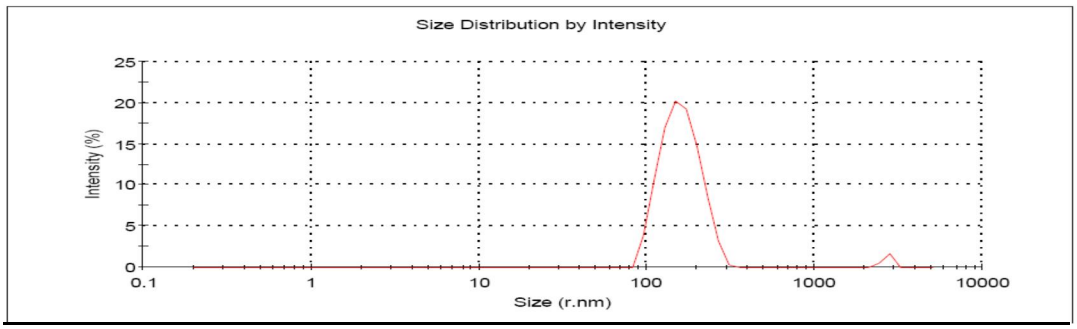


Fig. 15a Particle size of optimized formulation OPFG for Glibenclamide SEDDS

System

Temperature (°C):	25.0	Zeta Runs:	12
Count Rate (kcps):	182.3	Measurement Position (mm):	2.00
Cell Description:	Clear disposable zeta cell	Attenuator:	5

Results

		Mean (mV)	Area (%)	Width (mV)
Zeta Potential (mV): -24.8	Peak 1:	-24.8	100.0	4.16
Zeta Deviation (mV): 4.16	Peak 2:	0.00	0.0	0.00
Conductivity (mS/cm): 0.179	Peak 3:	0.00	0.0	0.00

Result quality : Good

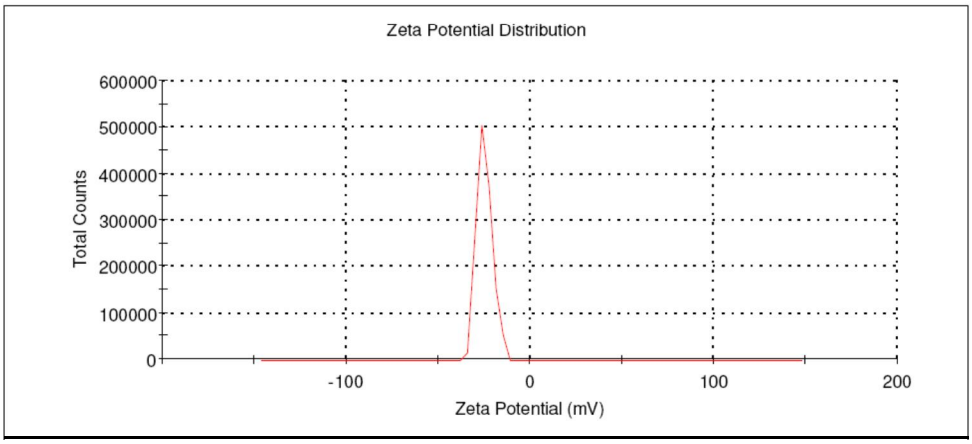


Fig. 15b Zeta potential of optimized formulation OPFG for Glibenclamide SEDDS

6.14 DRUG LOADING

The Atorvastatin and Glibenclamide SEDDS formulations were subjected to drug loading studies. The drug content was carried out by UV-visible spectrophotometer (Shimadzu UV-1700) and the drug loading was performed as per procedure described in section 5.12.11. A linear calibration curve was obtained at 247 nm for Atorvastatin calcium and 226.5 nm for Glibenclamide in the range of (2-20 µg/ml) with a correlation coefficient (r^2) of 0.999.The drug content of Atorvastatin calcium was calculated from the Beers Lambert's law equation Y = 0.045.concentration + 0.003 (r^2 = 0.999; p < 0.001)] and Y= 0.118.concentration+ 0.002 (r^2 = 0.999; p < 0.001) for Glibenclamide. The % drug loading for optimized formulation of Atorvastatin calcium (OPFA) and Glibenclamide (OPFG) was found to be 87.2% ± 2.25 and 89.3% ± 2.23 respectively. It was clearly inferred increase in Smix concentration enhances maximum drug load in SEDDS.

6.14 *IN VITRO* DISSOLUTION STUDIES

The *in vitro* drug release studies were performed as per procedure described under 5.12.12 for Atorvastatin calcium SEDDS and 5.12.13 for Glibenclamide SEDDS. The *in vitro* dissolution profile of Atorvastatin calcium optimized formulations OPFA, AF4, AF5, AF11 and AF13 carried out by USP II dissolution apparatus in phosphate buffer pH 6.8 and Glibenclamide optimized formulations OPFG, GF1, GF2, GF7 and GF12 in phosphate buffer pH 7.4 was significantly higher than with API and marketed tablet (Storvas 10 mg for Atorvastatin calcium and Daonil 5mg for Glibenclamide) as shown in the Table 21a and Table 21b and Fig.16a and Fig. 16b. It could be suggested that spontaneous micro-emulsification resulted in the faster rate of drug release into the aqueous phase in the form of small and mono dispersed droplets[139]. The drug content was calculated from the Beers Lambert's law equation of Y =0.012.concentration+0.001 (r^2 = 0.999; P < 0.001) for Atorvastatin calcium and Y= 0.018.concentration + 0.001 (r^2 = 0.999; P < 0.001) for Glibenclamide.

Table 21a : Cumulative percent release of Atorvastatin calcium from various formulations

Time in min	AF1*	AF5*	AF11*	AF13*	OPFA SEDDS	API	Marketed Tablet
0	0	0	0	0	0	0	0
5	29.56±0.69	28.89±0.88	27.45±0.59	25.56±1.25	26.21±0.74	38.69±1.24	33.21±2.03
10	34.58±2.08	38.56±0.63	33.46±1.28	32.45±0.19	39.3±0.23	47.56±0.75	45.23±1.12
20	52.56±1	55.33±2.02	56.59±0.56	57.53±0.73	58.36±0.45	65.22±1.12	60.33±2.21
30	74.23±1.59	73.52±1.94	75.56±1.50	74.87±0.22	72.66±0.32	80.45±1.23	79.54±1.64
40	76.89±1.38	76.26±0.55	77.62±1.20	78.66±0.16	79.5±0.18	86.23±1.56	85.62±0.54
50	84.98±1.27	82.56±1.16	83.32±1.30	84.98±0.02	86.72±0.16	89.21±2.73	86.74±2.21
60	91.26±2.74	90.21±1.48	90.36±0.17	91.63±0.44	91.3±0.55	92.34±1.23	90.69±1.72
75	92.27±1.78	92.24±2.55	92.48±0.56	93.56±1.22	94.5±0.49	93.86±0.62	92.66±1.54
90	95.85±1.30	96.16±0.72	97.28±1.13	98.56±0.44	99.75±0.31	95.64±1.26	93.31±1.18

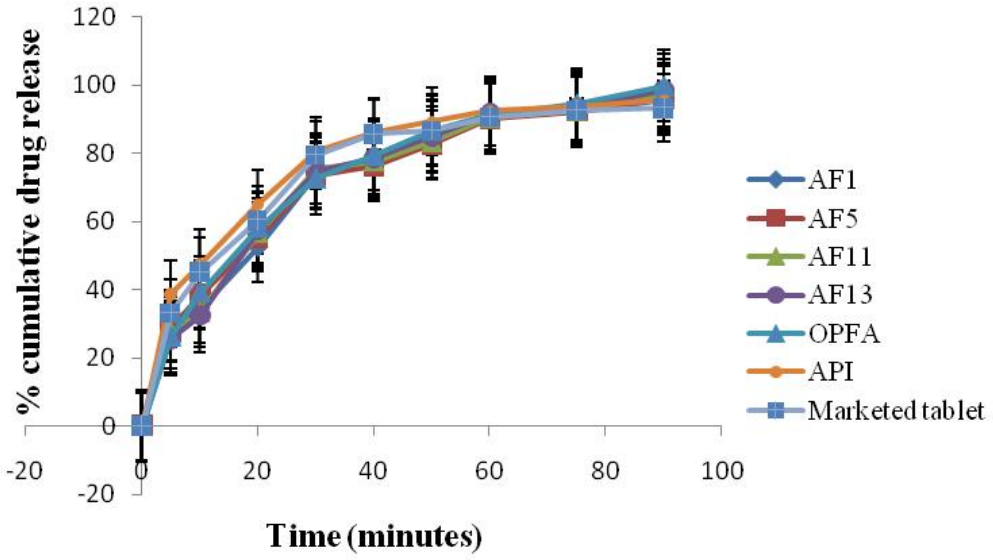


Fig 16a Dissolution comparison graph of API, marketed and optimized formulation of Atorvastatin calcium SEDDS

Table 21b : Cumulative percent release of Glibenclamide SEDDS from various formulations

Time in min	GF1*	GF2*	GF7*	GF12*	OPFG SEDDS	API	Marketed Tablet
0	0	0	0	0	0	0	0
5	91.2	90.8	92.1	94.1	95±3.51	38.7±1.98	35.31±2.22
10	92.4	93.4	94.3	95.3	96.8±1.97	55.9±2.24	52.4±3.71
20	94.6	95.7	95.4	96.5	97.2±3.14	87.3±3.21	85.5±3.26
30	95.2	96.1	97.8	97.3	99.7±2.74	93.8±2.67	92.3 ±3.47

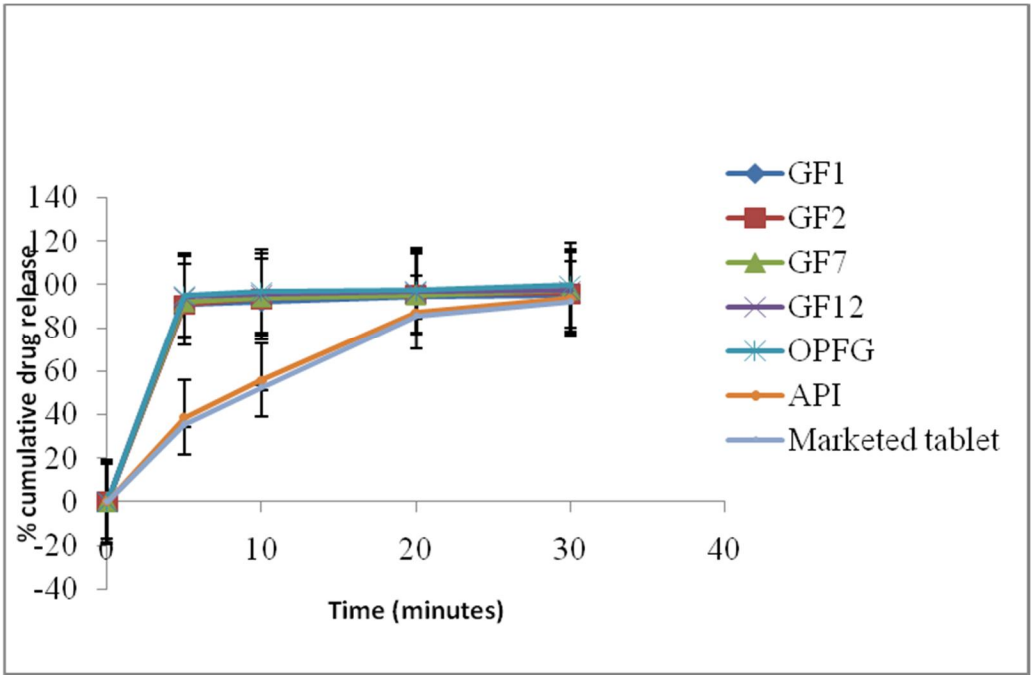


Fig 16b Dissolution comparison graph of API, marketed formulation and optimized formulations of Glibenclamide SEDDS

6.14.1 Kinetic modeling and Mechanism of drug release of optimized formulations

The dissolution data of optimized formulations OPFA and OPFG showed first order release kinetics with higher correlation coefficient R^2-0.9848 for Atorvastatin calcium and R^2- 0.9978 for Glibenclamide are shown in Table 22 and illustrated in Fig. 17a and Fig 17b. *In vitro* release kinetics data were computed using DD solver and the resultant data were fitted to the Korsmeyer-Peppas exponential equation to establish the mechanism of drug release. The exponent, n has been proposed as indicative of the release mechanism. The 'n' values for OPFA and OPFG was found to be 0.406 and 0.024 which suggested that drug release follows Fickian diffusion controlled mechanism for Atorvastatin calcium and Quasi Fickian diffusion for Glibenclamide.

Table 22 : Release kinetic study of optimized formulations for Atorvastatin calcium and Glibenclamide

FC	Zero order kinetic R^2	First order kinetic R^2	Higuchi Kinetic R^2	Korsmeyer-Peppas	
				R^2	n value
OPFA	0.9569	0.9848	0.9366	0.9701	0.406
OPFG	0.9569	0.9978	0.9519	0.8821	0.024

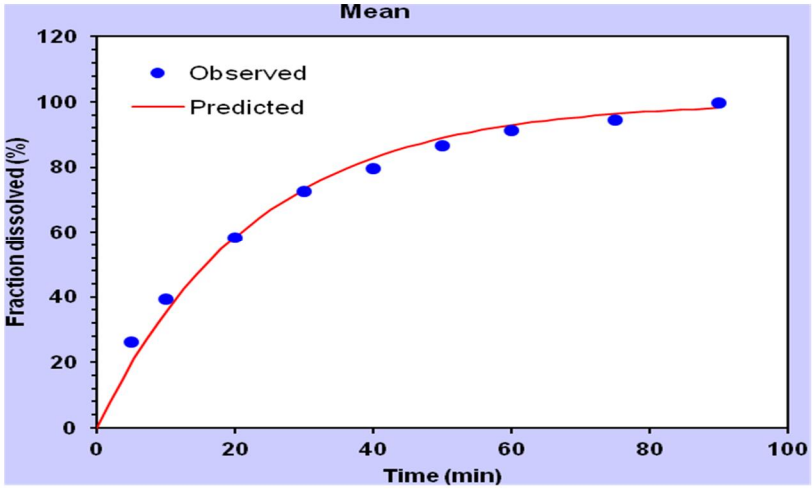


Fig.17a. Dissolution first order release kinetics of optimized formulation OPFA

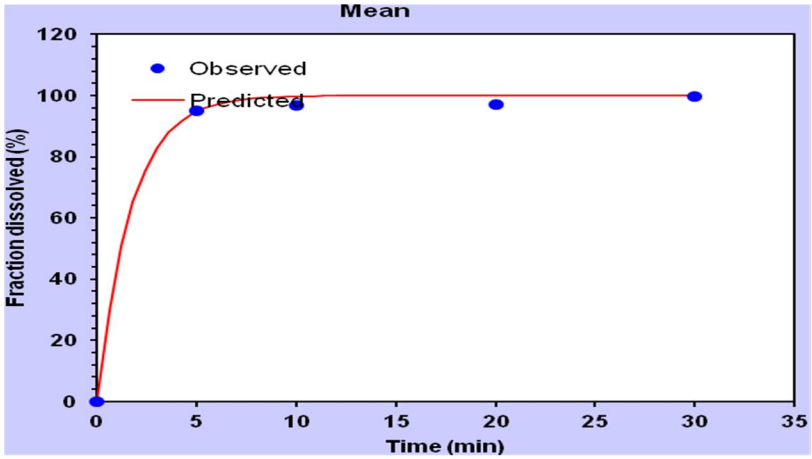


Fig.17b. Dissolution first order release kinetics of optimized formulation OPFG

6.15 *IN VITRO* DIFFUSION RELEASE STUDY

Diffusion study was carried out to study the release behavior of formulation from liquid crystalline phase around the droplet using dialysis technique. *In vitro* diffusion profile of Atorvastatin calcium from optimized SEDDS in phosphate buffer (pH 6.8) is given in Table 23a. It was observed that at the end of 12 hour, formulation OPFA SEDDS showed about 99.24% diffusion due to its nano range globule size and presence of surfactant/co-surfactant. In contrast, the marketed tablet (Storvas 10mg) showed about 98.18 % diffusion of the drug in 12 hours due to low aqueous solubility. *In vitro* diffusion profile of Glibenclamide from optimized SEDDS in phosphate buffer (pH 7.4) is given in Table 23b. It was observed that at the end of 24h, formulation OPFG SEDDS showed about 99.8% and 96.23% for the marketed tablet (Daonil 5mg) at the end of 2 hours (Table data only for 2 hours).

Table 23a : Percent cumulative drug absorbed through dialysis membrane of optimized Atorvastatin calcium SEDDS formulations

Time in hours	AF4*	AF5*	AF11*	AF13*	OPFA SEDDS	Marketed Tablet
0	0	0	0	0	0	0
0.5	82.19±1.23	84.93±1.54	83.45±0.76	82.31±0.78	89.32±2.17	81.25±2.25
1	92.19±0.78	93.42±2.78	92.64±1.23	91.89±0.98	92.22±0.91	90 ±1.14
2	93.75±1.84	94.23±1.66	93.62±2.46	93.16±1.19	93.43±1.56	92±1.98
4	94.94±2.21	94.45±2.56	94.89±0.78	94.23±2.56	95.36±2.45	94 ±2.54
6	96.28±0.73	96.82±0.84	96.4±0.92	96.45±0.74	96.39±1.47	95 ±2.69
8	97.67±0.94	97.14±2.41	97.54±1.47	97.67±1.64	98.56±0.95	96.9±1.85
12	98.45±1.86	98.25±1.78	98.23±2.82	98.21±2.47	99.24±2.26	98.18±0.99

Table 23b : Percent cumulative drug absorbed through dialysis membrane of optimized Glibenclamide SEDDS formulations

Time in hours	GF4*	GF5*	GF11*	GF13*	OPFG SEDDS	Marketed Tablet
0	0	0	0	0	0	0
0.5	97.3±2.73	96.4±2.57	95.3±1.96	96.5±0.64	97.2±0.95	82.92±1.97
1	98.6±0.97	98.1±0.72	97.4±0.72	98.4±2.21	99.3±1.74	90.32±2.19
2	99.2±1.43	99.5±0.87	98.6±1.65	99.6±1.93	99.8±2.12	96.23±1.41

6.16 STABILITY STUDIES

The optimized SEDDS of Atorvastatin calcium and Gibenclamide were loaded in soft gelatin capsules (Size 3).They were stored under cold condition (4-8°C) at refrigerator and room temperature (25°C) were subjected to stability studies to evaluate their stability and the integrity of the dosage form. The samples were also charged at elevated temperature of 50°C in stability chambers (Labtech India) with ambient humidity condition. It was analyzed from the results that there was no significant change in the % drug loading and particle size as given in Table 24a and Table 24b.

The formulations were found to be stable at cold, room temperature and at elevated temperatures when the samples were analyzed for its particle size and % drug loading after first and 6 months. It was also seen that the formulation was compatible with the soft gelatin capsule shells, as there was no sign of capsule she ll deformation. Furthermorethe formulation was found to show no phase separation and drug precipitation. Thus, the studies confirmed the stability of the developed formulation and its compatibility with soft gelatin capsules.

Table 24a : Stability studies of optimized Atorvastatin calcium SEDDS formulations

Temperature (°C)	Particle Size (nm)		% drug load	
	After 1 Month	After 6 month	After 1 month	After 6 month
Cold Temperature (2 -8°C)	173±2.23	176± 1.23	87.2±1.33	83.7±1.89
Room Temperature (25±2°C)	169.7±1.85	171.7±0.86	88.9±2.24	86.2±2.65
Elevated Temperature (50±2°C)	170±2.35	175.6±1.56	85.9±1.42	81.9±2.78

Data expressed as mean ± SD, n=3

Table 24b : Stability studies of optimized Glibenclamide SEDDS formulations

Temperature (°C)	Particle Size (nm)		% drug load	
	After 1 Month	After 6 month	After 1 month	After 6 month
Cold Temperature (2 -8°C)	174±2.23	178± 1.23	86.2±1.33	83.7±1.89
Room Temperature (25±2°C)	172±1.85	174.7±0.86	89.3±2.23	85.9±2.65
Elevated Temperature (50±2°C)	180±2.35	185.6±1.56	84.9±1.42	80.9±2.78

CHAPTER 7

DISCUSSION

7.1 SOLUBILITY STUDY

Atorvastatin calcium of BCS Class II drug is a cholesterol-lowering agent is widely used to treat hyperlipidaemias. The drug has poor water solubility in water and absolute bioavailability of only 14% which was proved by Lea *et al* [161] and Black *et al*[162] due to its presystemic clearance in the gastrointestinal mucosa or hepatic first-pass metabolism[163].The partition coefficient (Log P) for the drug is 6.36. Since it's a weak acid of pKa 4.46 the drug remains unionized in the pH of the stomach. Glibenclamide (GBD) of BCS Class II drug is a second generation sulfonylurea. It is orally used as a hypoglycemic agent to treat noninsulin-dependent (type II) diabetes mellitus[164, 165]. The aqueous solubility of GBD is low and highly pH-dependent in the physiological range because of its pKa value of 5.3. Low aqueous solubility gives rise to unsatisfactory dissolution profiles leading to potential problems of poor bioavailability and bioequivalence of the drug's dosage form[166, 167]. However, the oral absorption of both the drugs is limited by its poor solubility and bioavailability. The Atorvastatin calcium and Glibenclamide SEDDS were developed by performing the preliminary solubility studies to examine the highest solubilizing capacity of the drug in an oil phase, surfactant and co surfactant. For achieving the maximum solubility of the drug different lipid classes of triglycerides with the long chain, medium chain and synthetic triglycerides of different HLB values were utilized. The solubility of a drug in oils, surfactants and co surfactants was identified for optimum loading of the drugs in SEDDS formulations.

The Atorvastatin calcium was found to have maximum solubilizing capacity in sunflower oil which is a long chain triglyceride, capable of solubilizing the drug in a specific amount facilitate self-emulsification and increase the fraction of Atorvastatin calcium through the intestinal lymphatic system. In solubility studies, MCT's such as coconut oil are used since the modified and hydrolyzed vegetable oils[168] show more solubility and appreciable self-emulsifying property. The solvent capacity for hydrophobic drugs can be improved by using sunflower oil which is a triglyceride.

Peceol a novel a semi synthetic medium chain derivative was chosen as oil phase for Glibenclamide since it was found to have maximum solubility when compared to the other oils and in which the solubility was found to be minimium in sunflower oil as indicated in results of Table 12. Peceol is an efficient solubilizer for Glibenclamide which was proven by Patil Prashant P *et al*[169] in his research work. The labrasol which showed the highest solubilization capacity was selected as surfactant followed by capryol PGMC, labrafil 1944 CS and Labrafil 2125 for formulation of Atorvastatin calcium SEDDS as shown in Fig. 6a. A similar work has been published by Shafiq S *et al*[25] using poorly water soluble drug in which the highest solubility of the drug was in labrasol among all surfactants. Capryol PGMC (Propylene glycol monocaprylate type I) of HLB 6 is a water insoluble surfactant which is included in SEDDS formulations along with a cosolvent[170]. From Fig. 6b it was illustrated that labrasol was chosen as surfactant because it showed maximum solubility followed by labrafil 1944 CS, capryol PGMC and labrafil 2125 for formulation of Glibenclamide SEDDS. The medium length alkyl chain surfactant labrasol was selected as a surfactant for both the drugs since it has maximum solubilizing capacity and it was represented for drugs having poor intestinal absorption[171]. Transcutol HP showed maximum solubility for Atorvastatin calcium and Glibenclamide and it was selected as the cosurfactant for the formulation of SEDDS as indicated in Table 12.

The cosolvents such as ethanol and PEG are not included as for the development of SEDDS because they cause serious problems of evaporation from sealed gelatin capsules which results in the precipitation of drug inside the shell. The cosolvent transcutol HP is most preferred for the development of SEDDS since it is more stable and less volatile than ethanol when compared with other cosolvents[172] and it was proved to be good solubilizer for poorly water soluble drugs. Transcutol HP is a strong solubilizer with low toxicity has a long history of safe use as a solvent in many products including pharmaceuticals, cosmetics and food applications[b] . The self-emulsifying systems form fine oil/water emulsions with only gentle agitation, upon their introduction into aqueous media. The surfactant and co-surfactant get preferentially adsorbed at the interface, reducing the interfacial energy as well as providing a mechanical barrier to coalescence. The decrease in the free energy required for the emulsion formation consequently improves the thermodynamic

stability of the micro emulsion formulation[23, 174]. Therefore, the selection of oil and surfactant and the mixing ratio of oil to S/CoS, play an important role in the formation of the micro emulsion.

7.2 CONSTRUCTION OF PHASE DIAGRAM

In the present study, sunflower oil was tested for phase behavior studies with labrasol and transcutol HP for Atorvastatin calcium and peceol (oil) with labrasol and Transcutol HP as the S/CoS mixture. As observed from the ternary plot in Figures 7a and 7b, sunflower oil gave a wider micro emulsion region at 3:1 Smix ratio for Atorvastatin calcium and Peceol at 1:1 of Smix ratio. However, it was observed that increasing the surfactant ratio resulted in a loss of flowability and increase in surfactant toxicity. When co-surfactant was incorporated along with the surfactant in equal proportion at Smix ratio of 1:1 (Fig. 7b) a higher nanoemulsion region was observed in Glibenclamide SEDDS. This may be due to the addition of co-surfactant leading to further decrease in the interfacial tension, which will lead to increase in the fluidity of the interfacial film, thus increasing the entropy[175]. The % of oil, surfactant and cosurfactant selected for both the drugs were selected from the phase diagram and only those formulations which use the minimum and maximum concentration of Smix were taken for the formulation of SEDDS. Moreover, the self-emulsification ability of SEDDS depends on the formulation parameter variables such as surfactant/ cosurfactant and oil ratio, a polarity of the emulsion, globule size and charge on the droplets. The stability and efficiency of the drug is increased by self-emulsification property.

7.3 OPTIMIZATION OF SEDDS

Based on the ternary phase diagram and self-emulsification studies, sunflower oil (X_1), Smix- labrasol and transcutol HP (X_2) were chosen as components for Atorvastatin calcium SEDDS and peceol (X_1), Smix- labrasol and transcutol HP (X_2) were chosen for Glibenclamide SEDDS. To perform the 3^2 factorial design it was essential to identify the concentration of self-emulsification region from ternary phase diagrams of the SEDDS containing oil, surfactant and cosurfactant. The factorial design was carried out for optimization of two dependent variables such as globule size (Y_1) and % drug loading (Y_2) and concentrations of oil(X_1), Smix(X_2) were taken

as dependent variables. The experimental runs and the observed Y_1, Y_2 responses for 13 formulations were reported in Table 14a and Table 14b as generated by using Design Expert software version 10.0.2.0. The values of Y_1 and Y_2 observed responses ranged from 106.8 nm to 415 nm and 70.1% to 91.5% for Atorvastatin calcium. The Y_1 and Y_2 values were between 169.7nm to 616.3nm and 82% to 92.3% respectively for Glibenclamide. The model selection was based on the comparisons of several statistical parameters including standard deviation (SD), R-squared values, % correlation variation (% CV) and Sum of Squares (SS). The details are mentioned in the Table 16a and Table 16b which suggested special quadratic model for analyzing Y_1 and Y_2 responses, respectively. Adequate precision is a measure of the signal to noise ratio. A ratio greater than 4 is desirable. The ratio was found to be 7.629 and 16.864 for Y_1 and Y_2 responses, respectively for Atorvastatin calcium. For Glibenclamide the ratio was found to be 7.414 and 7.335 for Y_1 and Y_2 responses which indicates adequate precision. The analysis of variance (ANOVA) was applied for estimation of significance of the model. The ANOVA responses were summarized in Table 15a and Table 15b. A model is considered to be significant if the if the p-value (significance probability value) is less than 0.05 using 5% significance level. From the p-values presented in Table 15a and Table 15b it can be concluded that for responses Y_1 and Y_2, quadratic model were significant. From Table 17a the interaction terms X_1X_2, X_2^2 are significant terms for Atorvastatin calcium and both are significant model terms for globule size and % drug load. From Table 17b the main variable X_1 is significant model term for globule size in Glibenclamide. The polynomial regression equations as discussed in the results and analysis section represent the quantitative effect of independent variables (X_1 and X_2) and their interactions on the responses (Y_1 and Y_2). A positive sign in interpretation of the regression equations for both the drugs represented a synergistic effect, while a negative sign indicated an antagonistic effect. The theoretical values of Y_1 and Y_2 were obtained by substituting the values of X_1 and X_2 in the polynomial regression equation. The relationship between the dependent and independent variables for Atorvastatin calcium were further elucidated using 2D contour plots and 3D response curves which were represented by Figures 10a, 10b, 10c and 10d. Figures 11a, 11b, 11c and 11d showing the 2D contour plots and 3D response curves represented the

effect of variables of X_1, X_2 on the responses of Y_1 and Y_2, respectively. From Figure 10b of 3D response curve of Atorvastatin calcium it was clearly refined that when the concentration of Smix was increased from 30% to 70% the globule size was minimized. The same effect was observed in Figure 11b of Glibenclamide the particle size Y_1 response was decreased when the Smix concentration was increased from 30% to 50%. From Figure 10d it was illustrated the % drug loading of Atorvastatin calcium was increased when the level of oil concentration was increased from 40% to 80%. The % drug loading of Glibenclamide showed a significant effect when the concentration of oil was increased from 15% to 25% which is represented by Figure 11d. After studying the effect of the independent variables on the responses, the levels of these variables that give the optimum response were determined. The optimum formulation gives a minimum droplet size and higher % drug loading value along with optimum amount of oil and optimum amount of surfactant in the resultant SEDDS. Numerical optimization was achieved by Design expert software using desirability function which sets the minimum and maximum targets for the constraints. In the present study the experimental target was programmed with minimum globule size and maximum drug loading for the optimized SEDDS formulation and the results obtained are desirable. The selected values obtained for Atorvastatin calcium during the optimization process of X_1,X_2 were found to be 67.586% of oil, 52.529% of Smix and the predicted response were Y_1=153.651nm, Y_2= 88.582 % respectively. The optimized formulation of Glibenclamide consisted of 15.046 % of oil, 41.047 % of Smix with the predicted response of Y_1= 169.678 nm, Y_2=90.743 %. For confirmation a fresh formulation in triplicate was prepared at the optimum levels of the independent variables and the resultant SEDDS formulations were evaluated for the responses. The results of experimental value were in close agreement with predicted values for both the drugs as shown in Table 18 and the biasness were calculated which was found within the limits. The overlay plot of Atorvastatin calcium for two responses illustrated in Figure 12a showed the optimized concentration of oil (67.5761%) and Smix (52.5328% with the result of minimum particle size as 153.597 nm and 88.5782% as maximum drug loading for Atorvastatin calcium. The overlay plot of the predicted responses for Glibenclamide in Figure 12 b showed optimum concentrations 15.046% of oil, 41.047% of Smix with Y_1=169.678

nm, Y_2=90.743 for Glibenclamide. The desirability function plots of Atorvastatin calcium and Glibenclamide are illustrated in Figures 13a and 13b and the desirability function values were found to be 0.856 for Atorvastatin calcium, 0.921 for Glibenclamide which were found to be within the limits from 0 to 1. If any of the responses fall outside their desirability range, the overall function becomes zero.

7.4 SELF-EMULSIFICATION, DRUG PRECIPITATION, PHASE SEPARATION AND ASSESSMENT OF EMULSIFICATION TIME STUDIES

The self-emulsification was visually assessed to measure the apparent spontaneity of nanoemulsion formation. SEDDS when diluted in water were found to be non turbid and bluish transparent in appearance indicating spontaneous emulsification. All the resulting nanoemulsions were transparent with some opalescence in appearance and did not show any sign of phase separation. All the results of the nanoemulsion formulations were transparent and their absorbances were below 1 which showed good optical clarity as illustrated in Table 20a and Table 20b. The selection of surfactants and cosurfactants are determined by emulsification ability which depends on the physicochemical properties such as globule size, Zeta potential, turbidity measurement and PDI of the resulting nanoemulsion. All the formulations of both the drugs showed rapid emulsification time within a minute which proves the performance of the formulations for enhancing the dissolution profile. Thus it can be concluded that the absorption of the drug can be increased *in vivo* if the formulations have low emulsification time. The results are correlated with the findings of Warisnoicharoen *et al*[176] which concluded that emulsification is also influenced by the structure and chain length of the surfactant. Labrasol a hydrophilic surfactant having HLB value of 12 rendered very good nanoemulsions that required a short emulsification period.

7.5 TURBIDITY MEASUREMENTS

The rapid equilibrium reached by the dispersion and reproducibility of the process[29] is determined by turbidity measurements. The results obtained are within the limits for both the drugs. The emulsification efficiency is confirmed by a decrease in turbidity values which results in the corresponding decrease in droplet size. In the

formulation AF3 of Atorvastatin calcium the turbidity value was high of 210 NTU due to the larger droplet size of the emulsion formed of 290 nm which was shown in Table 14a and Table 20a. In the formulation GF5 of Glibenclamide SEDDS the turbidity value was high of 142 NTU due to the larger droplet size of the emulsion formed of 402.3nm which was shown in Table 14b and Table 20b. Labrasol is a high dispersible surfactant produces small droplet size and good PDI from all surfactants.

7.6 REFRACTIVE INDEX AND VISCOSITY MEASUREMENT

There was no significant difference in the refractive index values of the formulations tested. The refractive index values close to that of the water (1.333) prove the isotropicity of the formulations as indicated in Table 20a and Table 20b.

7.7 DROPLET SIZE

The globule size observed for all the formulation was less than 620 nm. The drug loading did not showed significant difference in the polydispersity values. The droplet size distribution is one of the most important characteristics of nanoemulsion for stability evaluation and is a critical step in the pathway of enhancing drug bioavailability. The smaller nano emulsion particle size leads to larger interfacial surface area, thus promoting rapid absorption and improved bioavailability.

7.8 ZETA POTENTIAL

The emulsion stability is directly related to the magnitude of the surface charge and the zeta potential is stability indicative parameter in colloidal system that is the system will resist aggregation. The reason for this behavior could be attributed to the strong repulsive Coulomb force between charged particles which counterbalances the vander Waals attraction force. Generally, an increase in electrostatic repulsive forces between micro emulsion droplets prevents the coalescence of micro emulsion droplets. High absolute (positive and negative) zeta potential values (above +30 or −30 mV) should preferably be achieved in most of the emulsions prepared in order to ensure the creation of a high-energy barrier against coalescence of the dispersed droplets[177]. The zeta potential of the optimized formulations of Atorvastatin calcium and Glibenclamide were found to be -31.8 mV to -24.8mV which were nearer the limits with good separation as indicated in the Fig.

15a, Fig. 15b. The zeta potential values were found to carry negative charges due to the presence of free fatty acids. Significant increase in the value of zeta potential was observed after drug loading, higher absolute values of zeta potential generally, indicated an increase of electrostatic repulsive forces between emulsion droplets preventing the coalescence droplets and increases in the stability. Among all the vehicles tested Labrasol (surfactant) and Transcutol HP (co-surfactant) proved to be the most promising vehicles for SEDDS formulation.

7.9 POLYDISPERSITY INDEX

The PDI for all the formulations were less than 0.5, formulation with combination of Smix showed lower PDI values as illustrated with the results given in Table 20a and Table 20b, thus indicating the uniform size distribution improving the performance of the spontaneous emulsification.

7.10 CLOUD POINT

The cloud point is an essential factor in the SEDDS consisting of non-ionic surfactants, and it is responsible for the successful formation of a stable microemulsion[32].When the temperature is higher than the cloud point, an irreversible phase separation will occur and the cloudiness of the preparation would have a bad effect on drug absorption because of the dehydration of the polyethylene oxide moiety. Hence, the cloud point for SMEDDS should be above 37°C which will avoid phase separation occurring in the gastrointestinal tract. The cloud point for all the formulation as shown in Table 20a and 20b tested was above 37°C. Therefore, it would suggest a stable micro emulsion can be formed at physiological temperature *in-vivo*.

7.11 DRUG LOADING

The maximum drug loading for Atorvastatin calcium SEDDS has been obtained ranging from 7.1 mg to 9.15mg, and for Glibenclamide ranged from 8.2 mg to 9.23 mg for all thirteen formulations. The results of predicted responses and measured responses of the % drug loading for the optimized formulations was very well matched for both the drugs as shown Table 18 showing minimum biasness and maximum drug loading of 8.72 mg for Atorvastatin calcium and 8.93 mg of

Glibenclamide can be incorporated safely in to the optimized formulations of OPFA and OPFG.

7.12 *IN VITRO* DISSOLUTION STUDY AND STABILITY STUDY

The dissolution study of API, marketed formulation and optimized formulations were performed in 6.8 pH phosphate buffer for Atorvastatin calcium. The comparison results in Fig.16a depicted that the optimized formulation above 90 % drug release in 30 min showed the pH independent release of Atorvastatin from SEDDS formulations. The rapid release of Atorvastatin from SMEDDS formulations could be attributed to the spontaneous formation of micro emulsion with a small droplet size, which permitted a faster rate of drug release into the aqueous phase, much faster than that of plain Atorvastatin. Thus, this greater availability of dissolved Atorvastatin from the SEDDS formulation could lead to higher absorption and higher oral bioavailability. Drug release from the all SEDDS formulation was found to be significantly higher as compared with that of plain Glibenclamide and marketed tablet as showed in Fig. 16b. All Glibenclamide SEDDS formulation released drug above 90% within 5 min as showed in Table 21b. It could be suggested that the SEDDS formulation resulted in spontaneous formation of a micro emulsion with a small droplet size, which permitted a faster rate of drug release into the aqueous phase, much faster than that of plain Glibenclamide and marketed tablet. Thus, this greater availability of dissolved Glibenclamide from the SEDDS formulation could lead to higher absorption and higher oral bioavailability. It was also showed that increase in surfactant concentration and decrease in oil concentration in formulation increase in drug release. The selected optimized formulations OPFA and OPFG from Table 24a and Table 24b were physically stable and did not showed any changes in the color and appearance of the formulation. Also there was no significant change in the drug content and release through the time of the stability study after the formulations being subjected to different stress conditions (cold temperature of 4-8°C and elevated temperature of 50±2°C) of temperatures. Thus it can be concluded that SEDDS formulation for Atorvastatin calcium and Glibenclamide were stable.

CHAPTER 8

SUMMARY AND CONCLUSION

The purpose of this study was to develop an oral administrable SEDDS of poorly water soluble drugs of Atorvastatin calcium and Glibenclamide under Biopharmaceutical classification system of class II classification. Solubility evaluation and ternary phase diagram were carried out to select excipients of SEDDS. The composition of Atorvastatin calcium loaded and Glibenclamide loaded SEDDS was optimized using 3^2 facorial design. The impact of the formulation parameters on mean globule size and percentage drug load were studied by applying the analysis of variance and regression models. Several formulation and process variables were evaluated and optimized by response surface methodology. The optimum formulation was prepared by response optimizer through desirability function and the experimental values were found to be in close agreement with the predicted values. Optimized formulation was further subjected to stability studies. Optimal Atorvastatin calcium SEDDS contains sunflower oil as oil phase, labrasol as a surfactant and transcutol HP as cosurfactant (Smix) in the ratio of 67.586% oil and 52.529% % w/w Smix formulates SEDDS with lower droplet size (169.7nm), PDI (0.2), and zeta potential (-31.8 mv) and percentage drug load (87.2%) values. The optimal glibenclamide SEDDS contains peceol as oil phase, labrasol as a surfactant and transcutol HP as co-surfactant (Smix) in the ratio of 15.046% oil and 41.047% %w/w Smix formulates SEDDS with lower droplet size (172 nm), PDI (0.244), and zeta potential (-24.8mV) and percentage drug load (89.3%) values. It was concluded that the smaller particle size and drug load more the release of drug which results in better bioavailability. The *in vitro* evaluation parameters such as emulsification time, viscosity determination, cloud point measurement, turbidity measurement, refractive index and spectroscopic optical clarity test were performed and the results were found within the limits for all formulations of two drugs. The stability studies revealed that there was no change in particle size and percentage drug load for the two drugs after 6 months. The *in vitro* drug release from optimized Atorvastatin SEDDS formulation were found to be 99.75% after 90 min and 99.7% after 30 min and for Glibenclamide SEDDS. It was extremely higher in comparison to the marketed formulation and API

suspension. *In-vitro* drug release studies closely indicate that optimized formulations obey first order kinetics and the mechanism of drug release was by fickian diffusion. The results further concluded that SEDDS can be explored as a potential drug carrier for dissolution enhancement of Atorvastatin and Glibenclamide and other poorly soluble drugs.

CHAPTER 9

IMPACT OF THE STUDY

The impact of the present research work is that self-emulsifying drug delivery systems are the newer approach to enhance the dispersibility and minimize the particle size of dispersed systems, thereby potentially increasing oral absorption for poorly water soluble drugs.

Atorvastatin calcium, a 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor belonging to BCS Class II category is a plasma lipid-regulating agent. Atorvastatin calcium has therapeutic application in hyperlipidemia and cardiovascular events. The oral bioavailability of Atorvastatin calcium is only 12% and poor bioavailability has been attributed to its poor solubility in water and high presystemic clearance (> 80%). The utility of SEDDS improved the dissolution and bioavailability of Atorvastatin calcium. The higher drug load would lead to better patient compliance by reducing the size of the final dosage form and thereby the side effects of Atorvastatin calcium like myotoxicity (myopathy, myositis, and rhabdomyolysis) and hepatotoxicity can be reduced.

Glibenclamide (GBM) belongs to BCS Class II category of poor solubility and poor bioavailability drug used for the treatment of non-insulin dependent Diabetes mellitus. The oral absorption is dissolution rate limited and requires enhancement in the solubility and dissolution rate for increasing its oral bioavailability. An optimal self-emulsifying drug delivery system (SEDDS) containing GBM was formulated for enhancement of solubility and dissolution rate and thereby increasing its bioavailability. The study revealed that higher amount of surfactants significantly increased dissolution of Glibenclamide while decreasing emulsion droplet size and emulsification time.

Overall, the study suggested that dissolution and oral bioavailability of Atorvastatin calcium and Glibenclamide could be improved by SEDDS technology. This concept must be extrapolated in drug-food interaction studies.

BIBLIOGRAPHY

1. Amidon GL, Lennernas H, Shah VP, Crison JR. A theoretical basis for a biopharmaceutic drug classification: the correlation of *in vitro* drug product dissolution *in vivo* bioavailability. Pharm Res 1995; 12(3):413-420.
2. Pouton CW. Formulation of poorly water-soluble drugs for oral administration: Physicochemical and physiological issues and the lipid formulation classification system. Eur. J. Pharm. Sci. 2006; 29: 278-287.
3. Craig DQM. The use of emulsifying systems as a means of improving drug delivery B.T Gattefosse 1993; 86.
4. Aungst BJ. Novel formulation strategies for improving oral bioavailability of drugs with poor membrane permeation or presystemic metabolism. Journal of Pharm Sci 1993; 82 (10): 979-987.
5. Serajuddin AT. Solid Dispersion of poorly water soluble drugs: early promises, subsequent problems and recent breakthroughs. Journal of Pharm Sci 1999; 88(10):1058-1066.
6. Shah NH, Carvagal MT, Patel CL, Infeld M H, *et al*. Self - emulsifying drug delivery systems with polyglycolyzed glycerides for improving *in vitro* dissolution and oral absorption of lipophilic drugs. Int J Pharm 1994; 106:15-23.
7. Strategies to Formulate Lipid-based Drug Delivery Systems [Internet] Available from: http://www.americanpharmaceuticalreview.com/Featured-Articles/3688.
8. Pouton CW, Porter CJH. Formulation of lipid-based delivery systems for oral administration: Materials, methods and strategies, Advanced Drug Delivery Reviews. 2008; 60 (6): 625-637, 673-691.
9. Zhang P, Liu Y, Xu, J. Preparation and Evaluation of Self-emulsifying drug delivery system of Oridonin. Int. J. Pharm. 2008; 355, 269-276.
10. Microencapsulation: Methods and Industrial Applications Second edition by Simon Benita. CRC Press, 2005; p.433.
11. Charman SA, Charman WN, Rogge MC, Wilson TD, Dutko FJ *et al*. Self-emulsifying drug delivery systems formulation and biopharmaceutic evaluation of an investigational lipophilic compound. Pharm. Res.1992; 9:87-93.

12. Patel PA, Chaulang GM, Akolkotkar A, Mutha SS *et al*. Self - emulsifying drug delivery systems: A Review. Res J Pharm Technol 2008; 1:313-23.
13. Pallavi M Nigade, Swapnil Patil, Shradha S Tiwari. Self - emulsifying drug delivery system: A Review. International Journal of Pharmacy and Biological Sciences. 2012; 2 (2): 42-52.
14. Vishvajit A Kamble, Deepali M. Jagdale, Vilasrao J Kadam. Self Micro Emulsifying Drug Delivery System. International journal of Pharma and bio sciences 2010; 2:2-9.
15. Prajapati BG, Patel MM. Conventional and alternative pharmaceutical methods to improve bioavailability of lipophilic drugs. Asian J Pharma 2007; 1(1):1-8.
16. Gershanik T, Benita S, Self-emulsifying lipid formulations for improving oral absorption of lipophilic drugs. Euro J Pharm Bio Pharm 2000; 50(1): 179-188.
17. Jannin, VJ, Musakhanian, and Marchaud D. Approaches for the development of solid and semi-solid lipid-based formulations. Advanced Drug Delivery Reviews. 2008; 60(6): 734-746.
18. Constantinides P. Lipid microemulsions for improving drug dissolution and oral absorption: physical and biopharmaceutical aspects. Pharmaceutical research, 1995; 12(11):1561-1572.
19. Devani M, Ashford M, Craig DQ. The emulsification and solubilization properties of polyglycolysed oils in self-emulsifying formulations. J Pharm Pharmacol.2004; 56:307-16.
20. Kale AA, Patravale VB. Design and Evaluation of Self-Emulsifying Drug Delivery Systems (SEDDS) of Nimodipine. AAPS PharmSciTech.2008;9 (1):191-6.
21. Kumar A, Sharma S, Kamble R. Self-emulsifying Drug Delivery System (Sedds): Future Aspects, Int. J. Pharm. Pharm. Sci.2010; 2(4):7-13.
22. Francoise Nielloud, Gilberte, Marti-Mestres. Pharmaceutical Emulsions and Suspensions. CRC Press. 2000; 2nd ed. p.341.
23. Groves M, and Galindez D De. The self-emulsifying action of mixed surfactants in oil. Acta pharmaceutica Suecica, 1976; 13(4):361.
24. Dixit, AR, Rajput SJ, Patel SG. Preparation and bioavailability assessment of SMEDDS containing Valsartan. AAPS Pharmscitech. 2010; 11:314- 321.

25. Shafiq S, Shakeel F, Talegaonkar S, Ahmad FJ, *et al*. Development and bioavailability assessment of Ramipril nanoemulsion formulation. Eur J Pharm Biopharm. 2007; 66(2): 227-43.
26. Bandivadekar MM, Pancholi SS, Shelke N. Preparation and characterization of solid SMEDDS by adsorbent techniques to improve dissolution profile of poorly aqueous soluble drug Ramipril, International research Journal of Pharmacy. 2011; 2(6): 85-90.
27. Carli F and Chielleni E. Pharmaceutical composition comprising a water/oil/water double microemulsion incorporated in a solid support, 2004; Google Patents.
28. Pouton CW. Self-emulsifying drug delivery systems: Assessment of the efficiency of emulsification. Int J Pharm. 1985; 27(2-3): 335-48.
29. Gursoy N. Excipient effects on *in vitro* cytotoxicity of a novel Paclitaxel self-emulsifying drug delivery system. J. Pharm. Sci. 2003; 92: 2411–2418.
30. Gershanik T, Benita S. Positively charged self - emulsifying oil formulation for improving oral bioavailability of Progesterone. Pharm Dev Technol, 1996; 1: 147-157.
31. Teeranachaideekul V, Junyaprasert VB, Souto EB, Muller RH. Development of Ascorbyl palmitate nanocrystals applying the nanosuspension technology. Int J Pharm 2008; 354: 227-234.
32. Itoh K, Tozuka, Y, Oguchi T & Yamamoto K. Improvement of physicochemical properties of N-4472 part I formulation design by using self microemulsifying system. International journal of Pharmaceutics 2002; 238:153- 160.
33. Dissolution methods. [Internet] Available from: https://www.accessdata.fda.gov/ scripts/ cder/ dissolution/
34. Sahana, DK, Mittal, G, Bhardwaj V, Kumar MN. PLGA nanoparticles for oral delivery of hydrophobic drugs: influence of organic solvent on nanoparticle formation and release behavior *in vitro* and *in vivo* using Estradiol as a model drug. Journal of pharmaceutical sciences 2008; 97:1530-1542.
35. Zambaux, MF, Bonneaux F, Gref R, Dellacherie E *et al.* Preparation and characterization of protein C-loaded PLA nanoparticles. Journal of controlled release. 1999; 60:179-188.

36. Date AA, Nagarsenker, MS. Design and evaluation of self-nanoemulsifying drug delivery systems (SNEDDS) for Cefpodoxime proxetil. International journal of pharmaceutics 2007; 329 (1-2):166-172.
37. Singh, AK, Akash Chaurasiya, Manish Singh, Satish C *et al*. Exemestane loaded self-microemulsifying drug delivery system (SMEDDS): development and optimization. AAPS Pharmscitech 2008; 9: 628-634.
38. R Nanda Kishore, PR Yalavarthi, HC Vadlamudi, KR Vandana. Solid self–microemulsification of Atorvastatin using hydrophilic carriers: a design. Drug Dev Ind Pharm 2015; 41 (7):1213–1222.
39. Leppert PS, Fix JA. Use of everted intestinal rings for *in vitro* examination of oral absorption potential. J Pharm Sci. 1994; 83(7): 976-81.
40. KR Vandana, PR Yalavarthi, C.R Sundaresan, RN Sriramaneni, *in-vitro* assessment and pharmacodynamics of Nimesulide incorporated Aloevera trans emulgel. Curr Drug Discov Tech. 2014; 11:162–167.
41. Pouton CW. Lipid formulations for oral administration of drugs: nanoemulsifying, self-emulsifying and self-microemulsifying drug delivery systems. Eur J Pharm Sci 2000; 11: S93-S98.
42. More HN, Hazare AA. Practical Pharmaceutics (Physical pharmacy), Manas Prakashan, Kolhapur, 1st Ed., 2004; 86-105.
43. Nekkanti V, Karatgi P, Prabhu R, Pillai R. Solid self-microemulsifying formulation for Candesartan cilexetil, AAPS Pharmsci., 2010;11: 9-17. [DOI] PMid: 20013081 PMCid:2850472
44. Herbert A Liberman., Martin M Rieger, Gilbert S Banker Pharmaceutical Dosage forms Disperse systems Volume 1 2nd edn. pp 438-439.
45. Doombos, DA. Optimization in pharmaceutical sciences, Pharm. Weekblad Sci 1981; Ed3: 549-577.
46. Myers RH, Montgomery DC. Response Surface Methodology: Process and Product Optimization Using Designed Experiments. 1995: New York: John Wiley & Sons.p.1.
47. Prindene P, Piccerelle P, Cautiire E, Kalantzis G, *et al*. Formulation and evaluation of o/w emulsions using experimental design. Int. J. Pharm. 1988; 163: 73-79.

48. Bodea A, Leucuta SE. Optimization of Propranolol hydrochloride sustained release pellets using factorial design. Int. J. Pharm. 1997; 154: 49-57.
49. Lipps, D M, Sakr, AM. Characterization of wet granulation process parameters using response surface methodology I. Top spray fluidized bed. J. Pharm. Sci. 1994; 83: 937-947.
50. Khan, MA, Bolton, S, Kislaloglu, MS. optimization of process variables for the preparation of Ibuprofen coprecipitates with Eudragit. Int. J. Pharm. 1994; 102: 185-191.
51. Box, GEP Hunter, WG, Hunter, JS. Design with more than one blocking variable. In Statistics for Experimenters. 1978; New York: John Wiley & Sons. pp.245-280.
52. Nachtshiem CJ. Tools for computer aided design of experiments. J Qual Techno.1987; 19:132.
53. Beatriz Zanchetta, Marco Vinícius Chaud, Maria Helena Andrade Santana. Self-Emulsifying Drug Delivery Systems (SEDDS) in Pharmaceutical Development. J Adv Chem Eng.5-3; 2-7.
54. Maryadele J O'Neil. Editor. The Merck index: An Encyclopedia of Chemicals, Drugs, and Biologicals. 13th ed. p. 148, 440.
55. Sean C. Sweet man. Martindale: The complete drug reference 36th ed. p. 798, 1218.
56. Atorvastatin, Glyburide. [Internet] Available from: http://www.drugbank.ca/ drugs/DB01076, DB01016.
57. Indian Pharmacopoeia Vol II; 2007; 5th ed. p.547-548. The Indian Pharmacopoeia Commission. Govt. of India, Ministry of Health & Family Welfare. Ghaziabad.
58. Micronase (glyburide) [Internet] available from: http://www.rxlist.com/ micronase-drug.htm
59. Raymond C Rowe, Paul J Sheskey, Marian E Quinn Editors. Handbook of pharmaceutical excipients. Pharmaceutical Press, 2009. 6th ed. p.721-722, 184-185, 199-200,614-615, 470-471,288-289.
60. Pasquale D *et al*. A study of sterilizing conditions for injectable oils. Bull Parenter Drug Assoc 1964; 18(3): 1–11.
61. Kupiec TC *et al.* Dry heat sterilization of parenteral oil vehicles. Int J Pharm Compound. 2000; 4(3): 223–224.

62. Indian standard specification for mustard oil IS: 546-1975. Second revision August 2007; p.5-7. Bureau of Indian standards. New Delhi.
63. Indian standard specification for Rice bran oil. IS: 3448-1984. Second revision. p. 4-6. Bureau of Indian standards. New Delhi.
64. Gibson L. Lipid-based excipients for oral drug delivery. In: Hauss, D. J., Oral lipid-based formulations: enhancing the bioavailability of poorly water soluble drugs. Informa Healthcare Inc., New York. 2008:1–31.
65. Strickley RG. Currently marketed oral lipid-based dosage forms: drugs products and excipients, in: Hauss, D J. Oral lipid-based formulations: enhancing the bioavailability of poorly water soluble drugs. Informa Healthcare Inc., New York. 2007: 1–31.
66. Strickley RG. Solubilizing excipients in oral and injectable formulations, Pharm Res. 2004; 21: 201–23.
67. www.colorcon.com. Retrieved on 14-3-2009
68. Xianyi Sha, Guijun Yan, Yunjuan Wu, Junchan Li. Effect of self-microemulsifying drug delivery systems containing Labrasol on tight junctions in Caco-2 cells. Eur. J. Pharm. Sci. 2005; 24: 477–486.
69. Ahmed Abdalla, Sandra Klein, Karsten Mader. A new self-emulsifying drug delivery system (SEDDS) for poorly soluble drugs: Characterization, dissolution, *in vitro* digestion and incorporation into solid pellets. Eur. J. Pharm. Sci.2008; 35(5): 457-464.
70. Sadika Akhter, Md. Ismail Hossain. Dissolution enhancement of Capmul PG8 and Cremophor EL based Ibuprofen Self Emulsifying Drug Delivery System (SEDDS) using Response surface methodology. International Current Pharmaceutical Journal. 2012; 1(6): 138-150.
71. Albertini B, Sabatino MD, Melegari C, Passerini N. Formulation of spray congealed microparticles with self-emulsifying ability for enhanced Glibenclamide dissolution performance. J Microencapsul. 2015; 32(2):181-92.
72. Bachhav YG, Patravale VB. SMEDDS of Glyburide: Formulation, *in vitro* evaluation, and stability studies. AAPS Pharm SciTech. 2009; 10(2):482-7.
73. Prabagar Balakrishnan, Beom-Jin Lee, Dong Hoon Oh, Jong Oh Kim. Enhanced oral bioavailability of Dexibuprofen by a novel solid Self-emulsifying drug

delivery system (SEDDS). European Journal of Pharmaceutics and Biopharmaceutics. 2009:72(3):539–545.

74. Mithun Mohanraor Bandivadeka, Shyam Sundar Pancholi, Ruchika Kaul-Ghanekar, Amit Choudhari. Self-micro emulsifying smaller molecular volume oil (Capmul MCM) using non-ionic surfactants: a delivery system for poorly water-soluble drug. Drug Development and Industrial Pharmacy. 2012; 38 (7): 883–892.
75. Bandivadekar M, Pancholi S, Kaul-Ghanekar R, Choudhari A. Single non-ionic surfactant based self-nanoemulsifying drug delivery systems: formulation, characterization, cytotoxicity and permeability enhancement study. Drug Dev Ind Pharm. May 2013; 39(5):696-703.
76. Shantanu Bandyopadhyay, Katare OP, Bhupinder Singh. Optimized self nano-emulsifying systems of Ezetimibe with enhanced bioavailability potential using long chain and medium chain triglycerides. Colloids and Surfaces B: Biointerfaces. 2012; 100:50-61.
77. Sachs Barrable K, Thamboo A, Lee SD, Wasan KM. Lipid excipients Peceol and Gelucire 44/14 decrease P-glycoprotein mediated efflux of rhodamine 123 partially due to modifying P-glycoprotein protein expression within Caco-2 cells. J Pharm Pharm Sci. 2007; 10(3):319-31.
78. Emad B Basalious, Nevine Shawky, Shaimaa M, Badr-Eldin. SNEDDS containing bioenhancers for improvement of dissolution and oral absorption of Lacidipine. I: Development and optimization. International Journal of Pharmaceutics 2010; 391: 203–211.
79. Bhattacharya A, Bajpai M. Development and Oral Bioavailability of Self Emulsifying Formulation of Ketoconazole. International Journal of Pharmaceutical Sciences and Nanotechnology. 2013; 5(4): 1858-1865.
80. Fulden Buyukozturk, James C BenneyanRebecca L Carrier. Impact of emulsion-based drug delivery systems on intestinal permeability and drug release kinetics. Journal of Controlled Release.2010; 142 (1): 22-30.
81. Cirri M, Roghi A, Valleri M, Mura P. Development and characterization of fast dissolving tablet formulations of Glyburide based on solid self microemulsifying systems. Eur J Pharm Biopharm. 2016; 104:19-29.

82. Manisha Devani, Marianne Ashford, Duncan QM. Craig. The emulsification and solubilisation properties of polyglycolysed oils in self-emulsifying formulations. Journal of Pharmacy and Pharmacology.2004; 56:307–316.
83. Elnaggar YSR, El-Massik MA, Abdallah OA. Self-nanoemulsifying drug delivery systems of Tamoxifen citrate: Design and optimization. Int. J. Pharm. 2009: 380: 133–41.
84. Sylvie Fernandez, Vincent Jannin Jean-David Rodier, Nicolas Ritter, Bruno Mahler. Comparative study on digestive lipase activities on the self emulsifying excipient Labrasol medium chain glycerides and PEG esters. Biochimica et Biophysica Acta 1771.2007; 633–640.
85. Erica Franceschinisa, Dario Voinovicha, Mario Grassib, Beatrice Perissuttia. Self-emulsifying pellets prepared by wet granulation in high shear mixer: influence of formulation variables and preliminary study on the *in vitro* absorption. International Journal of Pharmaceutics. 2005; 291: 87-97.
86. Gao P, Witt MJ, Haskell RJ, Zamora KM *et al*. Application of a mixture experimental design in the optimization of a self-emulsifying formulation with a high drug load. Pharm Dev Technol. 2004; 9(3):301-9.
87. Hashem FM, A Sawahli MM, Nasr M, Ahmed OA. Custom fractional factorial designs to develop Atorvastatin self-nanoemulsifying and nanosuspension delivery systems enhancement of oral bioavailability. Drug Des Devel Ther. 2015; 9: 3141-52.
88. Kadu PJ, Kushare SS, Thacker DD, Gattani SG. Enhancement of oral bioavailability of Atorvastatin calcium by self-emulsifying drug delivery systems (SEDDS). Pharm Dev Technol. 2011; 16(1):65-74.
89. Fariba Khan, Md. Saiful Islam, Monzurul Amin Roni, Reza-Ul Jalil. Systematic Development of Self-Emulsifying Drug Delivery Systems of Atorvastatin with Improved Bioavailability Potential. Scientia Pharmceutica. 2012; 80: 1027–1043.
90. Czajkowska Kosnik A, Szekalska M, Amelian A, Szymanska E. Development and Evaluation of Liquid and Solid Emulsifying Drug Delivery Systems for Atorvastatin. Molecules. 2015; 20(12):21010-22.
91. Ying Liu, Zhi qiang chen, Xin zhang, Nian ping feng *et al.* An Improved Formulation Screening and Optimization Method Applied to the Development of

a Self Micro emulsifying Drug Delivery System. Chem. Pharm. Bull.2010; 58(1): 16-22.

92. Mantri SK, Pashikanti S, Murthy KV. Development and characterization of self-nanoemulsifying drug delivery systems (SNEDDS) of Atorvastatin calcium. Curr Drug Deliv. 2012; 9(2):182-96.
93. Nirmal Marasini, Bijay Kumar Poudel, Tuan Hiep Tran, Han Gon Choi S. Statistical Modeling, Optimization and Characterization of Spray-Dried Solid Self Microemulsifying Drug Delivery system Using Design of Experiments. Chem. Pharm. Bull. 2013; 61(2) 184–193.
94. Venkatesh Miryala, Mallesh Kurakula. Self-nanoemulsifying drug delivery system (SNEDDS) for oral delivery of Atorvastatin- Formulation and Bioavailability studies. Journal of Drug Delivery & Therapeutics. 2013; 3(3): 131-142.
95. Mohsin K, Porter CJ, Pouton CW, Devraj R, Williams HD, Warren DB, *In vitro* assessment of drug-free and Fenofibrate-containing lipid formulations using dispersion and digestion testing gives detailed insights into the likely fate of formulations in the intestine. Eur J Pharm Sci.2013; 49(4):48-60.
96. Neelam Patel, Kinesh Patel, Lata Panchal, Pragna Shelat *et al*. Development and characterization of self micro emulsifying drug delivery system of Glibenclamide: a novel drug delivery system. Inventi Rapid-ndds. Inventi: pndds/449/12.
97. Pradeep Patil, Prasad Joshi, Anant Paradkar, Effect of Formulation Variables on Preparation and Evaluation of Gelled Self-emulsifying Drug Delivery System (SEDDS) of Ketoprofen. AAPS Pharm SciTech. 2004; 5 (3): 43-50.
98. Colin W. Pouton. Formulation of self-emulsifying drug delivery systems. Advanced Drug Delivery Reviews. 1997; 25:47-58.
99. Shailesh T Prajapati, Harsh A Joshi, Chhaganbhai N Patel. Preparation and Characterization of Self-Microemulsifying Drug Delivery System of Olmesartan Medoxomil for Bioavailability. Improvement. Journal of Pharmaceutics. http://dx.doi.org/10.1155/2013/728425.
100. Panner Selvam R, Kulkarni PK, Mudit Dixit. Preparation and Evaluation of Self-nanoemulsifying Formulation of Efavirenz. Ind J Pharm Edu Res. 2013; 47:1.

101. Xianyi Sha, Juan Wu, Yanzuo Chen, Xiaoling. Fang Self microemulsifying drug-delivery system for improved oral bioavailability of Probucol: preparation and evaluation. International Journal of Nanomedicine. 2012; 7:705–712.
102. Jessy Shaji , Shital Lodha. Response Surface Methodology for the Optimization of Celecoxib Self-microemulsifying Drug delivery System. Indian J Pharm Sci. 2008; 70(5): 585-590.
103. Shakeel F, Haq N, Alanazi FK, Alsarra IA. Self-nanoemulsifying performance of two grades of Lauroglycol (Lauroglycol-90 and Lauroglycol-FCC) in the presence of mixed nonionic surfactants. Pharmaceutical Development and Technology. 2014; 19 (7):799-805.
104. Sharma S, Bajaj H, Bhardwaj P, Sharma AD *et al*. Development and characterization of self emulsifying drug delivery system of poorly water soluble drug using natural oil. Acta Pol Pharm. 2012; 69(4):713-7.
105. Shen H, Zhong M. Preparation and evaluation of self-microemulsifying drug delivery systems (SMEDDS) containing Atorvastatin. J Pharm Pharmacol.2006; 58(9):1183-91.
106. Singh SK, Verma PR, Razdan B. Glibenclamide loaded self-nanoemulsifying drug delivery system: development and characterization. Drug Dev Ind Pharm. 2010; 36(8):933-45.
107. Smita K, Pawar, Pradeep R. Vavia. Rice Germ Oil as Multifunctional Excipient in Preparation of Self-Microemulsifying Drug Delivery System (SMEDDS) of Tacrolimus. AAPS Pharm Sci Tech. 2012; 13(1): 254-261.
108. Subramanian R, Wasan KM. Effect of lipid excipients on *in vitro* pancreatic lipase activity. Drug Dev Ind Pharm. 2003; 29(8):885-90.
109. Shyam Vithlani, Shruti Sarraf, Cheng Shu Chaw. Formulation and *in vitro* evaluation of self-emulsifying formulations of Cinnarizine. Drug Dev Ind Pharm. 2012; 38(10):1188-94.
110. Zhiyuan Wang, Jin Sun, Yongjun Wang, Xiaohong Liu *et al*. Solid self-emulsifying nitrendipine pellets: Preparation and *in vitro/in vivo* evaluation. International Journal of Pharmaceutics. Jan 2010; 383, (1–2):1-6.

111. Yeom DW, Song YS, Kim SR, Lee SG *et al*. Development and optimization of a self-microemulsifying drug delivery system for Atorvastatin calcium by using D-optimal mixture design. Int J Nanomedicine.2015; 10: 3865-77.
112. Yi Zhaoa, Changguang Wanga, Albert HL. Chowb, Ke Renc *et al*. Self-nanoemulsifying drug delivery system (SNEDDS) for oral delivery of Zedoary essential oil: Formulation and bioavailability studies. International Journal of Pharmaceutics 2010; 383: 70–177.
113. Liang-Zhong Lv, Chen-Qi Tong, Qing Lv, Xin-Jiang Tang *et al*. Enhanced absorption of hydroxysafflor yellow A using a self-double-emulsifying drug delivery system: *in vitro* and *in vivo* studies. International Journal of Nanomedicine. 2012; 7:4099–4107.
114. Jaiswal Parul, Aggarwal Geeta, Harikumar SL, Kaur Amanpreet. Bioavailability enhancdement of poorly soluble drugs by smedds: a review. Journal of Drug Delivery & Therapeutics; 2013; 3(1): 98-109.
115. RN Gursoy, S Benita. Self-emulsifying drug delivery systems for improved oral delivery of lipophilic drugs. Biomedicine and Pharmacotherapy 2004; 58: 173-182.
116. Sandeep Kumar Singh, Priya Ranjan Prasad Verma, Balkishen Razdan. Glibenclamide-loaded self nanoemulsifying drug delivery system: development and characterization. Drug Development and Industrial Pharmacy. 2010; 36(8): 933–945
117. Rawat M, Saraf S, Saraf S. Influence of Selected Formulation Variables on the Preparation of Enzyme-entrapped Eudragit S100 Microspheres. AAPS PharmSciTech. 2007; 8:E116.
118. Patil P, Joshi P, Paradkar A. Effect of Formulation Variables on Preparation and Evaluation of Gelled Self-emulsifying Drug Delivery Systems (SEDDS) of Ketoprofen. AAPS PharmSciTech. 2004; 5:42.
119. Chen ML. Lipid excipients and delivery systems for pharmaceutical development: A regulatory prospective. Adv Drug Deliv Rev. 2008; 60(6):768-77.
120. Holm R, Porter CJ Edwards GA, Mullertz A. Examination of oral absorption and lymphatic transport of Halofantrine in a triple-cannulated canine model after administration in self triglycerides. Eur J Pharm Sci. 2003; 20(1): 91-7.

121. Yap SP, Yyen KH. Influence of lipolysis and droplet size on Tocotrienol absorption from self emulsifying formulations. Int J Pharm. 2004; 281(1-2): 67-78.
122. Developing lipid based formulations for oral bioavailability enhancement. Formulation guidelines by gattefosse [Internet] Available from: (http://www.gattefosse.com)
123. Cornaire G, Houin G, Arellano C, Cloarec A Impact of excipients on the absorption of P-glycoprotein substrates *in vitro* and *in vivo*. Int J Pharm.2004; 278: 119-131.
124. David J. Hauss, Steven E Fogal James V, FicorilliCaswall A Price. Lipid-Based Delivery Systems for Improving the Bioavailability and Lymphatic Transport of a Poorly Water-Soluble LTB_4 Inhibitor. Journal of Pharmaceutical sciences.1998; 87(2):164-169.
125. Indian Pharmacopoeia Vol I 2007; 5^{th} ed. p. 135-138, 13-14. The Indian Pharmacopoeia Commission. Govt. of India, Ministry of Health & Family Welfare. Ghaziabad.
126. Indian standard specification for methods of sampling and test for oils and fats. IS 548- 1. p. 40-41, p. 47-52, p. 29-30. Bureau of Indian standards. New Delhi.
127. Singh S, Inamullah, Rai J, Choudhary N, Sharma S**.** Stability indicating uv-vis spectrophotometric method for estimation of Atorvastatin calcium and Fenofebrate in tablet dosage forms. Bulletin of Pharmaceutical Research 2012; 2 (3):159-66.
128. Namdeo R, Jadhav, Ramesh S, Kambar, Sameer J, Nadaf. Dual wavelength spectrophotometric method for simultaneous estimation of Atorvastatin Calcium and Felodipine from Tablet Dosage Form. Advances in Chemistry. 2014; Article ID 131974, 6 pages http://dx.doi.org/10.1155/2014/131974.
129. Abida Bilal, Rehman, Muhammad Sajid Hamid Akash, Khalid Hussain, Muhammad Ibrahim, Syed Saeedul Hussan. Development and Validation of Analytical Method for Qualitative and Quantitative Determination of Glibenclamide in Different Brands of Tablet Dosage form Using UV-Visible Spectroscopy. J Mol Genet Med 2013; 7:3.

130. Santosh Girani, Dhaval Patel, Mantesh Kavatekar, Ajay Shahapur, Vital Vijapure. Formulation and evaluation of matrix type transdermal therapeutic system containing Glibenclamide. European journal of pharmaceutical and medical research. 2016; 3(5): 556-569.
131. Navneet Dhillon, Kanav Midha, Manju Nagpal**,** Rakesh Pahwa. Formulation, Optimization and Characterization of Solid Dispersion of Glibenclamide. Pharm Methods. 2015; 6(2): 72-81.
132. Divyakumar Bora, Priyanka Borude, Kiran Bhise. Formulation and Evaluation of Self microemulsifying drug delivery system of low solubility drug for enhanced solubility and dissolution. Asian Journal of Biomedical and Pharmaceutical Sciences.2012; 15:7-14.
133. Craig DQM, Barker SA, Banning D. Booth SW. An investigation into mechanism of self emulsification using particle size analysis and low frequency dielectric spectroscopy. Int. J. Pharm. 1995; 114: 103–110.
134. Chouksey R, Pandey H, Jain AK, Soni H, *et al*. Preparation and evaluation of the self-emulsifying drug delivery system containing atorvastatin HMG-CoA inhibiter. Int J Pharm Pharm Sci. 2011; 3: 147–152.
135. Ajeet K Singh, Akash Chaurasiya, Manish Singh, Satish C *et al*. Exemestane Loaded Self-Microemulsifying Drug Delivery System (SMEDDS): Development and Optimization, AAPS Pharm Sci Tech 2008; 9:628-34.
136. Subramanian N, Ray S, Ghosal SK, Bhadra R, *et al*. Formulation Design of Self-Microemulsifying Drug Delivery Systems for Improved Oral Bioavailability of Celecoxib. Biol Pharm Bull. 2004; 27: 1993–1999. http://dx.doi.org/10.1248/bpb.27.1993.
137. Balakrishnan P, Lee BJ, Oh DH, Kim JO, Lee YI *et al*. Enhanced oral bioavailability of coenzyme Q10 by a novel solid self-emulsifying drug delivery system. Int J Pharm Sci. 2009; 374: 66-72.
138. Parmara N, Singlab N, Amina S, Kohlia K. Study of cosurfactant effect on nanoemulsifying area and development of lercanidipine loaded (SNEDDS) self nanoemulsifying drug delivery system. Colloids and Surfaces B: Biointerfaces 2011; 86:327–338.

139. Zhao Y, Wang C, Chow AH. Self nanoemulsifying drug delivery system (SNEDDS) for oral delivery of Zedory Essential oil: Formulation and bio availability studies. Int. J. Pharm. 2010; 383:170-177.
140. Kamalinder K Singh, Sharvani K Vingkar. Formulation antimalarial activity and biodistribution of oral lipid nanoemulsion of Primaquine. International Journal of Pharmaceutics 2008; 347:138.
141. Vipul Rokad, Chirag Nagda, Dhruti Nagda. Design and evaluation of solid self-emulsifying drug delivery system of Rosuvastatin calcium. Journal of Young Pharmacists Jul-Sep 2014; 6 (3):37-45.
142. Kim JY, Young SK. Enhanced absorption of Indomethacin after oral or rectal administration of Self emulsifying system containing Indomethacin torats, Int.J.Pharm.2000; 194: 81- 89.
143. Patil P, Vandana P, Paradkar P. Formulation of self emulsifying drug delivery system for oral delivery of Simvastatin: *In vitro* and *in vivo* evaluation. Acta pharma. 2007; 57: 111-122.
144. Maryam Kouchak, Mohammadreza Avadi, Mohammadreza Abbaspour, Alireza Jahangiri *et al*. Effect of different molecular weights of chitosan on preparation and Characterization of Insulin loaded nanoparticles by ion gelation method. Int. J. Drug Dev. & Res 2012; 4(2): 271-277.
145. Satish puttachari, Navanath V Kalyanea, Sarbaniduttagupta. Design and evaluation of self-micro emulsifying drug delivery systems of Acyclovir. Int J Pharm Pharm Sci, 2014; 6(4): 677-681.
146. Harshal DM, Tanvir S, Dheeraj B, Rajendra DW. Design and development of Solid micro emulsifying drug delivery system (SMEDDS) of Fenofibrate. Int J Pharm Pharm Sci. 2011; 3(4): 163-166.
147. Formulation Development and Bioavailability Evaluation of a Self-nanoemulsifying Drug Delivery System (SNEDDS) of Atorvastatin Calcium. 2013. [Internet] Available from https://www.semanticscholar.org/ 4985.
148. Yogeshwar G, Bachhav, Vandana B. Patravale. SMEDDS of Glyburide: Formulation, *In Vitro* Evaluation and Stability Studies. AAPS Pharm SciTech, 2009; 10 (2): 482-487.

149. Meena AK, Ratnam DV, Chandraiah G, Ankola DD *et al*. Oral nanoparticulate Atorvastatin calcium is more efficient and safe in comparison to Lipicure® in treating hyperlipidemia [J]. Lipids 2008; 43(3): 231-241.
150. Manavalan R, Ramasamy S. Physical Pharmaceutics. Vignesh Publisher Chennai. 2004: 288-299.
151. Ajith S Narang, David Delmarre, Danchen Gao. Stable drug encapsulation in micelles and microemulsions. International journal of Pharmaceutics. 2007; 345 (1-2): 9-25.
152. USP 2009 Vol 1. The United States Pharmacopoeial Convention. Rockville. USA. p. 1208-1210, 1291-1292, 1246-1247, 1335, 1366.
153. USP Pharmacopoeial Convention. USP twelfth revision 1942. The United States Pharmacopoeial Convention. Rockville. USA.
154. Indian standard specification for coconut oil BIS IS 542 1968. Bureau of Indian standards. New Delhi.
155. Indian standard specification for sunflower oil BIS IS 4277-1975. Bureau of Indian standards. New Delhi. p. 6.
156. Sergio Luis Costa Ferreira, Roy Edward Bruns, Erik Galvao Paranhos da Silva, Walter Nei Lopes dos Santos. Statistical designs and response surface techniques for the optimization of chromatographic systems. Journal of Chromatography A. 2007; 1158: 2–14.
157. Pharmaceutical dosage forms Disperse system Vol 1 second edition. Marcel Dekker 1998. Edited by Martin M Rieger, Gilbert S Banker, Herbert A Lieberman (inbunden, 1996) : 438-465..
158. Yalabik-Kas HS, Groves MJ. Zeta potential of droplets prepared from a self-emulsifying oil. Drug Develop Ind Pharm. 1984; 10:211–23.
159. Kevin S Chu, Allison N Schorzman, Mathew C Finniss, Charles J Bowerman. Nanoparticle drug loading as a design parameter to improve Docetaxel pharmacokinetics and efficacy. Biomaterials.2013; 34(33):1-13.
160. Derringer G, Suich. Simulataneous optimization of response variables. RJ. Qual. Technol. 1980; 12, 214–219.

161. Lea, AP, McTavish, D. Atorvastatin. A review of its pharmacology and therapeutic potential in the management of hyperlipidaemias. Drugs. 1997, 53: 828-847.
162. Black, AE, Hayes RN, Roth BD, Woo P *et al*. Metabolism and excretion of Atorvastatin in rats and dogs. Drug Metab. Dispos. 1999, 27; 916-923.
163. Lennernas H. Clinical pharmacokinetics of atorvastatin. Clin. Pharmacokinet. 2003; 42:1141-1160.
164. Wei H, Löbenberg R. Biorelevant dissolution media as a predictive tool for Glyburide a class II drug. Eur J Pharm Sci.2006; 29:45–52.
165. Neuvonen PJ, Kivisto KT. The effects of magnesium hydroxide on the absorption and efficacy of two Glibenclamide preparations. Br J Clin Pharmacol.1991; 32:215–20.
166. Lobenberg R, Amidon GL. Modern bioavailability, bioequivalence and biopharmaceutics classification system. New scientific approaches to international regulatory standards. Eur J Pharm Biopharm. 2003; 50:3–12.
167. Valleri M, Mura P, Maestrelli F, Cirri M, Ballerini R. Development and evaluation of Glyburide fast dissolving tablets using solid dispersion technique. Drug Dev Ind Pharm. 2004; 30:525–34.
168. Poonam Kailas Ghule, Sadik F Sayyad, Dr. Sanjay R. Chaudhari. Self microemulsifying drug delivery system: a method for enhancement of bioavailability. World journal of pharmaceutical research. 2016; 5(7):578-590.
169. Patil Prashant P, Kate Vaishali, Payghan Santosh. Potential Investigation of Peceol for formulation of Ezetimibe self nano emulsifying Drug Delivery Systems. Asian Journal of Biomedical and Pharmaceutical Sciences. 2016; 6(54): 21-32
170. Azeem A Mohammad Rizwan, Farhan J. Ahmad, Zeenat Iqbal, *et al.* Nanoemulsion components screening and selection: a technical note. AAPS Pharm Sci tech. 2009; 10: 69-76.
171. Eccleston, J. 1994. Microemulsions. In: Swarbrick, J. & Boylan, J C. (eds.) Encyclopedia of Pharmaceutical Technology. New York, USA: Marcel Dekker. pp. 375–421.

172. Borhade VB, Nair, HA, Hegde DD. Development and Characterization of Self-Microemulsifying Drug Delivery System of Tacrolimus for Intravenous Administration. Drug Development and Industrial Pharmacy. 2009; 35: 619-630.
173. Sullivan JR, Gad, SC, Julien, M. A review of the nonclinical safety of Transcutol®, a highly purified form of diethylene glycol monoethyl ether (DEGEE) used as a pharmaceutical excipient. Food and Chemical Toxicology. 2014; 72: 40-50.
174. Schulman JH, Montagne JB. Formation of microemulsions by amino alkyl alcohols. Ann N Y Acad Sci 1961; 92:366-371.
175. Lawrence MJ, Rees G D. Microemulsion-based media as novel drug delivery systems. Adv. Drug Deliv. Rev. 2000; 45: 89-121.
176. Warisnoicharoen W, Lansley, AB, Lawrence, MJ. Nonionic oil-in-water microemulsions: the effect of oil type on phase behaviour. International Journal of Pharmaceutics. 2000; 198: 7-27.
177. Yang SC, Benita S. Enhanced absorption and drug targeting by positively charged submicron emulsions. Drug Development Research. 2000; 50: 476-486.